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Inbreeding, heterosis, fertility, plasmon differentiation and Phytophthora resistance in Solanum verrucosum Schlechtd., and some interspecific crosses in Solanum

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Contents

1 Introduction

The common potato, *Solanum tuberosum* is the major field crop in the Netherlands and an important crop in many other countries. It belongs to subsection *Hyperbasarthrum* of the section *Tuberarium* of the genus *Solanum*. Representatives of this subsection occur in both wild and cultivated state from the south of the United States (40 °N) through Mexico and Central America to South America (45 °S). According to Hawkes (1963), whose classification will be followed here, almost 170 potato species are known, belonging to at least 17 taxonomic series. The subsection *Hyperbasarthrum* includes species with different ploidy levels, beginning with diploids and ending with hexaploids with 72 chromosomes.

Most of the cultivated potatoes occurring in the world belong to *S. tuberosum*. Six other species, however, are also cultivated though restricted to South America. It is known that the first potato grown in Europe has been introduced from South America in the second half of the sixteenth century.

Wild species of *Solanum* are present under widely varying ecological conditions: in cool high altitude areas, high-rainfall mountain regions as well as in dry valleys and deserts.

During their many years of cultivation the *S. tuberosum* varieties have shown a lack of resistance to various diseases, particularly late blight. Attention was consequently focused on the wild relatives to provide this resistance. According to Hawkes (1967) 'wild species can contribute genes to the cultivated potato for resistance to nearly every potato disease, and also may be used to extend its range of adaptation by providing resistance to drought, frost and other climatic extremes'.

Most of the wild species are diploids with 24 chromosomes. The direct crossing of such species with the cultivated varieties (48 chromosomes) is not expected to contribute greatly to breeding because of the sterility of the triploid hybrids. Consequently, doubling the chromosome numbers of such species, or crossing them with the hexaploids has been practised to provide better material for crossing with the cultivated varieties.

Since the discovery of a suitable method to extract haploids with 24 chromosomes from the cultivated varieties, the use of diploid species is becoming promising in direct crossing with such haploids. The extracted haploids are self-incompatible and mostly male sterile. Accordingly, self-compatible, male fertile wild species are to be preferred as partners in crossing with haploids.

All diploid species are generally self-incompatible, except five. Three of these five, *S. etuberosum*, *S. polyadenium* and *S. verrucosum* have been used in these studies,

together with several self-incompatible species and haploids from different taxonomic series within the subsection *Hyperbasarthrum*. The major aim has been the study of the possibilities of crossing these species and haploids with each other.

In particular, *S. verrucosum* has been extensively investigated. This is a Mexican species and species from that country are in general valuable sources of resistance to late blight. *S. verrucosum* is accepted as the ancestral species of series *Demissa* which probably constitutes a link between this series and series *Tuberosa* of South America.

There are two prerequisites in using wild species to improve cultivated varieties: firstly there must be sufficient variability to facilitate the selection of suitable starting material, and secondly such species have to be crossable with the cultivated ones. The variability within *S. verrucosum* has never been studied systematically and contradictory reports have even been published on its self-compatibility. It was thus necessary to start the investigations with a systematic study of the variability within *S. verrucosum*, including the effects of inbreeding and crossing and its resistance to various races of *Phytophthora infestans* (Mont.) de Bary.

Some *S. verrucosum* introductions were reported by Buck (1960) to have sterilizing plasmon, whereas the plasmon of one introduction was considered to be normal. If this statement is correct, introductions with non-sterilizing plasmons must be preferred in interspecific crossing. The crossability of 21 *S. verrucosum* introductions with several species and haploids was investigated to explore the correctness of the above statement and the possible existence of barriers to crossing or to gene exchange. This could provide information about the complications which may be expected in using this species, and thus also offer some indications as to how this species should be handled in breeding.

There are some hypotheses to explain unilateral incompatibility i.e. the phenomenon that crossing self-compatible and self-incompatible species is successful only in one direction, with the latter species as male partners. How far is this true in connection with the use of *S. verrucosum* and the other two self-compatible species? What are the results of crossing between self-compatible and self-incompatible populations? Are there any reproductive barriers between them? What are these barriers and the evolutionary forces behind their development? Can the hypotheses published on the unilateral relations simply account for the relation between self-compatible and self-incompatible species, their crossability and the expected behaviour of their hybrids, or a new hypothesis can be more satisfactory in this respect?

Reports on the studies of self-incompatibility in the cultivated diploid species do not always agree. Whereas some authors think of a simple system controlling incompatibility in these species, others do not. *S. phureja* and *S. stenotomum* are the two cultivated diploids used to illustrate this point.

2 Review of literature[1]

This chapter has been divided into different sections dealing with inbreeding (2.1), interspecies crossing (2.2) both subdivided for polyploids and diploids; a section on self- and cross-incompatibility (2.3) follows, and a final section (2.4) on *Phytophthora infestans* with particular reference to studies on *S. verrucosum* concludes this chapter.

2.1 Inbreeding in tuber-bearing Solanum

2.1.1 Inbreeding in tetraploids

Inbreeding in tetraploid varieties has received some attention in literature. In all cases harmful effects of inbreeding have been mentioned.

Several authors have reported on the effects of inbreeding (Salaman, 1910; Salaman & Lesley, 1922; Krantz, 1924, 1946; Krantz & Hutchins, 1929; Guern, 1940; Nečiporčuk, 1949; Hagberg & Tedin, 1951; Deshmukh & Verma, 1960; Pushkarnath, 1961; Mullin & Lauer, 1966; Paxman, 1969). Differences between varieties in their reaction to inbreeding have been mentioned by Krantz (1924) and Pushkarnath (1961).

With regard to inbreeding in tetraploids, the progeny may vary. Non flowering, non tuberiferous progeny occurs and inbred progeny differs in growth habit, vine growth, leaf size, and tuber characteristics. In addition short internodes, prostrate plants, simple-leaved types, seedlings deficient in chlorophyll, dwarfs and plants with tuber abnormalities are found (Salaman, 1910; Salaman & Lesley, 1922; Krantz, 1924, 1946; Krantz & Hutchins, 1929; Guern, 1940; Deshmukh & Verma, 1960; Pushkarnath, 1961).

Inbreeding greatly affects the fertility of the plants. Reduction in, or a total lack of pollen production and reduced seed set have been reported by Salaman (1910) and by Salaman & Lesley (1922). This reduction in fertility obviously restricts the continuation of inbreeding and limits selection to the seed setting plants (Salaman, 1910; Krantz, 1946; Krantz & Hutchins, 1929).

A series of experiments to study the effect of inbreeding by selfing and intraclonal crosses as compared with interclonal crosses in the Old Red Swedish variety and a clone of *S. tuberosum* ssp. *andigena* was undertaken by Hagberg & Tedin (1951). Hybrids showed higher values in germination percentage and energy, tuber yield and seed set per fruit as compared with the values reported after selfing and in intraclonal

1. For authors' names of species not mentioned in Table 1, see Hawkes (1963).

crosses, which varied among each other in the different experiments.

Paxman (1969) found the yield of the hybrids between *S. tuberosum* ssp. *tuberosum* and ssp. *andigena* to be the highest (153%), followed by hybrids within ssp. *tuberosum* (100%), within ssp. *andigena* (70%), selfed ssp. *tuberosum* (51%) and selfed ssp. *andigena* (46%).

Mullin & Lauer (1966) compared hybrids and inbreds crossed to two F_1 testers and found F_1, I_1 and I_2 to be about equal in general combining ability with respect to average yield and high yielding segregants, whereas I_3 and I_4 were less effective.

2.1.2 Inbreeding in diploids

Graham (1963) reported the self-compatible *S. verrucosum* to possess a buffering system against inbreeding effects. He pointed to efforts made to transfer *S. verrucosum* resistance to inbreeding to diploid species and haploids of *S. tuberosum*. Reports on effects of inbreeding in the self-incompatible species have been mentioned by some authors. Pushkarnath (1942) reported four out of eight 'varieties' of *S. chacoense* to be self-compatible due to the presence of fertility factors.

The bud pollination of *S. subtilius* and *S. caldasii* (both classified under *S. chacoense* by Hawkes, 1963) reported by Pushkarnath (1943) gave poor growing inbred progeny, with many yellow and variegated seedlings, most of which died before transplanting. Several inbreds did not flower or flowered only sparsely. Most flowers contained degenerated anthers. Recently, Hermsen (1969) found clear variations in vigour, growth habit, leaf size and shape, flowering, tuber yield, and pollen fertility in inbreds of *S. chacoense* obtained by extracting haploids from colchicine-doubled tetraploid *S. chacoense* CPC 1153; of the 62 haploids investigated, 26 were reported to have less than 20% good pollen.

Lamm (1945) found the selfed progeny of *S. rybinii* (= *S. phureja*) to vary in morphological characteristics. Pollen stainability ranged from 0–90% (the parent clone field no. 3918 showed 70% stainable pollen grains). The author assumed the presence of genotypical differences in chiasma frequency among the inbred plants. Off-types were observed in the inbreds. One of the plants showed most of its anthers to be pistilloids. He also found an inbred plant of *S. stenotomum* in which most of the flowers had six-lobed calyx and corolla and six anthers.

S. phureja CPC 979 was reported by Dodds (1956) to set fruits enclosing many seeds. Paxman (1958, 1960) tried to break the self-incompatibility of that *S. phureja* line by inbreeding, crossing between the most self-fertile inbreds and selection in the progeny on the basis of berry set after selfing. Although no direct reference was made to the fertility of the inbreds, it seemed that both male and female fertility had been affected. The report of crossing the best fertile inbreds suggested that different degrees of fertility were met with in the inbred population. Also low seed set was reported for I_1 and I_2 populations (36 and 42 seeds per berry, respectively), which should be considered as inbreeding depression because 250 seeds per berry were recorded for compatible crosses.

Inbreeding depression in related interclonal hybrids of *S. stenotomum* ssp. *goniocalyx* where 40% poor hybrid seedlings were found, has been reported by Dodds & Paxman (1962).

2.2 Interspecific crossing in Solanum

Hawkes (1958b) arranged the tuber-bearing potato species into five fertility groups which are intra-fertile but inter-sterile:

(1) species of series *Tuberosa*, *Commersoniana*, *Megistacroloba*, *Cuneoalata*, *Piurana*, *Conicibaccata*, (*Ciraeifolia*), *Acaulia*, *Demissa* and *Longipedicellata*;
(2) series *Polyadenia*;
(3) series *Bulbocastana*;
(4) series *Morelliformia*;
(5) species of series *Cardiophylla* and *Pinnatisecta*.

Several publications reported on the interspecific hybridization and barriers: Howard & Swaminathan (1952); Swaminathan & Howard (1953); Hawkes (1958a); Howard (1960); Graham & Dionne (1961); Grun (1961); Magoon et al. (1962). In the following mention will be made of interspecific hybrids being sterile owing to the interaction of male genome with female plasmon.

2.2.1 Plasmon-genic sterility at the polyploid level

Lamm (1941, 1945) found the hybrids between female *S. curtilobum* (2n = 60) and male *S. tuberosum* to be male fertile, whereas the reciprocal was male sterile with reduced female fertility. Male sterility and reduced female fertility of the hybrids male F_1 (octoploid *S. acaule* × *S. tuberosum*) in *S. tuberosum* plasmon and the fertility of the reciprocal was reported by Lamm (1953). The author referred to a similar case of sterility found by Ivanov (1939) of hybrids of *S. antipoviczii* (= *S. stoloniferum*) in *S. tuberosum* cytoplasm.

Irregularity of meiotic behaviour in hybrids between *S. demissum* and *S. tuberosum* was reported by Schnell (1948) to be due to the possible abnormal function of chromosomes in the cytoplasm. Male sterility based on gene-cytoplasm interaction was mentioned by Dionne (1961b) in the hybrids of female *S. demissum* with males *S. phureja*, *S. tuberosum* and *S. chacoense*. Pushkarnath & Kishore (1963) pointed to the possible presence of cytoplasmic sterility in *S. spectabile* (= *S. hougasii*) and *S. demissum* crosses. Ross (1966) reported pollen sterility of *S. stoloniferum* – *S. tuberosum* hybrids due to plasmatic effects. An uncertain case found by Johnstone (1941) might be interpreted as a case of gene-cytoplasm interaction. Johnstone found no improvement in fertility of the sterile hybrid *S. tuberosum* × *S. demissum* by doubling the chromosome number. The sterility might be due to interaction of *S. demissum* genes with *S. tuberosum* plasmon, but dominance of male sterility in the *S. tuberosum* female used by Johnstone would lead to the same results.

2.2.2 Plasmon-genic sterility at the diploid level

The first investigations pointing to an interaction of genes and cytoplasm in diploid *Solanum* were carried out by Koopmans (1951, 1952, 1954, 1955, 1959). Both interaction of *S. chacoense* genes with *S. rybinii* (= *S. phureja*) cytoplasm and of *S. chacoense* cytoplasm with *S. rybinii* genes led to the appearance of abnormal sex organs. The abnormalities were different, depending on the direction of the cross and the abnormality of the female. A nearly continuous range of abnormalities was found. Thus no simple segregation ratios could be established.

The presence in *S. chacoense* of a recessive gene leading to sterility in *S. phureja* cytoplasm was reported by Grun & Radlow (1960). Grun et al. (1962) found deformed flowers to be conditioned by a recessive gene (*df*) from *S. chacoense* in *S. phureja* and *S. stenotomum* plasmons, indehiscent anthers to be controlled by the interaction of dominant genes (*In*) in *S. chacoense* plasmon (indehiscence) and a recessive gene (*pl*) determining 'pollenless' in *S. phureja* and *S. stenotomum* plasmons.

A wide spread of the *In* genes was reported by Grun & Aubertin (1963) and five of them were discovered, any of which leading to indehiscence in sensitive plasmon. Such genes were found in *S. phureja*, *S. stenotomum* and *S. kurtzianum* (Grun & Aubertin, 1965) whereas *S. chacoense* showed to possess the sensitive plasmon. Some plasmons of *S. chacoense* were found to have dominant restorer genes epistatic to the *In* genes.

Sterility of hybrids between haploids and some diploid (mainly cultivated) species was found to be due to gene-cytoplasm interaction (Peloquin & Hougas 1961; Hougas & Peloquin 1962; Howard 1968).

Plasmon-genic sterility was reported also in hybrids with *S. verrucosum* plasmon (Buck, 1960; Grun et al., 1962). Buck (1960) used *S. verrucosum* PI 195171 and PI 195172 as females in crosses with the diploid species *S. chacoense*, *S. macolae* (= *S. kurtzianum*), *S. phureja*, *S. simplicifolium* (= *S. microdontum* ssp. *gigantophyllum*), *S. stenotomum* and *S. toralapanum*. He found the F_1 hybrids to be male sterile. Backcrossing with male parents gave male sterile progenies, whereas backcrossing with *S. verrucosum* segregated 1 sterile : 1 fertile. Male sterility was assumed to be controlled by the interaction of one dominant gene from self-incompatible species with *S. verrucosum* cytoplasm. On the other hand the hybrids between female *S. verrucosum* PI 161173 and *S. macolae*, *S. phureja*, *S. simplicifolium* and *S. tarijense* had pollen grains of 'normal appearance' and therefore the cytoplasm of this *S. verrucosum* introduction was considered by Buck to be normal.

Grun et al. (1962) used *S. verrucosum* PI 160228 in crosses with different species. They observed the same type of sterility as found by Buck. The authors named this type of sterility 'lobed sterility' (*Lb*). The microspores were reported to have 4-lobed appearance in this type of sterility. Genes leading to this 'lobed sterility' were detected in *S. chacoense*, *S. stenotomum*, *S. kurtzianum*, *S. famatinae* (= *S. spegazzinii*), *S. simplicifolium* and *S. vernei*.

When crossing *S. verrucosum* CPC 2514 and CPC 2515 with different species, Marks (1965a) found two hybrids to show exceptional behaviour. These were with

males *S. chomatophilum* and *S. tuquerrense* (both in the series *Piurana*). The hybrids showed breakdown near flowering time. It is possible that such behaviour is due to gene-cytoplasm interaction.

2.3 Self- and cross-incompatibility in haploids and diploid Solanum species

Self- and cross-incompatibility in *Solanum* has been subjected to extensive studies. The gametophytic system with either one locus or two loci has been reported to occur.

Self- and cross-incompatibility was found to be inherited according to the one locus gametophytic system in *S. chacoense*, *S. caldasii*, *S. subtilius*, *S. jujuyense* and EPC 143 (*S. subtilius*) (all belong to *S. chacoense*), by Pushkarnath (1942, 1945) and Pal & Pushkarnath (1942, 1944). The same system was reported by Dodds & Paxman (1962) to occur in *S. rybinii*, *S. kesselbrenneri*, *S. ascasabii* (the three are *S. phureja*) and *S. stenotomum* where the maximum number of the intra-incompatible inter-compatible groups found was four. Pandey demonstrated the one-locus gametophytic system for the following species and hybrids: *S. megistacrolobum* (Pandey, 1960a), *S. kurtzianum*, *S. simplicifolium* and *S. michoacanum* (Pandey, 1962b), *S. vernei*, *S. sparsipilum*, *S. soukupii*, *S. chacoense* × *S. soukupii*, *S. simplicifolium* × *S. soukupii* and reciprocal, *S. simplicifolium* × *S. chacoense* and *S. sparsipilum* × *S. simplicifolium* (Pandey, 1962c). Furthermore this one-locus system was found to operate in hybrid of haploids US–W 246 and US–W 253 (Cipar et al., 1960), in haploids ssp. *andigena* – *S. phureja* hybrids (Cipar, 1963, 1964), in *S. phureja* PI 225682, PI 195198, *S. stenotomum* PI 230513 and the hybrids between *S. phureja* and ssp. *andigena* haploid US–W 253 (Cipar et al., 1964a), in hybrids between different *S. phureja* and *S. stenotomum* introductions with the haploids US–W 42 and US–W 253 (Cipar et al., 1967).

The presence of a two-loci gametophytic system was reported by Pandey (1960b, 1962a, 1962c) in *S. ehrenbergii* (= *S. cardiophyllum* ssp. *ehrenbergii*), *S. pinnatisectum* and *S. bulbocastanum*. The two loci *S* and *R* in *S. pinnatisectum* were discovered by Pandey (1962a) to interact in a complicated way. Dominance relations exist among the *S* alleles. *R* alleles are hypostatic to *S* alleles, but *R* alleles in homozygous condition are epistatic over *S* alleles. Pandey assumes a sporophytic determination of the growth determining unit of the incompatibility complex and gametophytic determination of the specificity reaction. The hybrid plants were found to consist of six intra-incompatible inter-compatible groups, one of which ($R_{IC}R_{IC}$) being female incompatible with the other five groups.

An incompatibility behaviour deviating from the normal system has been reported by Carson & Howard (1942) in crosses between *S. rybinii* and *S. boyacense* (both *S. phureja*), by Pal & Pushkarnath (1944) in *S. aracc-papa* (see Hawkes 1963) and *S. rybinii*, by Bains (1954) in *S. rybinii*. The latter author believes that the incompatibility system in the diploids is more complicated than is assumed in most reports. Irregularity of incompatibility behaviour was found in the hybrid *S. rybinii* × *S. subtilius* by Pushkarnath (1953b), in *S. phureja* CPC 979 by Dodds (1956) who was

not able to classify the inbred progeny in the expected incompatibility groups and who referred to similar observations from Marks in *S. chacoense*. Pandey (1962b) found crosses between *S. megistacrolobum*, *S. sanctae-rosae* and *S. toralapanum* to show the one locus system, but with irregularities; three compatible groups were detected in reciprocal crosses between *S. simplicifolium* and *S. phureja* whereas crosses of *S. infundibuliforme* with *S. phureja* and ssp. *goniocalyx*, crosses between *S. vernei* and *S. simplicifolium*, and *S. gourlayi* × *S. leptophyes* showed irregular expression of incompatibility behaviour to such an extent that Pandey mentions an 'almost complete breakdown of expression'. The irregularity of the incompatibility behaviour caused Pushkarnath (1953b) to assume the presence of independent incompatibility systems in series *Tuberosa* and in series *Commersoniana*. Also independent incompatibility genes were reported by Pushkarnath (1959) to operate within series *Cardiophylla*, *Pinnatisecta*, *Commersoniana* and *Tuberosa*.

Cases of reciprocal differences have been reported by some authors. Emme (1936; cited by Lamm 1945) found one-way incompatibility between *S. aracc-papa* and *S. bukasovii*; Kovalenko & Sidorov (1939; see Lamm, 1945) found *S. ajanhuiri* to be crossable only as female partner, and Bukasov (1933; cited by Pushkarnath, 1953a) found one-way incompatibility in *S. aracc-papa* and *S. bukasovii*. Choudhuri (1944) reported *S. rybinii* and *S. phureja* to accept freely *S. simplicifolium* pollen, whereas the reciprocal did not. The presence of an *R* locus independent of *S* alleles was reported by Pushkarnath (1953a) in *S. aracc-papa*. This *R* locus, when present in the style in homozygous or heterozygous condition leads to prevention of fertilization by pollen grains carrying incompatibility alleles. Such *R*-carrying plants can be used only as male partners. A similar *R* locus acting in the ovary resulting in the cease of fertilization has been assumed by Malheiros–Gardé (1959a) to operate beside the *S* genes. Pandey (1957) suggested the *R* gene to have sporophytic effect in the pollen, so that specificity of *S* alleles is removed.

All reports indicated above clearly show that much is still to be elucidated in the incompatibility phenomenon in *Solanum*.

2.4 **Phytophthora infestans**

Black et al. (1953) proposed a designation of races of late blight and the resistance genes from *S. demissum*. On that basis, 13 races known at that time were named. This nomenclature was accepted internationally. Sixteen races could be identified, the most complex being Race 1.2.3.4. The genes discovered at that time were four: R_1, R_2, R_3, R_4. Later more complex races were recorded by Eide et al. (1959) indicating the existence of two more *R* genes, R_5 and R_6. Presence of *R* genes was reported in *S. stoloniferum* by Schick et al. (1958) and McKee (1962). Recently, Malcolmson & Black (1966) discovered three additional *R* genes in seedlings with *S. demissum* ancestry; they were designated R_7, R_8 and R_9.

The possibilities and historical significance of the use of wild species in breeding for resistance to *Phytophthora* were reported by Rudorf et al. (1950), Toxopeus (1964),

Mastenbroek (1966) and Ross (1966).

The resistance to *Phytophthora* in *S. verrucosum* has been reported by several authors. Reddick (1940) stated that Swiss immigrants had sent *S. verrucosum* home from Mexico in 1850. According to Correll (1962), Alphonse de Candolle assumed *S. verrucosum* to be cultivated for many years near Geneva, but because of the small tubers and the 'unexpected' susceptibility to *Phytophthora*, its cultivation was abandoned in spite of the excellent flavor of the tubers. So it seems that the resistance of *S. verrucosum* to *Phytophthora* has been known for a long time. Resistance of *S. verrucosum* to *Phytophthora* was reported by Hekkel (1898), Stuart (1905), Bukasov (1936), Reddick (1940), Castronovo (1950), Rudorf (1950), Rudorf et al. (1950), Rudorf & Schaper (1951), Wriedt (1955), Lebedeva (1959), Black & Gallegly (1957), Graham (1963) and Toxopeus (1964). Resistance and susceptible *S. verrucosum* were reported by Castronovo (1950), Rudorf et al. (1950), Wriedt (1955), Black & Gallegly (1957) and Graham (1963). The *S. verrucosum* material tested by Kotova (1966) showed susceptibility to *Phytophthora*.

Unfortunately in almost all the reports no mention has been made of the introductions of *S. verrucosum* used in the studies.

3 Material and Methods

3.1 Plant Material

The plant material used in the investigations is listed in Table 1; additional data are referred to at the appropriate places in the text. The material was most kindly put at the author's disposal by R. W. Ross (Potato Introduction Station, Wisconsin, USA), J. G. Hawkes (University of Birmingham, England), G. E. Marks (John Innes Institute, Hertford, England), H. Ross (Max Planck Institut, Köln, W. Germany), J. G. Th. Hermsen (Institute of Plant Breeding, Wageningen, the Netherlands) and N. van Suchtelen (Foundation for Agricultural Plant Breeding, Wageningen, the Netherlands).

Where two or more numbers are in use for the same seed collection (e.g. *S. verrucosum* PI 251756 = Haw 1542 = CPC 2644) the number given by the seed donor is used in the text.

3.2 Methods

Seeds were sown early in March in seed pans (25 × 25 × 8 cm), placed in a heated glasshouse (about 18 °C). About three weeks later the seedlings were transplanted, 3 cm apart, into other pans of the same size, with 64 seedlings per pan. They were kept there for a few weeks and then planted individually in pots 11 cm in diameter, kept outdoors in cold frames covered during rainy spells or when night frost was expected. When the seedlings in the small pots had grown up to 10 or 15 cm, they were

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Explanation to Table 1.

Cor : D. S. Correll
Gra : K. M. Graham
Haw: J. G. Hawkes
Hje : J. P. Hjerting
Och : C. Ochoa

CCC : Colección Central Colombia, Colombia
CPC : Commonwealth Potato Collection, England
EBS : Erwin Bauer Sortiment, W. Germany
MPI : Max Planck Institut, W. Germany
PI : USDA Plant Introduction Number, USA
WAC: Wageningen Potato Collection, Netherlands
WRF : Wisconsin Research Foundation, USA

AH: Primary Haploid from *S. tuberosum* ssp. *andigena*
TH : Primary Haploid from commercial varieties and unnamed clones of ssp. *tuberosum*
SH : Secondary Haploid from haploid-haploid mating
1. For particulars of species, see Ross & Rowe (1965)
2. Abbreviations according to Simmonds (1963)

Table 1. The *Solanum* species and clones used in the experiments.

Series	Species and clones[1]		Numbers
Etuberosa	*S. etuberosum* Lindl.	*etb*[2]	PI 245939 = Cor 134
Bulbocastana	*S. bulbocastanum* Dun.	*blb*	PI 275188 = Haw 1585
Pinnatisecta	*S. jamesii* Torr.	*jam*	52 T from Poland × Haw 1143–4
	S. cardiophyllum Lindl.	*cph*	WRF 1274
	S. trifidum Corr. = *S. michoacanum* (Bitt.) Rydb.	*trf*	PI 283065 = Gra 308 × 299
Commersoniana	*S. chacoense* Bitt.	*chc*	CPC 1153
	S. tarijense Hawkes	*tar*	Haw 58
Piurana	*S. chomatophilum* Bitt.	*chm*	CPC 3515
Polyadenia	*S. polyadenium* Greenm.	*pld*	PI 275239 = Haw 1569
Cuneoalata	*S. infundibuliforme* Phil.	*ifd*	PI 283077 = EBS 1782
Megistacroloba	*S. megistacrolobum* Bitt.	*mga*	PI 210034 = Hje 1028
	S. raphanifolium Gard. et Hawkes	*rap*	Haw 694
Demissa	*S. verrucosum* Schlechtd.	*ver*	
	PI 160228 = Cor 14217b, PI 161128 = Cor 14252 = CPC 2514, PI 161173 = Cor 14252 = CPC 2515, PI 195171 = CPC 1340.2, PI 195172 = CPC 54.4, PI 251756 = Haw 1542(343) = CPC 2644, PI 255544 = Gra 327, PI 275255 = Haw 1527, PI 275256 = Haw 1528(341), PI 275257 = Haw 1532, PI 275258 = Haw 1546, PI 275259 = Haw 1548, PI 275260 = Haw 1658, PI 310966 = Ugent 1289, Haw 756, Haw 2246, Haw 1350(349), CPC 1339, CPC 2247 = Haw 1047, CPC 2623a, EBS 2632		
Tuberosa			
wild species:	*S. canasense* Hawkes	*can*	PI 265864 = EBS 1831
	S. kurtzianum Bitt. et Wittm.	*ktz*	Haw 4003
	S. leptophyes Bitt.	*lph*	PI 210056 = Hje 1592
	S. microdontum ssp. *gigantophyllum* (Bitt.) Hawkes et Hjerting	*gig*	Haw 42
	S. multidissectum Hawkes	*mlt*	PI 210052 = Hje 1407
	S. sparsipilum (Bitt.) Juz. et Buk.	*spl*	PI 210039 = Hje 1051
	S. vernei Bitt. et Wittm.	*vrn*	PI 230468 = CPC 2413
cultivated species:	*S. phureja* Juz. et Buk.	*phu*	
	PI 225682 = CCC 193, PI 225698 = CCC 157, PI 225702 = CCC 226, PI 243461 = CCC 88		
	S. stenotomum Juz. et Buk.		
	ssp. *stenotomum*	*stn*	WAC 425, WAC 780 = Och 2333
	ssp. *goniocalyx* (Juz. et Buk.) Haw.	*gon*	WAC 204 × 257
	S. tuberosum L. ssp. *tuberosum*	*tbr*	
	haploids: US–W1, US–W4, US–W42, US–W166, TH 62–15–3, TH 63–71–38, TH 63–78–77, TH 64–41–2, TH 64–69–14, TH 66–1–25, TH 66–29–3, TH 66–30–35, TH 66–31a, TH 66–36–2, TH 67–17–2, SH 66–110–2, SH 66–117–2		
	S. tuberosum L. ssp. *andigena*	*adg*	
	(Juz. et Buk.) Hawkes haploids: AH 66–88–14, AH 66–94–28, AH 65–41–2 (spontaneous)		
	S. tuberosum – *andigena* haploid: SH 66–114–2		
German material, probably of complex origin:			
	haploids H 114, H 141 and tetraploid MPI 19268		
Varieties and tetraploid selections:			
	Prof. Broekema, Profijt, Libertas, Gineke, Radosa, Humalda, Oberarnbacher Frühe, Dr. Mac Intosh, Black 5030(34), Black 3751(5), Black 3036e		

finally transplanted to their permanent site, either in 17 cm pots, or in the field. The pots were placed in peat on normal benches in insect-free temperature-controlled glasshouses, spaced to facilitate individual handling of the plants and left there from about early June till the end of the growing season (September–October).

In the last year (1969) the seeds were treated early in January with 0.2 % gibberellic acid for 24 hrs to break the dormancy period (Spicer & Dionne, 1961), allowed to germinate in petri dishes, and the germinating seeds were put directly into pans, 3 cm apart.

All plants growing in the glasshouses were trained to sticks and pruned when necessary, except when stem heights had to be measured. Where grafting onto tomato plants was necessary, it was carried out when the seedlings were about two months old; the plants showing visible signs of growth were transplanted into the soil of the glasshouses, 50 cm apart. The grafted plants were tied upright with raffia to sticks or ropes.

In the first field experiment, in 1966, where comparisons between *S. verrucosum* inbred numbers and the hybrids were made, a randomized complete blocks design with three replicates was used. In each plot 6 × 8 plants were left to grow 50 cm apart (144 plants per inbred or hybrid population). For *S. verrucosum* inbred PI 195172–237–10 only 42 plants were grown in each plot and the surplus holes were planted with other *S. verrucosum* seedlings. The border rows and the belt were planted with a cultivated variety.

In the field experiment of 1968, designed to study the inheritance of short stem in *S. verrucosum* CPC 1339, the plants of both parents and F_2 populations were grown on randomized rows, in duplicate, 40 cm apart, the rows at distances of 65 cm.

In the glasshouses, the plants were usually selfed one to three days after opening of the flowers, the flowers were labelled but not bagged, as contamination is improbable in insect-free glasshouses. Generally, selfing included five flowers per plant; it was repeated several times over one or two seasons as long as no berry set was obtained. Female fertility was measured according to the number of seeds per berry.

In crossing self-compatible species, flowers were emasculated in the late bud stage, one or two days before opening, and pollen from male parents was applied to the stigma the same day or one day after opening of the emasculated flowers. In crossing with the self-incompatible species and haploids as females, the flowers were not emasculated during the first season (1966) but, when self set seeds were obtained instead of the expected hybrids, emasculation was carried out in the following seasons.

The stainability test of pollen grains was applied between 10 a.m. and 3 p.m. (the most reliable period), about 24 hrs after anthesis. Staining was done using lactophenol acid fuchsine: 20 cc phenol, 20 cc lactic acid, 40 cc glycerine, 20 cc water, and 8 cc acid fuchsine 1 %.

In the first season (1966) two or more flowers per plant were used and 200 pollen grains were checked per flower. In the following seasons a mixture of pollen grains from two or three flowers per plant was used. In all cases the staining check was repeated at least twice, and the percentages of pollen stainability presented in this

report are averages of the pooled data. Only completely rounded, well-stained, and separated pollen grains were considered good ones. Male fertility was determined indirectly as the percentage of pollen stainability.

The hanging drop method as used by Mortenson et al. (1964) was applied to check the germination ability of pollen grains *in vitro*. For this purpose a mixture of pollen grains from different flowers was used and at least 200 grains were counted at the place on the slide where germination was best.

The pollen tube growth in styles was checked by staining with acid fuchsine light green stain (Darlington & La Cour, 1966); 48 hrs after pollination the pistils were fixed in acetic alcohol (1:3) and kept in the stain at 60 °C for 24 hrs in a water bath. All pollen tube growth tests were made in 1969 on emasculated flowers.

In counting plant chromosomes in root tip cells, the roots were kept in 8-hydroxyquinoline for about 24 hrs, fixed in Carnoys' 2 for 24 hrs, hydrolysed in 1*N* HCl at 60 °C for 8 min in a water bath, stained in Feulgen and then squashed in 1 % acetocarmine.

The number of chloroplasts in the leaf epidermis was counted after staining with iodine.

To study microspores, mature anthers were fixed in a mixture of absolute alcohol (6 parts), chloroform (3 parts) and glacial acetic acid (1 part) for 24 hrs, followed by hydrolysis in 1*N* HCl for 8 min at 60 °C in a water bath, stained with 2 % orcein and squashed in 1 % orcein.

For the study of meiosis, young anthers were fixed, kept in the refrigerator and checked at the end of the season or in winter. Fixation was carried out with a mixture of 3 parts absolute alcohol and 1 part propionic acid, previously saturated with iron acetate. Squashing as described by Swaminathan et al. (1954), in a drop of 1 % propiono-carmine followed.

All tubers were harvested separately and if needed in the following season were stored at 4 °C.

For the tests on late blight, slices of potato variety Bintje were inoculated with four races of *Phytophthora infestans* (4, 1.4, 1.2.3.4, 1.3.4.7.8) and two incompletely identified isolates (B19, 331). One week after incubation at 15 °C, the slices were almost completely covered with the fungus. The slices were then washed with distilled water and spore suspensions were made with 3, 10 or 20 spores per mm^3.

The day and morning prior to inoculation, the 64-seedling trays with the 8–12 cm high seedlings (with 6–8 leaf internodes) were moistened and in the afternoon the plants were sprinkled with about 20 cc of the suspension per tray in such a way that leaves and stems were entirely covered.

The sprinkled trays were then placed in metal pans containing water and covered with polyethylene metal-framed caps to maintain high humidity. They were kept in rooms at 16 or 18 °C. To avoid plant damage due to humidity, the caps were removed after 24 hrs for a period of 48–72 hrs. Trays were then dampened and covered again to ensure an ideal environment for the growth of the fungus.

The seedlings were assessed for late blight 7–10 days after inoculation. Resistant

seedlings (no sporulation) were maintained.

Statistical analysis, where necessary, was carried out according to Snedecor (1965) and Briggs & Knowles (1967). The χ^2- test was used to check the fit of the observed numbers to the expected ratios even in cases of small numbers in the smaller classes. In such cases, the observed numbers showed good fit to expectations. Accordingly the use of χ^2 in such cases is not contradictory to the general use of this test, as the ratios which fit in χ^2 test also fit when the binomial distribution is used.

Inbreeding and heterosis have been expressed in percentages. The effect of inbreeding is calculated from the formulae

$[(\text{mean } I_n - \text{mean } I_{n+1})/\text{mean } I_n] \times 100$ or $[(\text{mean } F_1 - \text{mean } F_2)/\text{mean } F_1] \times 100$.

Mean I_n = mean value for the character under study in the population derived from the original introduction by selfing n generations. Mean I_{n+1} = mean value of the population which has been obtained by selfing I_n. A positive value for inbreeding effect is called 'gain', a negative value 'depression'.

The term heterosis is used for the hybrids to indicate their positive deviation from the 'mid parent' value. It is calculated from

$[(\text{mean } F_1 - \text{mid parent value})/\text{mid parent value}] \times 100$.

The data of the parent populations (remnant seeds) or selfed progenies from the real parents (single plants) used in the crossings were used to calculate the mid parent values.

4 Inbreeding effects, heterosis and fertility within S. verrucosum

Inbreeding effects on *S. verrucosum* have been investigated along three lines: firstly inbreeding without selection (four CPC introductions), secondly comparisons between selected and unselected populations in several inbred generations (PI 195172 and PI 275258), and thirdly the effect of inbreeding on hybrid populations. Hybrids between introductions of *ver* were sometimes incorporated to compare the effect of inbreeding with that of crossing.

4.1 Variability within and between inbreds and hybrids of S. verrucosum

4.1.1 Variation in the four CPC introductions; CPC 1339 (I_2), 2247 (I_1), 2514 (I_1) and 2644 (I_1)

The material for this experiment which was conducted in the glasshouse consisted of the CPC introductions 1339 (I_2), 2247 (I_1), 2514 (I_1) and 2644 (I_1). Each seed sample was divided into two lots. The first was sown for the 1966 studies and the second was stored to enable comparison of two inbred generations in 1967. Several characters were studied in each population.

Leaf dimensions were measured. They included length (L) and width (W) of the second lateral leaflet from the top of the rachis, together with the length of the terminal leaflet without and with its rachilla (T and D respectively).

The largest simple leaf was used for seedlings' measurements, while in full-grown plants the largest compound leaf on the middle of the stem was used. Leaves at the top of the stem were avoided because of their relatively small sizes and those at the bottom were not used because of the difficulty of standardization.

Three of the four leaf measurements were found by Simmonds (1964a) to be discriminative for both subspecies *tuberosum* and *andigena* of *S. tuberosum*. The L, W and D values used in this publication coincide respectively with the e, f and d symbols used by Simmonds.

Wide variations within each inbred population were found for nearly all investigated characters. Plants with abnormally stunted growth (Plate 1: two plants below) and plants with curled leaves were observed. Such plants showed no signs of disease. Pollen stainability varied from 10 to 100% (Fig. 1). The number of seeds per berry, set after selfing, ranged from 2 to more than 200 (Fig. 2). Variability in leaf measurements was also quite large. It is clear that the original clones from which the CPC populations were derived by selfing must have been very heterozygous, although

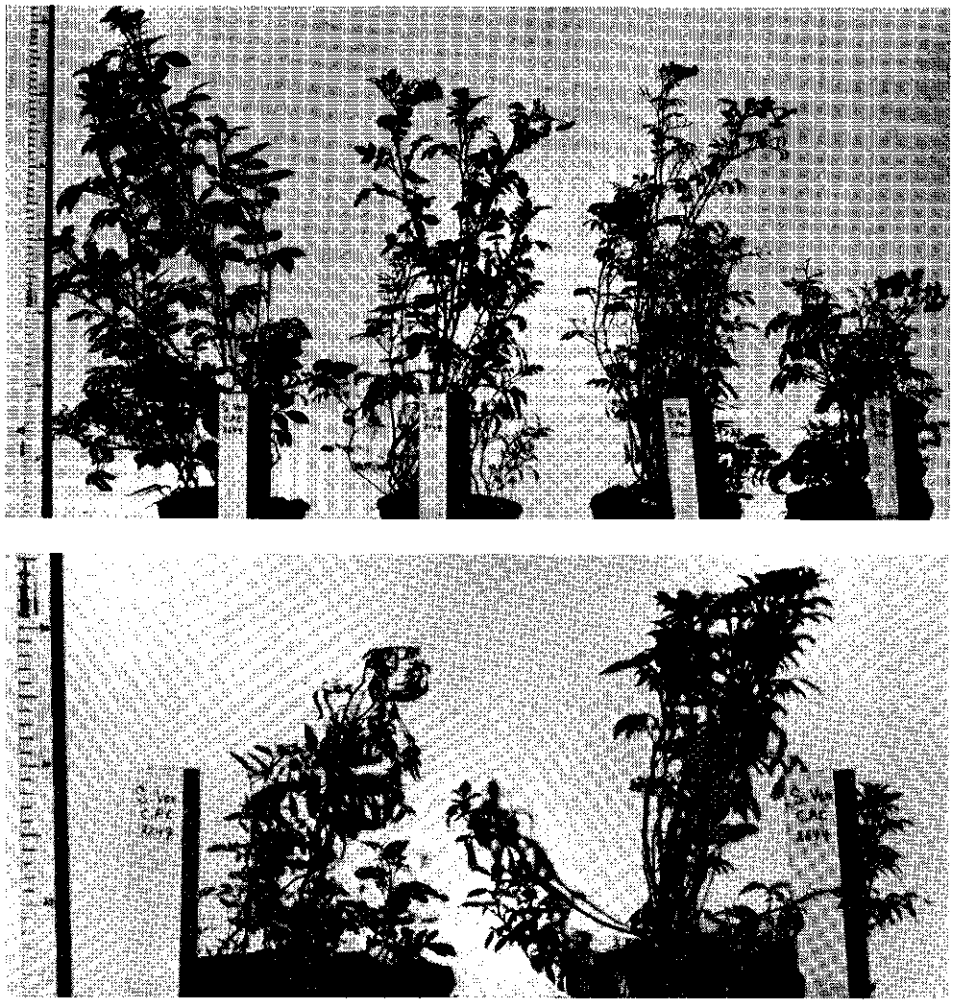


Plate 1. Plants of *S. verrucosum* (above from left to right) CPC 2644, 2247, 2514 and 1339 with normal growth and two stunted sisters (below). Notice the short stem of CPC 1339.

environmental variation of the characters studied should also be taken into account.

The mean values for some vegetative and generative characters are given in Tables 2 and 3. Some remarkable differences between the population means were observed. The low emergence rate of CPC 2644 might be due to the fact that this seed sample was harvested in 1959, whereas the seeds from CPC 1339, 2247 and 2514 were harvested in 1961, 1962, and 1963, respectively. Emergence rate after one week seems to be affected by the age of the seed.

Differences were observed in mean plant height of the populations. After one month both CPC 1339 and 2644 were significantly shorter-stemmed than 2514 and 2247, whereas by the end of the season, only CPC 1339 had retained its typically

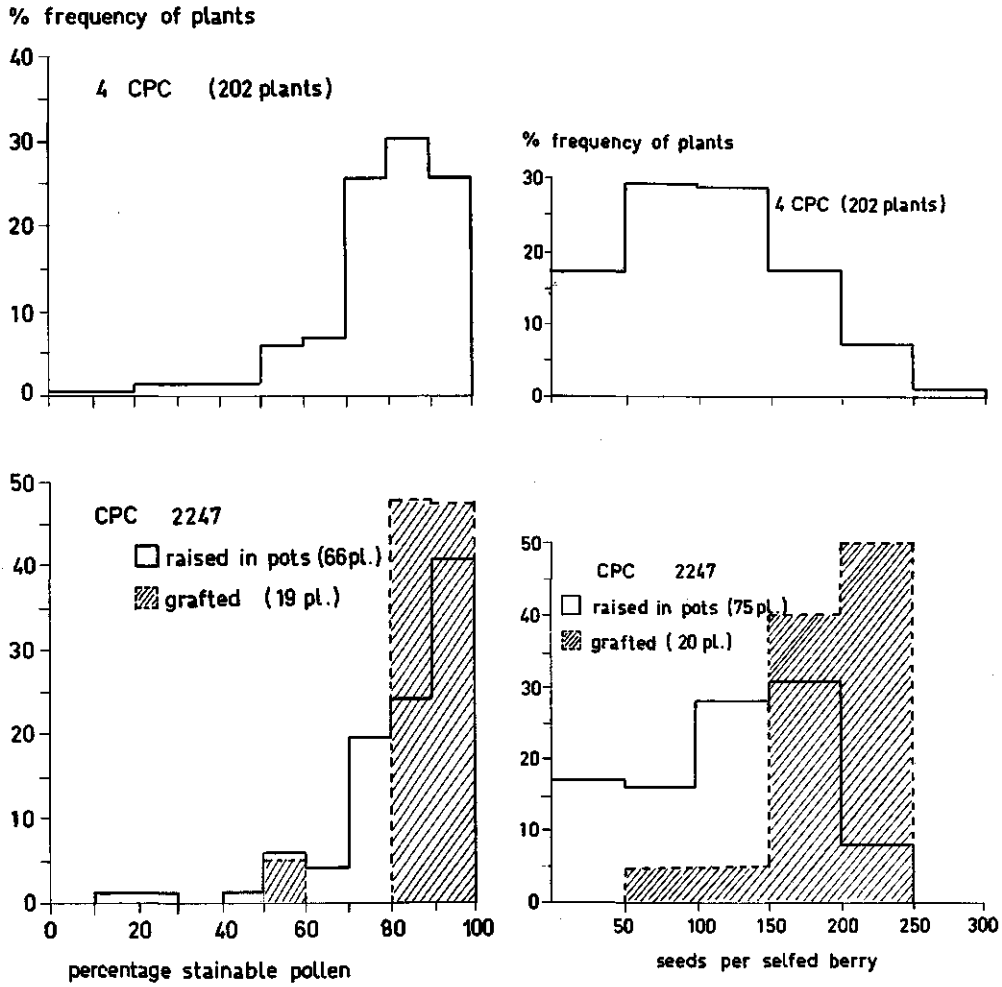


Fig. 1. Frequency distributions of percentage stainable pollen. Upper histogram: pooled data of CPC 1339–I_2, 2247–I_1, 2514–I_1 and 2644–I_1. Lower histogram: data of CPC 2247–I_1 raised in pots and grafted (glasshouse 1966).

Fig. 2. Frequency distributions of seeds per selfed berry. Upper: pooled data of CPC 1339–I_2, 2247–I_1, 2514–I_1 and 2644–I_1. Lower: data of CPC 2247–I_1 grown in pots and grafted (glasshouse 1966).

short stem. CPC 2247 ranked first for mean final plant height, tuberization, male and female fertility; CPC 1339 last. CPC 1339 was also exceptional in flowering later and in having larger leaves. However, mean berry set by selfing was twice as high in CPC 1339 and 2247 as in CPC 2514 and 2644.

The effects of grafting *S. verrucosum* CPC 2247 onto tomato can be read from Tables 2 and 3 and Figs. 1 and 2. Grafting caused a more vigorous growth and larger leaves, together with better mean fertility with less variation between the checked plants. The mean values for the L, W, and T leaf measurements, plant height, percen-

Table 2. Mean values for some vegetative characters of four CPC introductions of *Solanum verrucosum* (data of 1966). I_1 and I_2 are first and second inbred generations, respectively.

	Emergence %		Number of plants used	Plant height		Compound leaf measurements		Tuber yield per plant		Tuberiferous plants (%)
	after a week	final		after a month (mm)	at end of season (cm)	length (mm)	width (mm)	number	weight (g)	
CPC 1339 I_2	10.7	86.7	63	10.4	36.2	21.7	11.7	3.8	1.4	73
CPC 2514 I_1	70.0	78.3	46	15.3	52.0	13.8	7.0	10.7	4.5	93
CPC 2644 I_1	2.3	22.3	28	10.6	54.9	17.6	8.4	8.3	5.2	93
CPC 2247 I_1	26.9	86.0	86	16.0	58.5	18.7	10.6	15.0	10.0	99
Do. grafted			22		130.4	40.1	22.4			

Table 3. Mean values for some generative characters of the four CPC introductions of *Solanum verrucosum* mentioned in Table 2 (data of 1966).

	Number of selfed flowers	Number of berries set	Berry setting %	Number of seeds per		Stainable pollen grains (%)
				pollination	berry	
CPC 1339 I_2	6.3	5.1	82.4	78	94	75
CPC 2514 I_1	7.7	2.6	35.0	40	108	80
CPC 2644 I_1	8.2	3.3	44.7	48	115	79
CPC 2247 I_1	4.9	4.2	87.1	111	124	82
Do. grafted	6.8	6.0	88.2	168	190	88

tage of stainable pollen, percentage of berry set, number of seeds per berry and number of seeds per pollination of the non-grafted plants amounted to respectively 47%, 47%, 52%, 45%, 93%, 99%, 65% and 66% of the corresponding mean values of the grafted plants. Though berry set scarcely differed, flowering was promoted by grafting: more flowers were available throughout a longer period (flowering started somewhat later than in potted plants).

There is no reason to assume effective genetic differences between grafted and non-grafted plants. Both were taken at random from the same seed lot and almost all grafts succeeded. Consequently any differences are presumed to be largely due to grafting effects.

4.1.2 Variation in I_3 populations and $I_2 \times I_2$ hybrids from PI 195172 and PI 275258 (glasshouse)

I_3 and $I_2 \times I_2$ hybrid populations were studied to compare the effects of inbreeding and hybridization. The populations were grown mainly in the field, but for comparison plants from each population were also raised in the glasshouse.

I_2 seeds of the populations PI 195172–237 and PI 275258–119 and – 121 of *S. verrucosum* were sent by K. M. Graham to the late H. J. Toxopeus. Graham made the selection on the basis of resistance to *Phytophthora*. Four plants from PI 195172–237 (Nos. 6, 8, 9 and 10) were used as parents in crosses with two plants from both PI 275258–119 (Nos. 4 and 6) and PI 275258–121 (Nos. 1 and 6). In addition, six of the eight plants were selfed and from the other two seeds were taken from berries set spontaneously (spontaneous seeds). These seeds can be regarded as resulting from natural selfing, because all plants raised from them proved to be pure *S. verrucosum*.

4.1.2.1 The inbred populations (I_3) from PI 195172–237 (glasshouse)

Mean values for several vegetative and generative characters are presented in Tables 4 and 5 (upper parts). The differences between the means reached the level of significance in quite a number of cases (The F-test was used to check significance).

The inbreds seem to differ in growth-rhythm, as apparent from the mean values for stem height after one month and at the end of the season.

Length and width of simple leaves ran parallel, which was clear from Table 4 and from the small variation in W/L-ratio: 0.66–0.72. This does not hold true for the compound leaves.

The low percentage of tuberiferous plants in Inbred No. 6 (Table 4) and the low male and female fertility of Inbred No. 10 (Table 5) are worthwhile mentioning.

Figure 3 shows frequency distributions of pollen stainability in the four inbred populations from PI 195172–237. The female fertility distributions are presented in Fig. 4. Inbred No. 9 shows a greater variability than the other inbreds. The low fertility of Inbred No. 10 is also clear from Figs. 3 and 4.

Table 4. Average values for some vegetative characters in eight inbred and six hybrid populations of *Solanum verrucosum* grown in the glasshouse in 1966.

		Emergence (%)		Number of plants used	Stem height		Leaf measurements				Tuber yield		Tuberiferous plants (%)
							simple leaves		comp. leaves				
		after a week	final		after a month (mm)	at end of season (cm)	length (mm)	width (mm)	length (mm)	width (mm)	number of tubers	weight (g)	
PI 195172–237– 6	spont.	0.0	93.0	34	12.8	66.7	34.7	24.9	16.9	9.7	3.3	1.3	35
– 8	self.	3.5	90.2	34	14.6	57.9	33.2	23.1	15.4	9.2	6.8	2.2	74
– 9	self.	0.5	87.5	38	24.1	61.0	27.6	19.9	17.3	9.1	3.2	1.1	71
–10	self.	0.6	86.2	10	21.7	60.6	35.1	23.2	15.1	7.5	4.4	2.4	90
Average PI 195172		1.2	89.2	—	18.3	61.6	32.6	22.8	16.2	8.8	4.4	1.8	68
PI 275258–119– 4	self.	3.0	61.3	34	13.2	54.1	31.2	21.0	24.9	9.9	11.1	5.5	100
– 6	self.	53.8	83.8	30	19.3	48.7	34.6	23.8	21.1	9.1	14.6	7.3	97
–121– 1	self.	48.7	92.7	34	17.0	62.7	38.1	25.3	20.6	10.3	8.8	6.9	97
– 6	spont.	34.7	82.7	34	12.8	62.3	38.6	24.9	17.7	9.9	7.6	5.4	91
Average PI 275258		35.0	80.1	—	15.6	56.9	35.6	23.8	21.1	9.8	10.5	6.3	96
195172–237–6 × 275258–121– 5		52.7	88.3	34	22.5	73.0	42.3	29.2	25.1	13.3	8.6	4.0	94
–8 × – 5		54.6	89.4	34	26.1	59.0	40.1	28.5	22.5	11.8	14.7	8.1	97
275258–119–4 × 195172–237– 9		23.9	84.3	21	17.9	62.5	39.4	28.5	25.5	11.7	9.2	5.8	90
–6 × – 9		88.0	94.0	34	20.4	52.9	41.4	29.8	21.5	9.9	9.3	6.4	100
–121–1 × –10		70.0	94.7	34	20.3	55.8	40.2	27.4	18.7	9.9	12.2	6.5	97
–6 × –10		61.3	94.7	34	16.8	57.5	40.7	27.7	18.5	10.1	7.3	3.7	85
Average hybrids		58.4	90.9	—	20.6	60.1	40.7	28.5	22.0	11.1	10.2	5.8	94

The figures added to the original PI introduction number refer to seedling number in the two consecutive inbred generations. Self. = plants grown from seeds obtained by artificial selfing of parents; Spont. = plants raised from seeds of berries set spontaneously on the parents.
Significant differences (using F test) between means are indicated by].

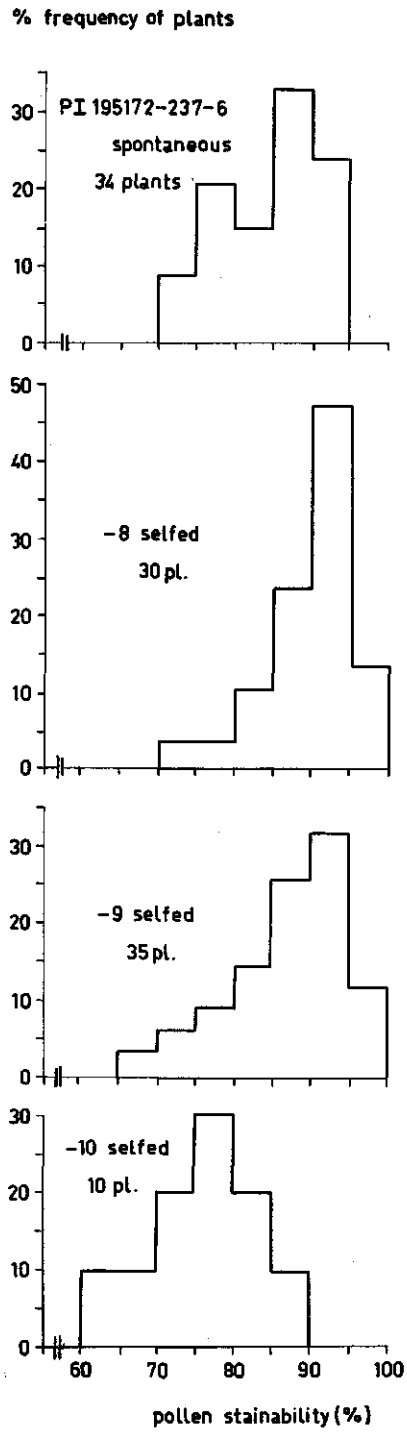


Fig. 3. Frequency distributions for pollen stainability of four inbred populations from PI 195172–237 (glasshouse 1966).

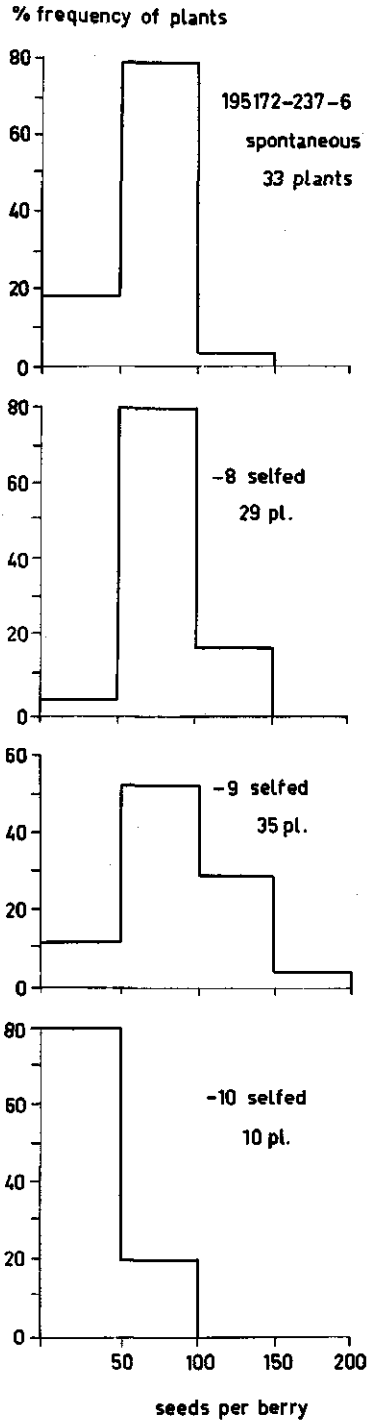


Fig. 4. Frequency distributions for seeds per selfed berry of four inbred populations from PI 195172–237 (glasshouse 1966).

Table 5. Number of flowers selfed, berry and seed set and percentage stainable pollen grains for the same inbred and hybrid populations as mentioned in Table 4.

		Flowers selfed	Berries set	Berry setting (%)	Seeds per pollination	Seeds per berry	Stainable pollen (%)
PI 195172–237– 6	spont.	5.9	5.0	89	62	69	85
– 8	self.	6.1	4.6	77	64	83	90
– 9	self.	6.5	5.8	92	79	85	88
–10	self.	5.7	3.5	62	23	35	76
Average PI 195172		6.1	4.7	80	57	68	84
PI 275258–119– 4	self.	5.5	4.7	85	135	155	75
– 6	self.	5.3	2.7	54	68	113	81
–121– 1	self.	6.9	3.7	58	49	81	79
– 6	spont.	6.7	4.3	71	56	82	78
Average PI 275258		6.1	3.9	67	77	108	78
195172–237–6 × 275258–121– 5		6.8	3.6	54	65	109	84
–8 × – 5		7.7	3.2	43	53	121	83
275258–119–4 × 195172–237– 9		5.4	4.7	88	128	139	81
–6 × – 9		6.9	3.6	52	52	96	74
–121–1 × –10		7.4	4.4	67	66	94	84
–6 × –10		6.9	4.6	72	57	82	87
Average hybrids		6.9	4.0	63	70	107	82

4.1.2.2 The inbred populations (I_3) from PI 275258–119 and – 121 (glasshouse)

Four inbred populations from PI 275258, two from each of Nos. 119 and 121, were raised on the same bench as the inbreds from PI 195172–237. The results are presented in the middle parts of Tables 4 and 5 and in Figs. 5 and 6. They are similar to those

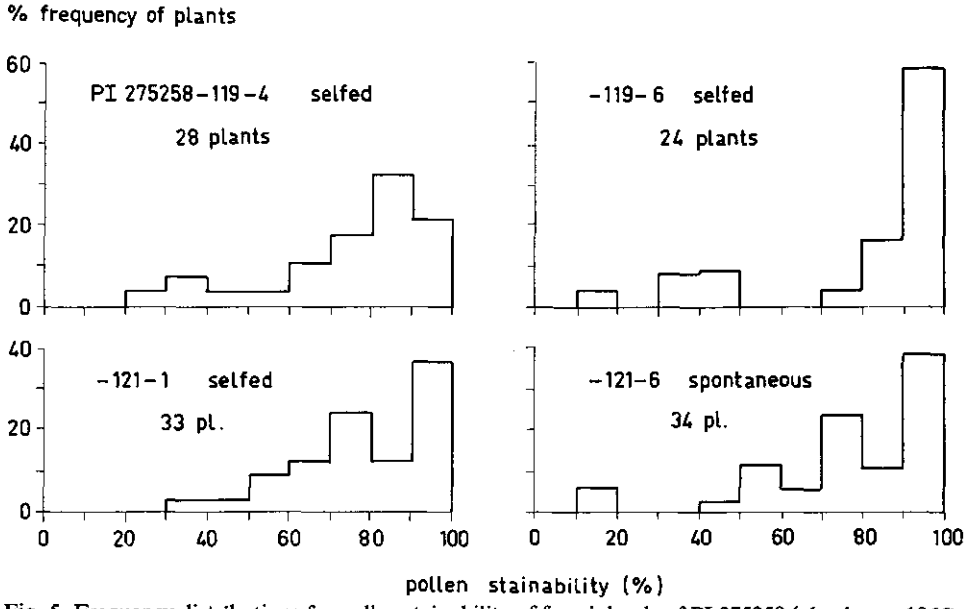


Fig. 5. Frequency distributions for pollen stainability of four inbreds of PI 275258 (glasshouse 1966).

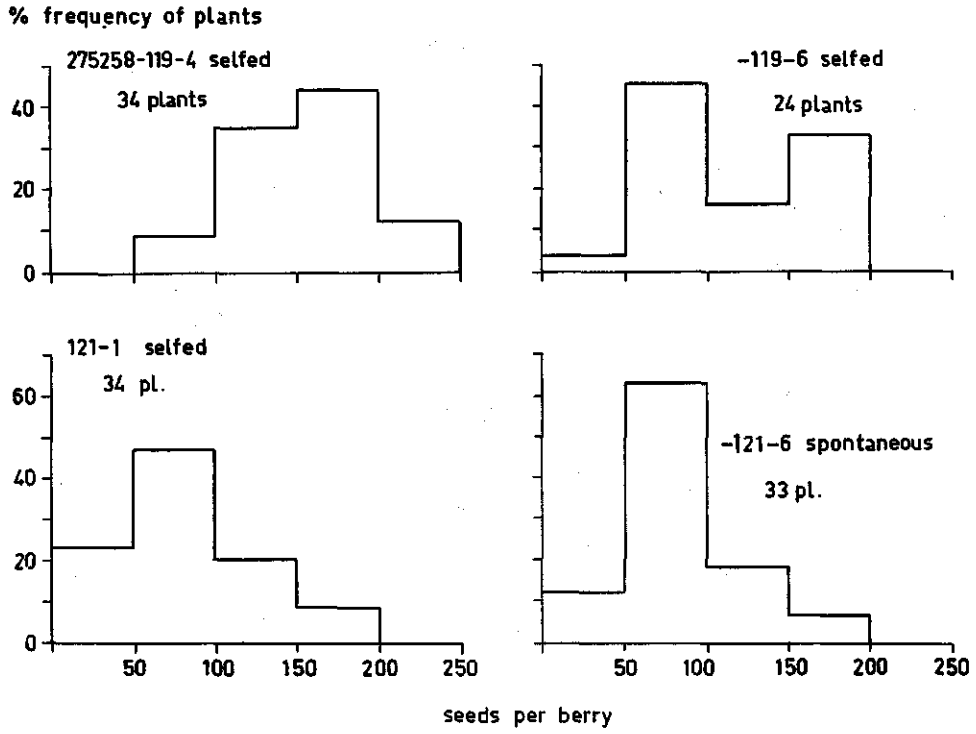


Fig. 6. Frequency distributions of seeds per selfed berry of four inbreds of PI 275258 (glasshouse 1966).

for the inbred populations from PI 195172–237: clear-cut conclusions about differences in growth-rhythm are hardly possible because quickness of emergence may interfere with stem height in the early growth stages.

Apart from the corresponding features of the inbreds from PI 195172 and PI 275258, some remarkable differences were found. Although inbreds of PI 275258 emerged quicker, their emergence was lower than that of PI 195172 inbreds owing to Inbred 119–4. In general PI 195172 had smaller leaves, longer stems, a lower tuber yield, more non-tuberiferous plants, a better berry set and higher pollen stainability, but a clearly lower seed set per pollination and per berry.

A comparison between Figs. 5 and 3 reveals that the inbred populations from PI 275258 had a greater within-population variability for pollen stainability than those from PI 195172, whereas the over-all means did not differ much (see also Fig. 7). A comparison between Figs. 6 and 4 also shows that female fertility within the inbreds of PI 275258 varied more than within those of PI 195172. But in contrast with male fertility, over-all female fertility was clearly higher in the PI 275258 inbreds.

4.1.2.3 The $I_2 \times I_2$ hybrids involving PI 195172 and PI 275258 (glasshouse)

Six hybrid populations, two with inbreds of PI 195172 and four with inbreds of PI 275258 as females, were grown to compare the effect of selfing (inbreds) and crossing

Plate 2. Hybrid *S. verrucosum* PI 195172–237–6 × PI 275258–121–5 compared with parent PI 195172–237–6 spontaneous in seedling stage.

(hybrids). The data on the hybrids are included in Tables 4 and 5 (lower parts).

In general the hybrids germinated earlier and showed larger seedlings than inbreds (Plate 2). This difference in vigour became less pronounced with age. However, most of the hybrids continued to show a better performance with all their plants surviving, contrasting with the inbred populations, in which 0–12.5% of the plants died during the season without any detectable cause. Hybrid plants flowered earlier and more profusely, whereas some inbred plants showed poor flowering and a few of them did not flower at all during the first season.

As apparent from Tables 4 and 5, the hybrid populations showed higher values for most of the characters as compared with their parents, though exceptions did occur. The differences between populations reached the level of significance not only when hybrids and inbreds were compared, but also among the hybrid populations. Hybrids showed better male fertility than inbreds of *ver* PI 275258, but did not show improvement over inbreds of *ver* PI 195172 (Fig. 7).

As demonstrated in Fig. 8, female fertility of the hybrids did not differ greatly from parent PI 275258 inbreds, whereas a clear improvement of seed set was found when compared with parent PI 195172 inbreds. The reciprocal hybrids are given separately in Fig. 8. Rather small reciprocal differences in seed set were found, but these certainly do not suggest a maternal effect. So it may be assumed that the relatively high

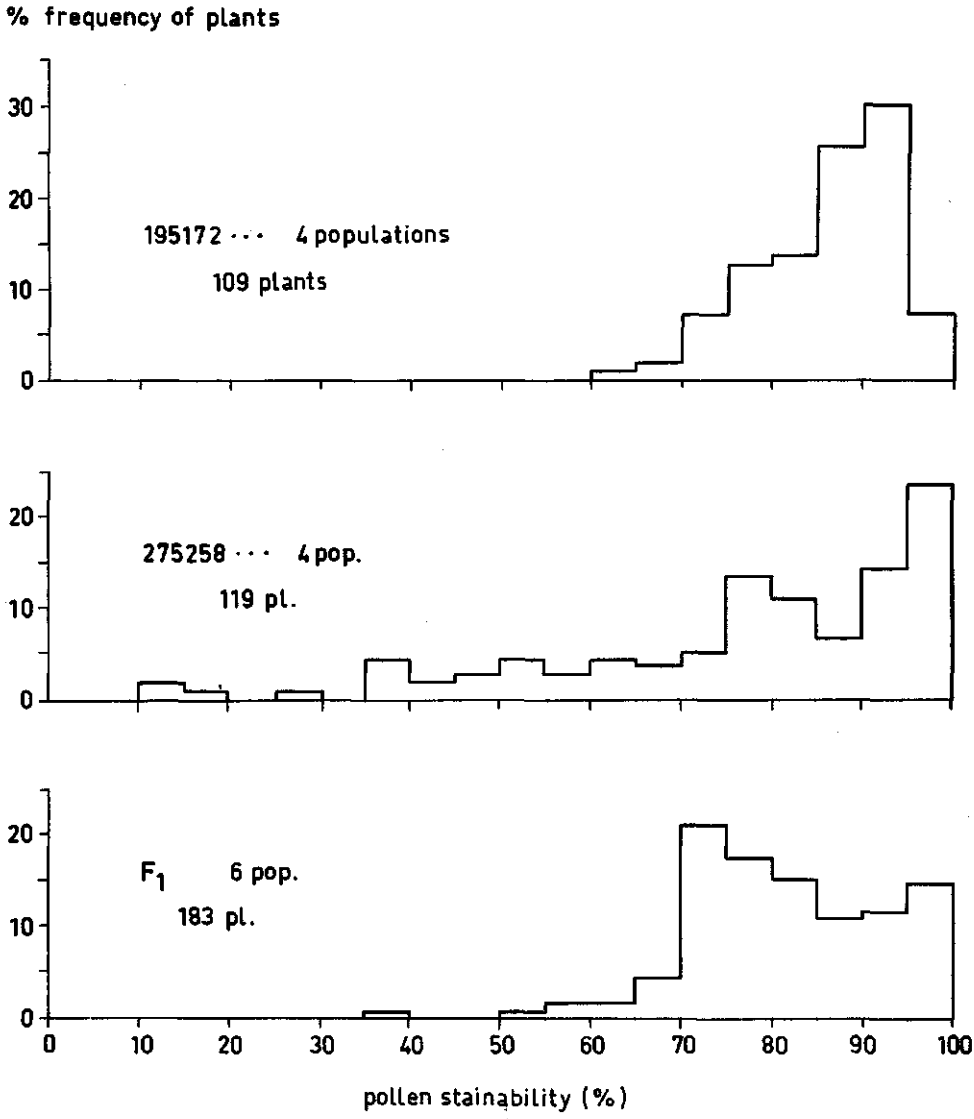


Fig. 7. Frequency distributions of pollen stainability of four inbreds of PI 195172, four of PI 275258 and six hybrid populations. Pooled data (glasshouse 1966).

seed set in the inbreds of PI 275258 is a genetically controlled character.

All hybrids showed heterosis for leaf measurements and number of tubers per plant (Table 6). For the remaining characters some of the hybrids showed heterosis, whereas other hybrids showed means below their 'mid parent' values. The characters expressing high values for heterosis were those widely differing in the parental populations, e.g. seed set and tuber yield.

All hybrid plants proved to be self-compatible.

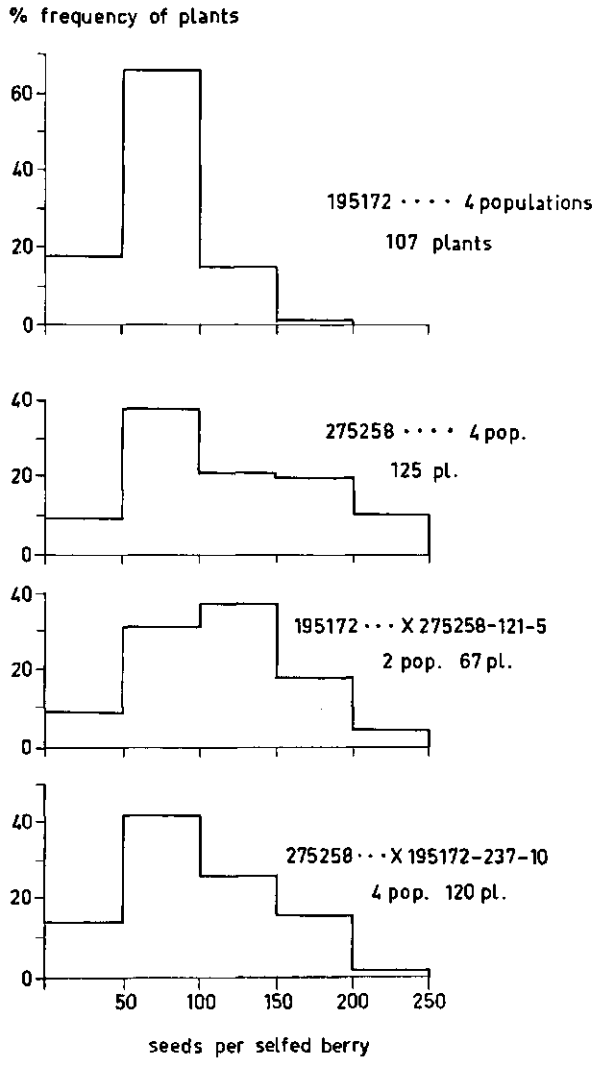


Fig. 8. Frequency distributions of seeds per berry for eight inbred populations from two PI numbers of *S. verrucosum* and six of the hybrids. Pooled data (glasshouse 1966).

Table 6. Range of deviations from mid parent values for some characters of the four hybrids in the last lines in column 1 of Tables 4 and 5.

	Deviations %		Deviations %
Emergence of seedlings (%)	+ 5.9 to +13.2	Percentage stainable pollen	−12.6 to +12.6
Height after a month	− 6.2 to + 4.8	Percentage berries set	−28.7 to +12.1
Height at end of season	−10.4 to + 8.6	Seeds per pollination	−28.8 to +82.1
Length of simple leaf	+ 9.8 to +34.0	Seeds per berry	− 3.0 to +61.7
Width of simple leaf	+13.0 to +39.3	Number of tubers per plant	+ 9.7 to +87.9
Length of compound leaf	+ 4.6 to +20.7	Weight of tubers per plant	−11.0 to +65.5
T value	− 0.5 to +11.0		

4.1.3 Variation in I_3 populations and $I_2 \times I_2$ hybrids from PI 195172 and PI 275258 (field)

The same eight inbred verrucosums and the same six hybrids as used in the glasshouse in 1966 (see 4.1.2) were in the same year also grown in the field, in a randomized complete blocks design with three replicates. In three inbred populations a few plants died after transplantation to the field (2%, 2% and 10% respectively).

Eleven plants per replicate, chosen at random, were studied, making a total of 33 plants for each inbred and each hybrid population, except in the studies of berry and seed set where ten plants were checked from each population. The spontaneously set berries were collected for each plant and five of them were used for seed counting. The results are presented in Table 7 and in Figs. 9 to 14. The F-test was applied to check the significance of the differences between the populations.

The results from the field experiment with the inbred populations from PI 195172–237, PI 275258–119 and PI 275258–121 were similar to those obtained in the glasshouse. However, the absolute values and the rank of the means are different: compare final plant height (Tables 4 and 7), percentage of stainable pollen and seeds per berry (Tables 5 and 7).

In both the glasshouse and the field experiments plant height and male fertility tended to be higher in the inbreds from PI 195172 than in those from PI 275258 but

Table 7. Average values for five characters in eight inbred and six hybrid populations of *Solanum verrucosum* grown in the field in 1966. See Tables 4 and 5.

		End of season plant height (cm)	Inflorescences per plant	Stainable pollen (%)	Number of spontaneous berries per plant	Number of seeds per spontaneous berry
PI 195172–237– 6	spont.	37.6	43	69	42	134
– 8	self.	37.5	40	76	49	103
– 9	self.	42.1	42	85	67	142
–10	self.	39.0	43	85	95	118
Average PI 195172		39.0	42	79	63	124
PI 275258–119– 4	self.	31.7	26	73	27	200
– 6	self.	35.9	30	84	36	213
–121– 1	self.	41.0	42	63	60	157
– 6	spont.	39.2	35	76	52	149
Average PI 275258		37.0	33	74	44	180
195172–237–6 × 275258–121– 5		41.4	47	87	151	222
–8 × – 5		42.6	52	80	114	154
275258–119–4 × 195172–237– 9		41.9	47	71	107	196
–6 × – 9		37.1	45	76	86	171
–121–1 × –10		35.8	39	77	55	139
–6 × –10		38.2	43	79	77	164
Average hybrids		39.5	46	78	98	174

Berry and seed set have been estimated from 10 plants of each population; the other three characters from 33 plants.
Significant differences (using F test) between means are marked by].

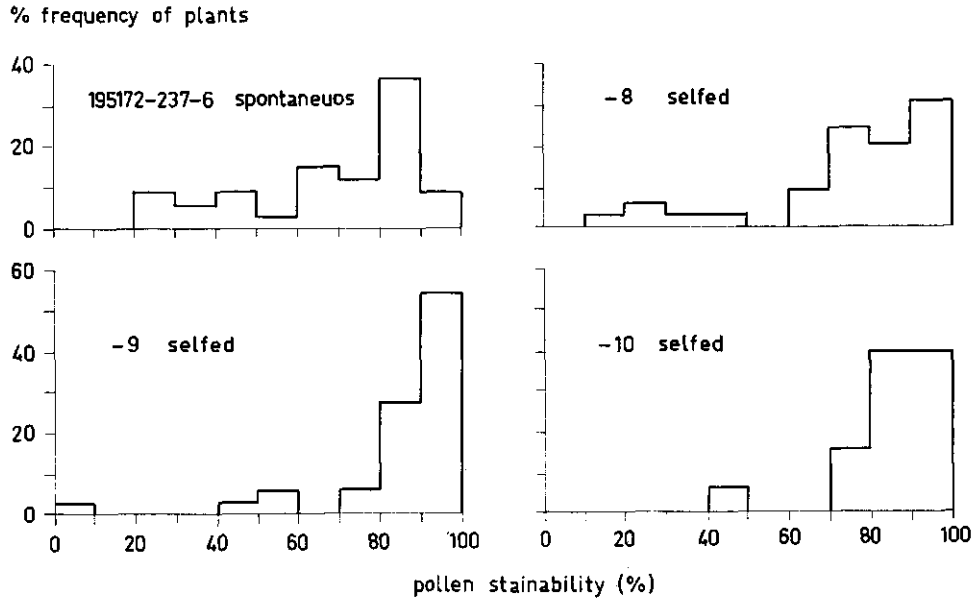


Fig. 9. Frequency distributions of pollen stainability of four inbreds of PI 195172–237 (field 1966). Thirty-three plants checked from each population.

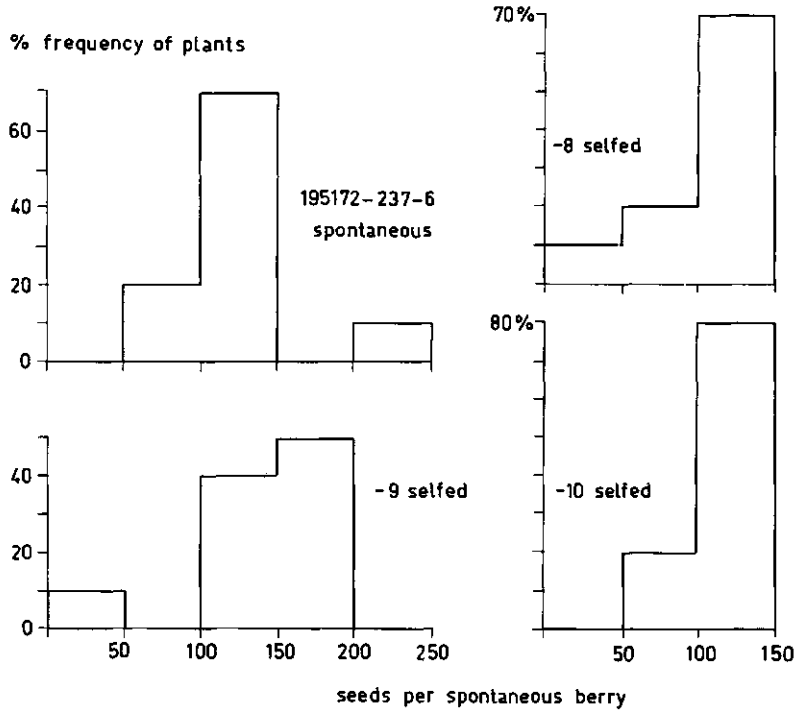


Fig. 10. Frequency distributions of seeds per spontaneous berry of four inbred populations of PI 195172–237 (field 1966). Data from ten plants per population.

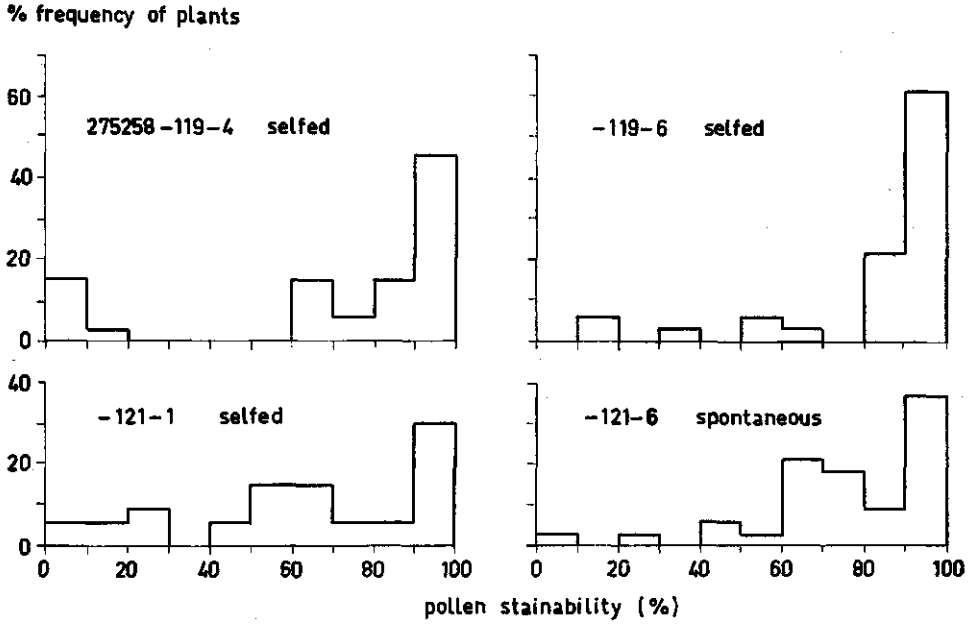


Fig. 11. Frequency distributions for pollen stainability of four inbred populations from PI 275258 (field 1966). Each population is represented by 33 plants.

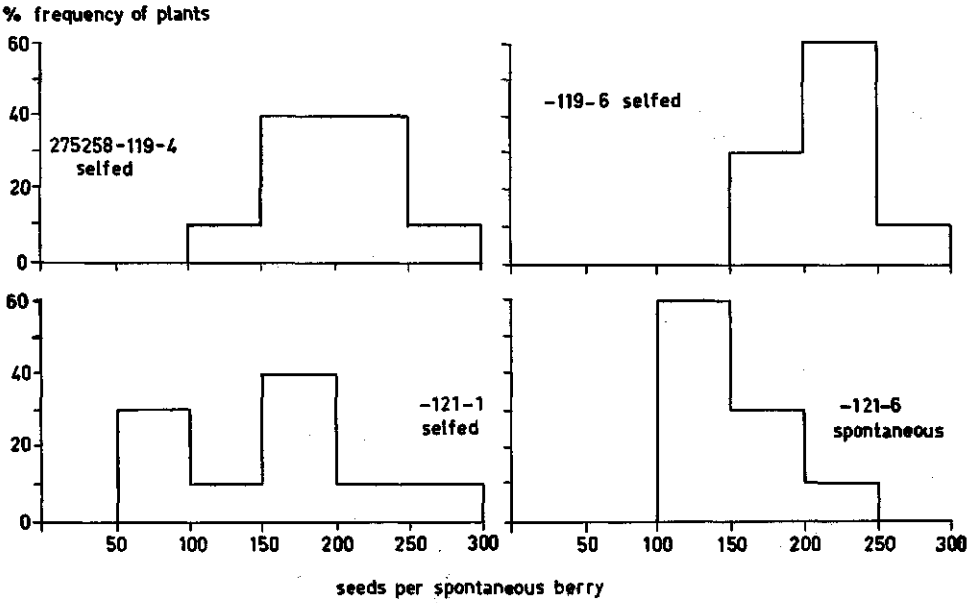


Fig. 12. Frequency distributions of seeds per spontaneous berry for four inbreds of PI 275258 (field 1966). Ten plants checked per population.

female fertility tended to be lower. The variation between the means of the inbred populations from PI 275258 is generally larger than that between the means of the PI 195172 inbred populations, perhaps because the latter originate from one I_1 plant and the former from two I_1 plants.

The large variability of spontaneous berry set in both groups of inbreds and in the hybrids is striking. The spontaneous berry set will be dealt with in more detail in 5.3.

Inbreds tended to react more strongly to varying environmental conditions than hybrids, as was apparent from their heights; this was less obvious for pollen stainability owing to the greater variability of this character (Fig. 13), and it was not observed under the more controlled conditions of the glasshouse experiments (Fig. 7).

Plant height tended to be less variable than pollen stainability under both glasshouse and field conditions. In the 14 inbred and hybrid populations studied, the

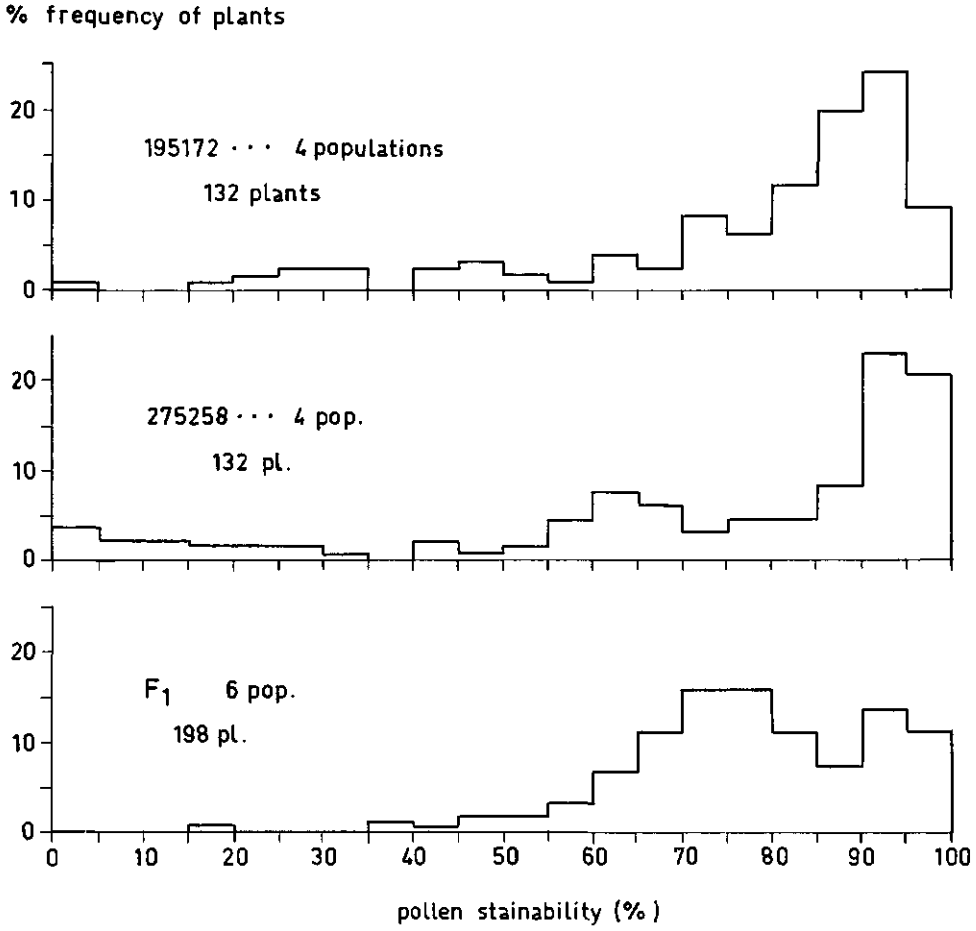


Fig. 13. Frequency distributions for pollen stainability in four inbreds of PI 195172, four of PI 275258 and six hybrid populations. Pooled data (field 1966).

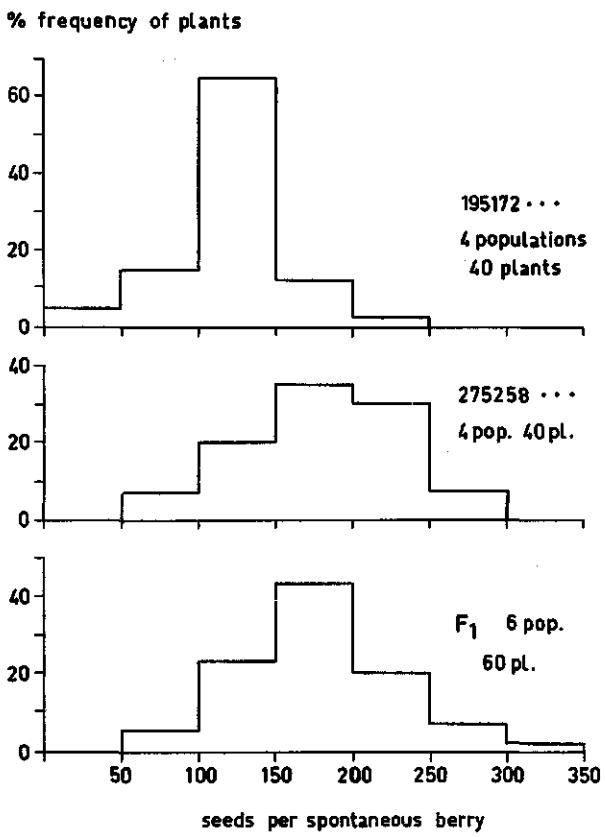


Fig. 14. Frequency distributions for seed set in inbreds of PI 195172 and PI 275258 and six hybrid populations. Pooled data (field 1966).

correlation coefficients for plant height and pollen stainability between greenhouse and field results were 0.59 and 0.21, respectively (r for plant height is statistically significant).

The frequency distributions for pollen stainability and seed set of inbreds and hybrids are given in Figs. 9 to 14. The comparison between the distribution of percentages of stainable pollen and number of seeds per spontaneous berry for inbreds from PI 195172 and PI 275258 revealed that the variability within the first was smaller than within the second. In the glasshouse experiments (see Figs. 3 to 8) this tendency was even clearer, owing to the smaller over-all variability.

The hybrid plants were more vigorous, had longer stems, flowered earlier and more profusely than inbred parent populations. In addition, the variation within the hybrid populations was generally less than within inbred populations. The mean values for different characters of the hybrid populations were in most cases higher than those for the inbreds (Table 7). The most striking difference was that between hybrids and inbreds in berry set which, on the average, was twice as high in the hybrids. However, differences between the hybrids were also observed and reached the level of significance in some characters.

Table 8. Range of deviations from mid parent values for some characters of the four hybrids in the last lines of column 1 in Table 7. See also Table 6.

	Deviations %		Deviations %
Plant height	−10.5 to +13.6	Number of spontaneous berries per plant	−29.0 to +127.8
Number of inflorescences per plant	− 8.7 to +37.4	Seeds per spontaneous berry	− 3.8 to + 23.0
Percentage of stainable pollen	−10.3 to + 4.6		

Hybrids showed better seed set when compared with inbreds of PI 195172 (Fig. 14) but this was not so in comparison with inbreds from PI 275258 (except for berries containing over 300 seeds).

The heterosis for the four hybrids of which the parent inbred populations were available is presented in Table 8. Differences were observed between hybrids and between various characters of the same hybrids. A comparison of Tables 7 and 8 reveals that the more the parents differ, the higher in general is the heterosis (cf., plant height and number of spontaneous berries).

4.2 **Effects of inbreeding and crossing in S. verrucosum.**

The material used for the 1967 studies on inbreeding and crossing effects consisted of:

(1) populations selfed for one or more generations (I_1, I_2 etc.),
(2) hybrid populations obtained by crossing different *S. verrucosum* introductions,
(3) F_2 populations from selfed F_1 plants.

4.2.1 Comparing I_1 and I_2 of CPC 2247, 2514 and 2644

Remnant I_1 seed of that used in 1966 was available of CPC 2247, 2514 and 2644. Seed of the next inbred generation (I_2) was produced by selfing all I_1 plants raised in 1966. Depending on the size of the population, 2, 4 or 5 seeds were picked at random from each plant and blended for each CPC population. In this way I_2 populations were expected to be good representatives of I_1 parents, and both I_1 and I_2 could be compared under equal environmental conditions in 1967. The data of mean values of I_1 and I_2 plants and inbreeding percentages for several characters are presented in Table 9.

Of the 36 inbreeding percentages, 28 were negative (13 significant) and 8 were positive (one significant). Stem height, measured three times, showed different reaction in the three CPC introductions, both in growth-rhythm and effect of inbreeding. The significant gain (16%) for final stem height in CPC 2247 resulting from inbreeding, is striking; so is the significant depression for male fertility in CPC 2644 (which, for the other characters, is least sensitive to inbreeding).

Table 9. Effects of inbreeding as apparent from the average values for different characters in three CPC introductions (glasshouse 1967).

	CPC 2247			CPC 2514			CPC 2644		
	I_1	I_2	inbreeding (%)	I_1	I_2	inbreeding (%)	I_1	I_2	inbreeding (%)
Number of plants used	48	48		36	50		48	48	
Stem height after one month (mm)	15.4	10.8	−30**	13.2	11.5	−13	8.6	9.6	+12
after two months (mm)	51.2	43.1	−16**	54.8	55.5	+ 1	33.7	35.9	+ 7
at end of season (cm)	78.9	91.3	+16**	87.8	75.5	−14**	79.3	76.7	− 3
Simple leaf length (mm)	40.9	41.4	+ 1	33.5	32.9	− 2	30.7	29.6	− 3
width (mm)	31.1	30.8	− 1	22.8	21.8	− 4	20.2	19.3	− 4
Compound leaf length (mm)	30.2	29.0	− 4	33.4	27.6	−17**	30.1	26.7	−12**
T value (mm)	51.8	50.8	− 2	61.5	56.8	− 8**	64.5	61.0	− 5
Percentage stainable pollen	81	75	− 8	90	84	− 6**	88	66	−24**
Number of seeds per berry	206	191	− 8	171	139	−19*	189	199	+ 5
Number of tubers per plant	9.6	5.4	−44**	7.0	4.9	−30*	6.4	7.4	+16
Weight of tubers per plant (g)	6.8	4.2	−38**	7.9	3.3	−58**	5.5	6.4	+16
Percentage of tuberiferous plants	96	92	− 4	97	74	−24	92	81	−11

*, **: significant differences at 0.05 and 0.01 level; −: depression; +: gain.

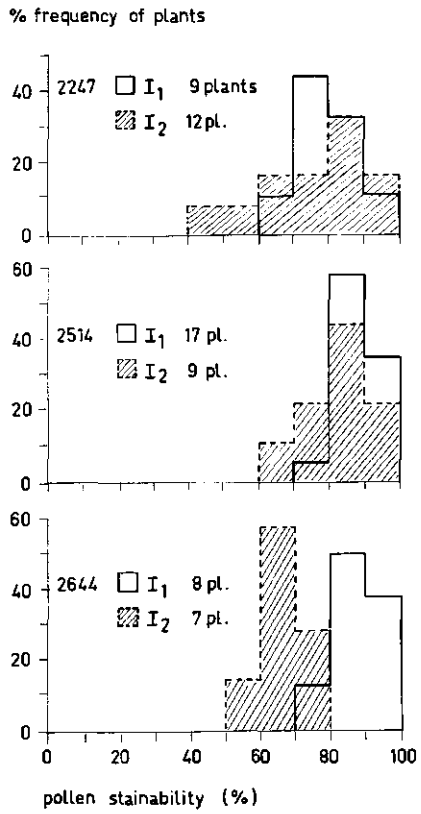


Fig. 15. Frequency distributions for stainability of pollen grains in I_1 and I_2 populations of CPC 2247, 2514 and 2644 (glasshouse 1967).

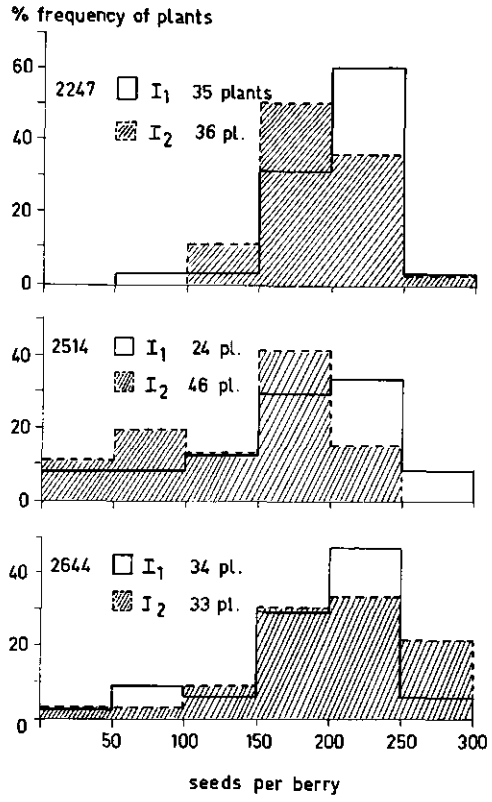


Fig. 16. Frequency distributions for female fertility in I_1 and I_2 populations from CPC 2247, 2514 and 2644 (glasshouse 1967).

Fertility generally showed inbreeding depression, significant for three out of six values. Male fertility tended to vary less but to be more sensitive to inbreeding than female fertility (Figs. 15 and 16).

Rather remarkably, the variability of CPC 2247 in this experiment was higher for male fertility but lower for female fertility as compared with the other two introductions.

Generally speaking, CPC 2514 appeared most sensitive to inbreeding, eleven characters showing inbreeding depression, seven of which were significant.

4.2.2 Inbreeding and heterosis in populations involving CPC 1339

For the 1967 studies, various 1966 seed lots were available:

(1) I_2 seed (remnant of 1966) of CPC 1339,

(2) I_3 seed of CPC 1339 obtained by selfing the I_2 plants grown in 1966 and blending

equal numbers of seed from each plant,
(3) seed from crosses made in 1966 between CPC 1339–I_2 and both CPC 2247–I_1 and PI 195172–237–9–13 (I_3).

These populations served to compare the effects of inbreeding and crossing. The characters studied are given in Table 10, together with the inbreeding and heterosis percentages.

A comparison between I_2 and I_3 of CPC 1339 revealed significant effects of inbreeding for five characters, of which only the effect on flowering time was positive.

For both hybrids, heterosis was calculated using the averages of parent populations CPC 1339–I_2, CPC 2247–I_1 and PI 195172–I_3 to calculate the 'mid parent' values. Many characters showed heterosis, except tuber yield, but a higher percentage of hybrid plants produced tubers.

Frequency distributions of male and female fertility are given in Figs. 17 and 18. These figures also show the positive effects of crossing for both characters. In addition they demonstrate that hybrids have a lower variability than inbreds, especially for pollen stainability. Thus hybridization had a remarkably favourable effect on both

Table 10. Effects of inbreeding in CPC 1339, and the heterosis in its hybrids with PI 195172–237–9–13 and CPC 2247 (glasshouse 1967).

		CPC 1339			CPC 1339 I_2–36 × PI 195172–237–9–13		CPC 2247 × CPC 1339	
		I_2	I_3	percent. inbreeding	F_1	percent. heterosis[1]	F_1	percent. heterosis[1]
Number of plants		50	50		48		52	
Stem height								
after 1 month	(mm)	8.6	6.1	−29**	10.8	+ 2	7.4	−38
after 2 months	(mm)	26.0	20.1	−23**	32.1	− 5	34.4	−11
at end of season	(cm)	58.2	61.0	+ 5	80.2	+22	88.2	+29
Simple leaf length	(mm)	28.3	25.3	−10*	34.5	+11	35.1	+ 1
width	(mm)	20.7	17.6	−15**	25.1	+14	25.1	− 3
Compound leaf length	(mm)	36.3	38.0	+ 5	33.8	+15	38.8	+16
T value	(mm)	58.8	56.9	− 3	59.4	+ 5	60.4	+ 9
Days to opening 1st flower		98	110	+12**	89	− 6		
% stainable pollen		81	82	+ 1	92	+ 8	89	+10
Seeds per berry		123	115	− 7	180	+44	229	+39
Haulm weight	(g)	103.5	93.3	−10				
Number of tubers		2.0	1.7	−15	2.1	+ 2	4.5	−29
Weight of tubers	(g)	1.5	1.1	−27	1.6	−28	2.7	−54
% tuberiferous plants		8	14	+75	50	+60	79	+39

*, **: significant differences at 0.05 and 0.01 level; −: depression; +: gain.

1.No significances were calculated. However, a comparison of the mean values of hybrids with those of parent populations, and the percentages of heterosis with the percentages of inbreeding, as well as the fact that the variability within hybrid populations was less than within parent populations would indicate that several of the differences between hybrids and parents are significant.

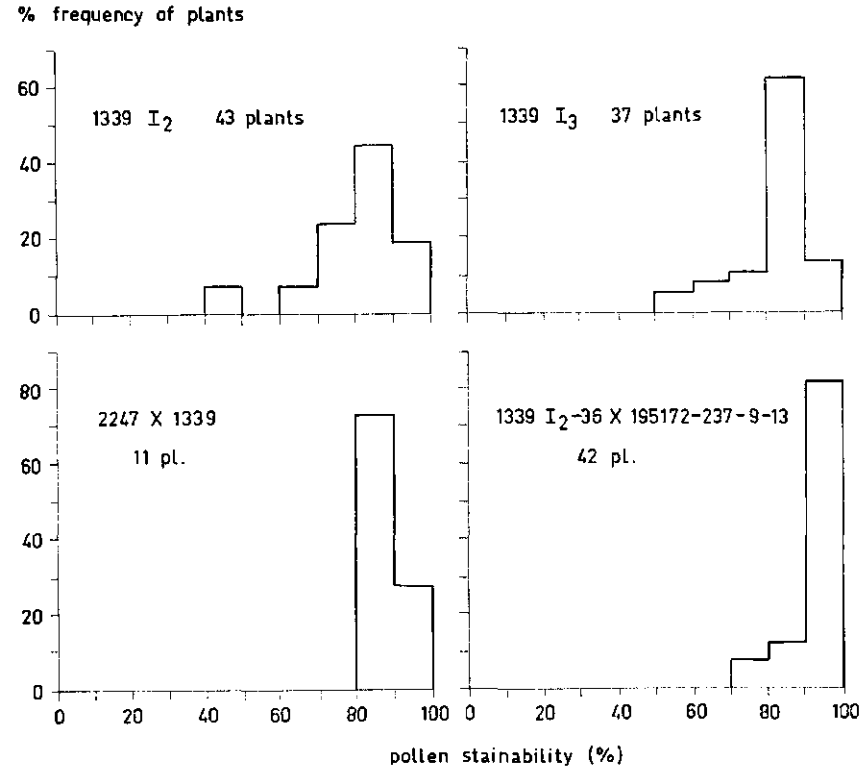


Fig. 17. Frequency distributions for pollen stainability in two inbred populations of CPC 1339, and hybrids of CPC 1339 with CPC 2247 and PI 195172–237–9–13 (glasshouse 1967).

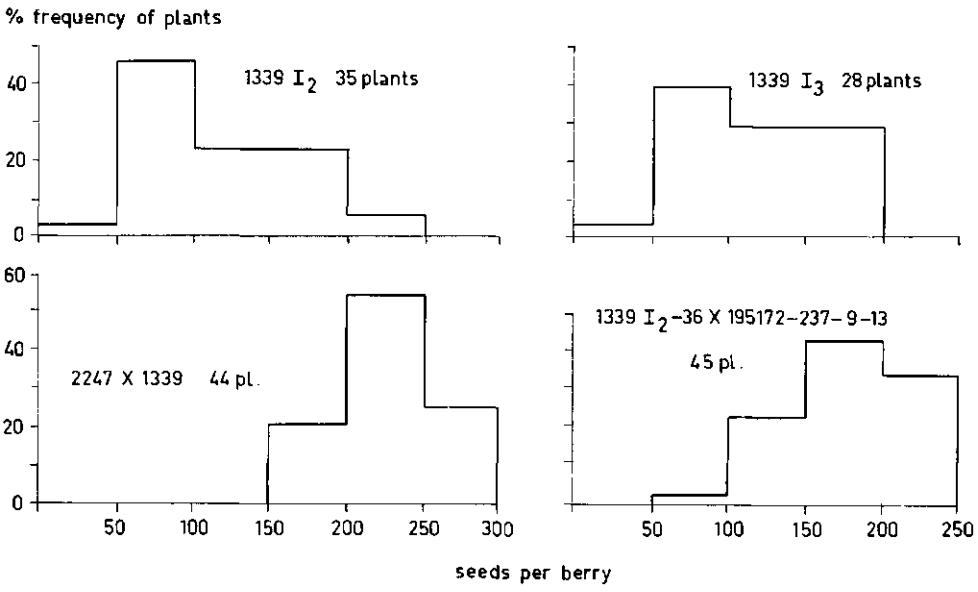


Fig. 18. Frequency distributions for female fertility in I_2 and I_3 of CPC 1339, and hybrids of CPC 1339 with CPC 2247 and PI 195172–237–9–13 (glasshouse 1967).

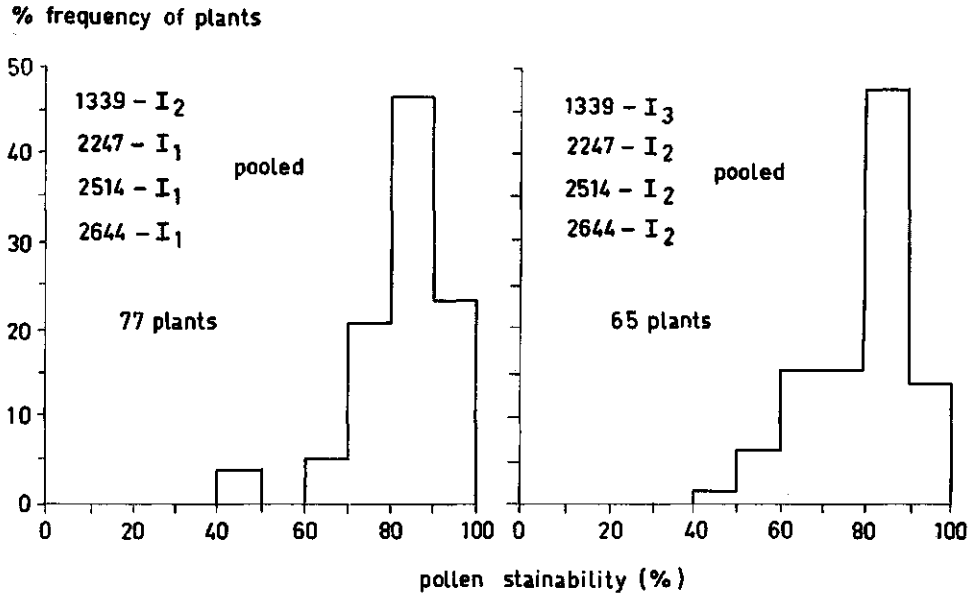


Fig. 19. Frequency distributions of male fertility. Pooled data of four CPC introductions 1339, 2247, 2514 and 2644 (glasshouse 1967).

male and female fertility.

Pollen stainability is a rather variable character, which is affected by both genotype and environment, so that flowers from one plant checked at the same time may give different percentages of stainable pollen. A clear demonstration of environmental influence can be observed by comparing the upper histogram in Fig. 1 (1966) with Fig. 19 (1967). Here no genetic differences can have been responsible as in 1967 remnant seed of 1966 has been used. The 1967 results looked better: higher mean stainability, smaller variation and no individuals in the classes below 40%.

The pooled data in Fig. 19 for pollen stainability in the four CPC introductions also show the effect of one more generation of inbreeding.

4.2.3 Comparison between some populations of PI 275258 and PI 195172 in various inbred generations

In 1967, the following populations of *S. verrucosum* were studied in the glasshouse:

PI 275258: I_0^2, seed received from Sturgeon Bay, USA
I_3, seed obtained from selfing PI 275258–119–4
I_4 mix, a mixture of 5 seeds from each of 34 selfed I_3 plants

PI 195172: I_0^2, seed received from Sturgeon Bay, USA

2. The symbol I_0 does not indicate a non-inbred generation but simply the least-inbred population available.

I_3, seed obtained from selfing PI 195172–237–9
I_4, seed from one selfed plant of I_3 = PI 195172–237–9–9
I_4 mix, a mixture of 5 seeds from each of 35 selfed I_3 plants
I_5 mix, a mixture of 10 seeds from each of 16 selfed I_4 plants

4.2.3.1 Populations of PI 275258

The data of PI 275258 populations are given in Table 11. The comparisons between I_0 on the one hand and I_3 and I_4 mix on the other showed that inbreeding led to depression in the majority of the characters. The most striking depressions were for stem height, some leaf measurements, and fertility. I_0 *v.* I_3 and I_0 *v.* I_4 showed that inbreeding in both populations are not essentially different, except for tuber production where I_3 exhibited a distinct depression and I_4 mix an obvious gain.

Table 11. Effects of inbreeding as apparent from average values of some characters in three populations of *Solanum verrucosum* PI 275258 (glasshouse 1967).

		Average values per plant			Inbreeding (%)		
		I_0	I_3	I_4mix	I_0 *v.*I_3	I_0 *v.*I_4mix	I_3 *v.*I_4mix
Number of plants		38	13	48	—	—	—
Stem height							
after one month	(mm)	11.8	4.1	7.9	−65.6**	−33.1**	+ 94.6**
after two months	(mm)	42.8	37.5	46.0	−12.3	+ 7.4	+ 22.4**
at end of season	(cm)	79.2	70.8	56.9	−10.5*	−28.1**	− 19.7**
Simple leaf length	(mm)	31.7	26.5	29.0	−16.4**	− 8.5	+ 9.4
width	(mm)	20.4	17.6	18.4	−13.5*	− 9.8	+ 4.2
Compound leaf length	(mm)	28.2	29.3	25.9	+ 4.0	− 8.3	− 11.8
T value	(mm)	58.8	66.4	59.3	+12.9*	+ 0.8	− 10.7*
% stainable pollen		85	72	61	−15 **	−28 **	− 14
Seeds per berry[1]		183		107		−41 **	
Number of tubers		6.4	1.3	10.9	−80.0**	+70.3**	+738.5**
Weight of tubers	(g)	6.5	1.8	7.0	−72.3**	+ 7.7	+288.9**

*, **: significant differences at 0.05 and 0.01 level; −: depression; +: gain.
1. In I_3 only two plants were selfed, so that no data could be included. However, it is expected that seed of I_3 would not differ much from that of I_0, as in 1966, when conditions had been less favourable, sister plants had produced 155 seeds per berry (see 4.1.2.2).

A comparison between I_3 and I_4mix revealed six significant differences, only two of which were a depression.

As appears from Fig. 20, a drop in male fertility and an increase in the variability is observed from $I_0 \rightarrow I_3 \rightarrow I_4$mix. Fig. 21 shows the clear decrease of female fertility in I_4mix but the variation is less than in I_0.

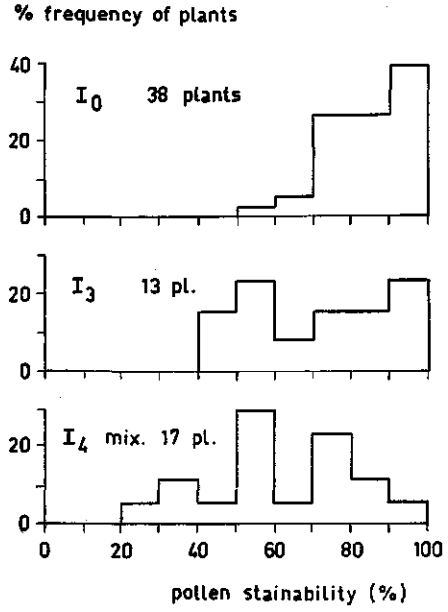


Fig. 20. Frequency distributions for stainability of pollen grains in three populations from PI 275258 (glasshouse 1967).

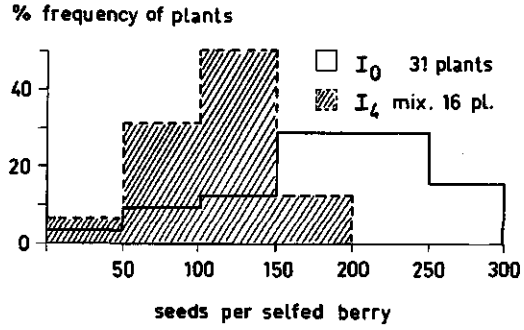


Fig. 21. Frequency distributions for seed set in two populations from PI 275258 (glasshouse 1967).

4.2.3.2 Populations of PI 195172

The data of the PI 195172 populations are presented in Table 12 and Figs. 22 and 23. A general decrease in some leaf measurements and stem height in early stages of growth was observed to accompany inbreeding. However, a significant gain appeared for end of season stem height and female fertility.

The comparison between I_3 and I_4 showed reactions somewhat different from those in other cases of inbreeding. Here there was a significant gain for stem height after one month and a significant depression for final stem height, T value, and female fertility. This is not surprising in view of the one plant origin of I_4.

The comparison between I_4 and I_4mix is interesting. Both populations are in the same inbred generation. The difference shows how a single plant can deviate from its population.

Table 12. Effects of inbreeding as apparent from average values for some characters in five populations from PI 195172 (glasshouse 1967).

		Average values per plant					Inbreeding (%)				
		I_0	I_3	I_4mix	I_4	I_5mix	I_0 *v.* I_3	I_0 *v.* I_5mix	I_3 *v.* I_4	I_4 *v.* I_4mix	I_4 *v.* I_5mix
Number of plants		45	50	50	50	50	—	—	—	—	—
Stem height after one month	(mm)	15.5	12.6	10.0	15.7	7.8	−18.8**	−49.7**	+24.7**	−36.4**	−50.2**
two months	(mm)	42.9	41.5	44.5	40.4	32.0	− 3.4	−25.4**	− 2.7	+10.2	−20.8**
at end of season	(cm)	64.3	73.0	86.6	66.1	78.6	+13.5**	+22.2**	− 9.5*	+31.2**	+19.0**
Simple leaf length	(mm)	32.8	33.6	29.7	36.2	28.8	+ 2.4	−12.2**	+ 7.6	−18.0**	−20.3**
width	(mm)	23.4	23.3	20.7	25.1	20.0	− 0.2	−14.5**	+ 7.7	−17.7**	−20.3**
Compound leaf length	(mm)	17.5	22.6	22.3	20.5	19.9	+29.7*	+13.7	− 9.3	+ 8.5	− 3.2
T value	(mm)	51.0	54.6	61.8	50.5	56.8	+ 7.0	+11.4	− 7.5*	+22.4**	+12.5*
Percentage of stainable pollen		83	90	87	89	91	+ 8	+ 9 **	+ 1	− 2	+ 2
Number of seeds per berry		75	125	128	95	109	+66 **	+45 **	−24 **	+34 **	+15 *
Number of tubers		4.0	2.1	2.1	1.9	1.3	−47.5*	−67.5	−10.5	+10.5	−31.6
Weight of tubers	(g)	3.4	2.6	2.1	1.9	2.3	−23.5	−32.4	−26.9	+10.5	+21.1

*, **: significant differences at 0.05 and 0.01 level; −: depression; +: gain.

Figure 22 demonstrates the gain in male fertility when different inbred populations are compared with I_0, as well as a decrease in variability. Here the gain was not sufficient to reach the level of significance, except when I_0 was compared with I_5mix.

The gain in female fertility is obvious in Fig. 23, but the differences in variability are not clear.

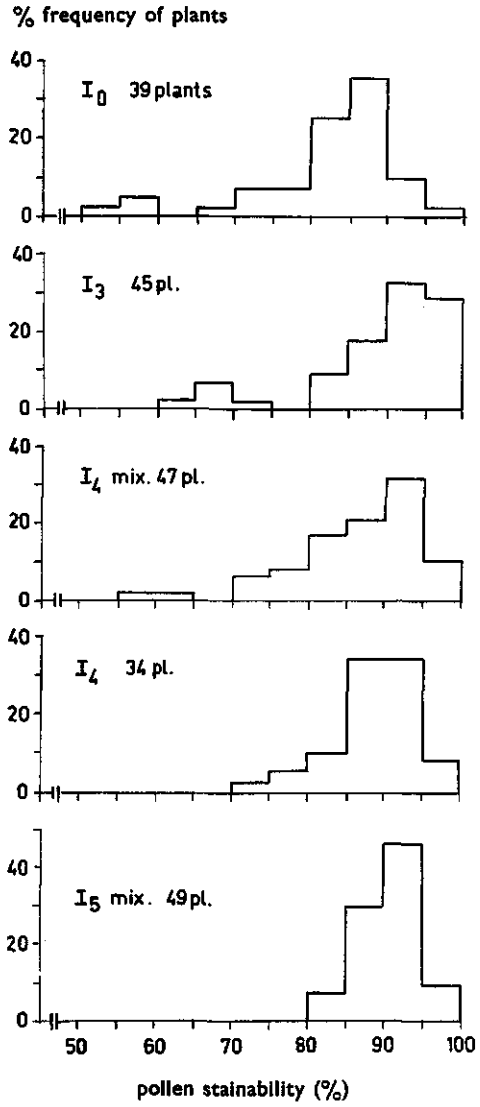


Fig. 22. Frequency distributions for pollen stainability in five populations from PI 195172 (glasshouse 1967).

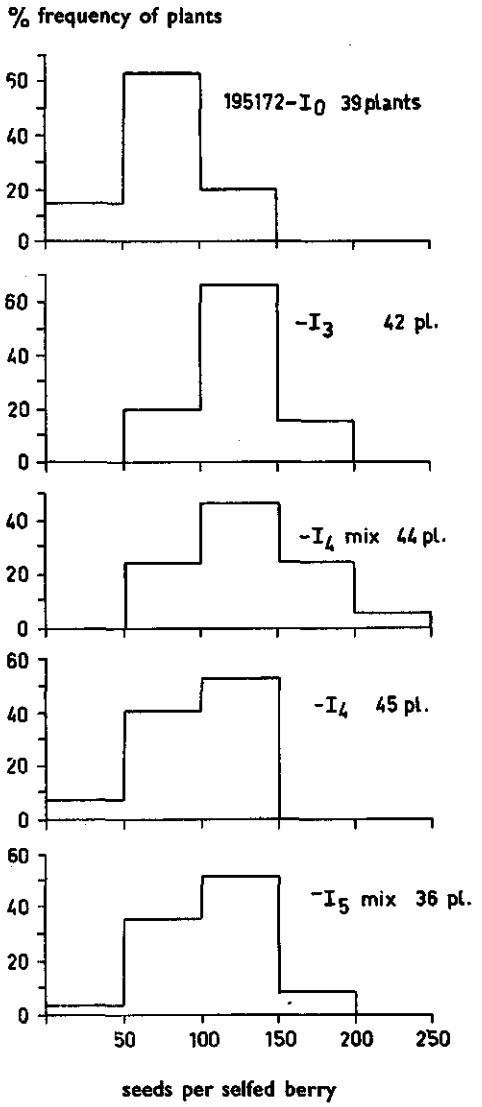


Fig. 23. Frequency distributions for seed set in five populations from PI 195172 (glasshouse 1967).

A comparison of the two populations PI 195172 and PI 275258 (Tables 11, 12 and Figs. 20, 21, 22, 23) shows clearly divergent reactions, particularly in final stem height and fertility: in PI 195172 a significant gain, in PI 275258 a clear depression. One may wonder if some unconscious selection for other characters in PI 195172 has taken place, accompanying that for resistance to *Phytophthora*.

4.2.4 Inbreeding effects in two related hybrids

Inbreeding was studied in the hybrids of PI 275258–121–1 × PI 195172–237–10 and PI 275258–121–6 × PI 195172–237–10. The female parents traced back to one plant in the preceding generation.

For this experiment some F_1 seeds were available together with the corresponding F_2 seeds, the latter obtained by blending an equal number of seeds from at least 32 selfed F_1 plants of each hybrid. Thus F_1 and F_2 could be compared for both hybrids in the same year (1967) under the same glasshouse conditions.

As apparent from Table 13, nearly all effects were significant depressions. Tuber yield in the second population was an exception and F_2 populations flowered later.

Male fertility did not show much reaction, except for somewhat larger variation in F_2 visible from Fig. 24. The depression in female fertility is clearly demonstrated in Fig. 25.

Table 13. Effects of inbreeding on some characters studied in two related *Solanum verrucosum* hybrids and their F_2 populations (glasshouse 1967).

	PI 275258–121–1 × PI 195172–237–10			PI 275258–121–6 × PI 195172–237–10		
	F_1	F_2	inbreeding(%)	F_1	F_2	inbreeding(%)
Number of plants used	100	100		100	100	
Stem height after one month (mm)	15.9	9.8	−44**	14.2	12.0	−16**
two months (mm)	14.1	10.4	−26**	12.7	10.3	−19**
at end of season (cm)	80.0	69.9	−13**	76.7	71.4	− 7**
Simple leaf length (mm)	31.1	26.4	−15**	29.5	27.7	− 6
width (mm)	20.8	18.4	−11*	19.6	18.8	− 4
Compound leaf length (mm)	35.3	29.7	−15**	33.5	27.6	−17**
T value (mm)	75.8	61.8	−18**	76.8	66.6	−13**
Days to opening of first flower	77	82	− 6**	78	82	− 5**
Percentage of stainable pollen	81	80	− 1	78	76	− 2
Number of seeds per berry	147	98	−33**	117	98	−16**
Number of tubers per plant	6.2	4.3	−31**	3.3	5.7	+73**
Weight of tubers per plant (g)	7.2	3.6	−50**	4.2	4.9	+17

*, **: significant differences at 0.05 and 0.01 level; −: depression; +: gain.

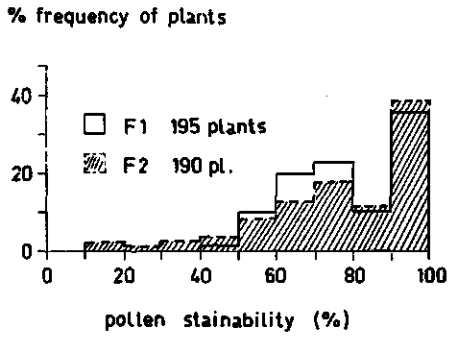


Fig. 24. Frequency distributions for male fertility in two related F_1 and corresponding F_2 populations of the hybrids PI 275258–121–1(6) × PI 195172–237–10. Pooled data (glasshouse 1967).

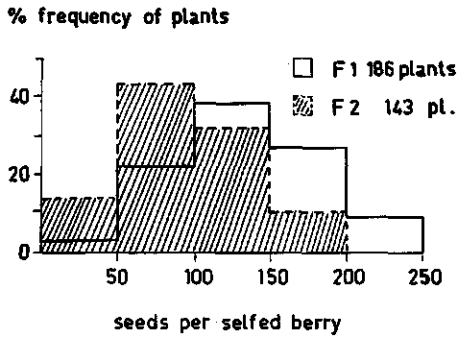


Fig. 25. Frequency distributions for female fertility in the two F_1 and two F_2 populations mentioned in Fig. 24. Pooled data (glasshouse 1967).

5 Types of male sterility, self-compatibility and inheritance of certain characters in S. verrucosum

Certain characters were met with in some of the 21 introductions of *S. verrucosum*, which needed further study. Of these, some will be discussed in this chapter and where possible the nature of their inheritance will be explained. Reference will be made to the fertility and self-compatibility of *S. verrucosum*.

5.1 Fertility and types of male sterility in fifteen introductions of S. verrucosum

In testing self-compatibility and fertility in fifteen introductions of *S. verrucosum* not mentioned in the previous chapter, three types of male sterility (see 5.1.1,2,3) and a pollen wall change (see 5.1.2) were detected.

5.1.1 Male and female fertility and 'ordinary' male sterility

In 1967 and 1968 small populations (the largest consisting of 42 plants) of *S. verrucosum* introductions were raised in the glasshouse. Some plants from each introduc-

Table 14. Stainability of pollen grains and seed set in 15 *Solanum verrucosum* introductions checked in two seasons.

Introduction	Year of test	Stainability of pollen grains		Seed set per berry	
		average percentage	number of plants checked	average number	number of plants checked
PI 161173	1968	78	4	95	7
PI 195171	1968	73	7	128	13
PI 255544	1968	72	4	249	6
PI 275255	1968	80	5	87	7
PI 275256	1968	87	3	91	5
PI 275259	1968	85	10	145	13
PI 275260	1968	80	10	236	10
PI 310966	1968	82	5	139	6
Haw 756	1967	86	22	165	13
Haw 1350	1967	71	42	83	1
Haw 1528	1967	87	30	85	6
Haw 1532	1968	58	3	97	3
Haw 2246	1968	35	3	148	5
CPC 2623a	1968	74	1	143	6
EBS 2632	1967	88	14	63	3

tion were checked for male and female fertility (Table 14). With two exceptions (Haw 1532 and Haw 2246) they showed good male fertility and the percentage of stainable pollen grains was always above 70%. Pollen stainability in both Haw 1532 and Haw 2246 could be determined for only three plants from each population so that the results could not be considered decisive (if more plants had been checked, the stainability percentage would probably have been different).

The plants were selfed. Where reasonable numbers of pollinations could be made plants set berries freely, the berries contained reasonable numbers of seeds as apparent from Table 14. Differences in seed set, however, were manifest: in some introductions it was four times that in others, contrary to pollen stainability which showed relatively low differences.

The 'good' pollen grains were rounded and stained richly. They had smooth walls, with up to three germination pores (Plate 13–1). The 'bad' pollen grains were shrunken, irregularly shaped and stained either faintly, incompletely, or not at all. The sterility of such 'normally bad' pollen will be indicated as 'ordinary' sterility to differentiate it from other types of male sterility discovered in *S. verrucosum* and interspecific hybrids.

5.1.2 'Bubble sterility' and 'blunt spine pollen wall'

The second type of male sterility was found in Haw 2246. The three plants checked in 1968 showed reduced pollen stainability and some grains showed breakdown of the cytoplasm; the majority stained faintly and looked bubbled. The latter phenomenon had not been observed before. This type of sterility will be referred to as bubble sterility (proposed symbol *bl*).

Pollen of two of the three plants showed many coarse blunts on their walls, among both fertile and bubble sterile pollen grains (Plate 3). This will be called 'blunt spine pollen wall' (proposed symbol *bs*).

In addition, the cytoplasm of the blunt spiny pollen seemed to consist of granuled particles which might have been the cause or the result of the spines. Plant 6, which showed this character in 1968, was selfed. Nine plants of the progeny were checked in 1969. All showed blunt spine appearance, pointing to the homozygosity of plant 6 for

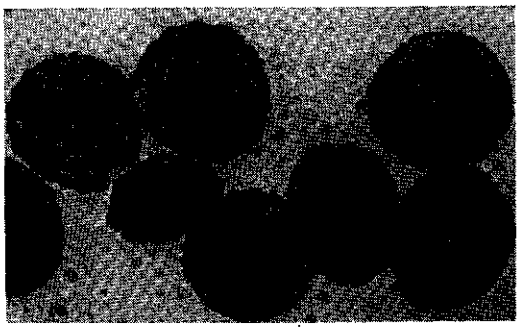


Plate 3. Bubble sterility accompanied by blunt spine appearance of pollen walls.

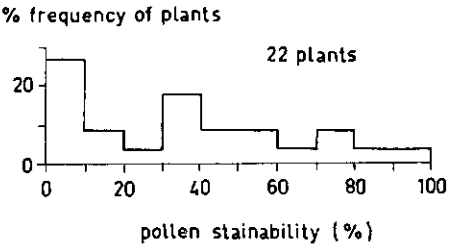


Fig. 26. Frequency distributions for pollen stainability in the selfed progeny of plant 2 of *S. verrucosum* Haw 2246 raised in 1969.

this character.

Plant 2, showing 25% stainable pollen with normal smooth walls in 1968, was selfed, and 22 plants of the progeny were checked in 1969. These showed a wide and almost continuous range of pollen stainability, from 0 to more than 90% (Fig. 26), pointing to a polygenic control. The sterile pollen grains were generally characterized by a bubbled appearance. From the 22 plants checked, 7 showed grains with blunt spine walls, whereas 15 plants had smooth pollen walls. This ratio suggests 'blunt spine pollen wall' to be a recessive character and 15 *Bs*–: 7 *bsbs* fits the theoretical ratio of 3:1 ($\chi^2 = 0.55$, P = 0.50–0.30), pointing to a monogenic control. Hence parent plant 2 must have been heterozygous for the blunt spine pollen wall character (*Bsbs*).

5.1.3 'Undivided microsporocyte' type of sterility

The other type of male sterility observed in pure *S. verrucosum* plants was found in sib-mated PI 255544 and in the selfed progeny from one plant of Haw 1542. In the sterile plants, the anthers were slightly thicker and shorter (1 mm) and more yellowish than those of fertile plants. The sterile anthers shed poorly. Their contents consisted of big rounded structures (Plate 13–5) which stained very faintly or irregularly.

Some of these anthers were studied cytologically. In the squashed preparations, the big round structures were found to be pollen mother cells and not pollen grains. Each contained four nuclei still enclosed within a common wall. The check of mature anthers showed the occurrence of some change in the original cell wall of the PMC: it had become thinner and showed four weak points or lobes resembling pores of normal pollen grains (Plate 4).

Hence a blockage in the normal pathway of pollen formation seems to occur in this type of sterility. It prevents microspore cell wall formation within the PMC. Nuclear division proceeds normally, giving rise to four nuclei enclosed in a thin aberrant wall. Neither dyad nor tetrad cell walls have been observed. This type of male sterility will be named 'undivided microsporocytes' (proposed symbol *um*). It is also dealt with under 6.6.5.1.

In some of the anthers a few undivided microsporocytes contained more than four nuclei. In four plants the number of PMC's with more than four nuclei was 4, 6, 1 and 4 against 68, 92, 102, and 71 microsporocytes, respectively, with only four nuclei or, generally speaking, less than 5%. Such microsporocytes were generally bigger and

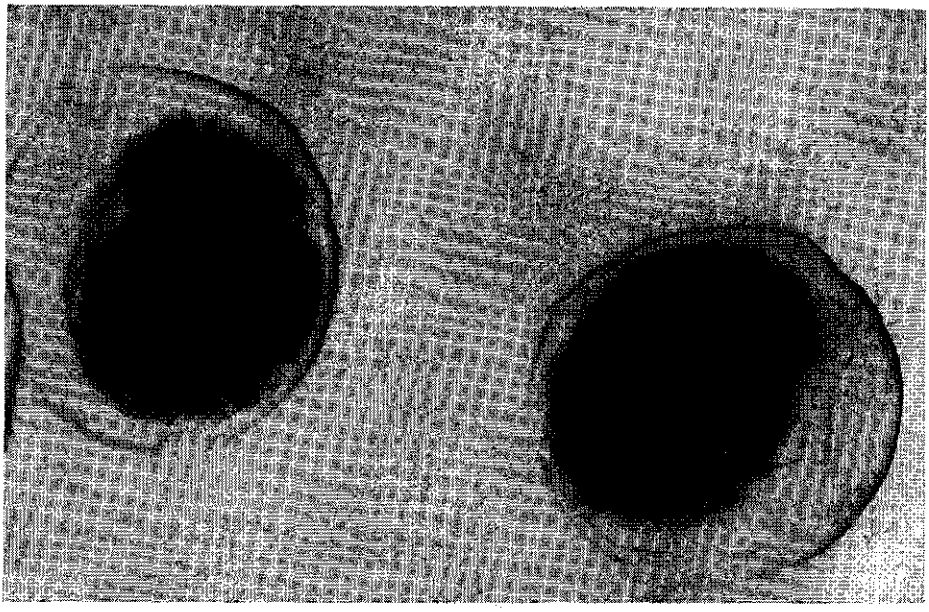


Plate 4. Undivided microsporocytes showing the presence of four nuclei without microspore cell wall development. The original cell wall of the pollen mother cell underwent a change which caused it to resemble a pollen grain wall.

irregular. The additional nuclei were sometimes of the same size as the other four and sometimes smaller (micro nuclei). From 1 to 7 additional nuclei were observed but 1 was most frequently found.

In few anthers some PMC's showed an elongated shape unlike the normally rounded ones. Some of these were splitters from a thread consisting of more of such microsporocytes. With one exception they contained four nuclei; the exception enclosed 9 nuclei.

In the squashed anther preparations showing undivided microsporocytes, but also in anthers showing other types of sterility (see Chapter 6), giant cells were observed. These are believed to be tapetal cells.

In a few of the *um*-sterile plants, as well as in others of interspecific origin but showing the same type of sterility, some 'embryo sac' -like giants and 'embryo'-like structures were observed in the contents of the shedding anthers. Their presence in such male sterile plants with undivided microsporocytes might question their identity as real 'embryos' and 'embryo sacs', but one of these giants (Plate 5) greatly resembled an 'embryo'.

As previously mentioned, the sterility type 'undivided microsporocytes' was observed in PI 255544 and Haw 1542. In 1968, from eight plants checked from sibmated PI 255544, three showed this type of sterility. Hybrids between two of these plants (Nos. 4 and 5) with a sister fertile plant (No. 1) were raised in 1969. These

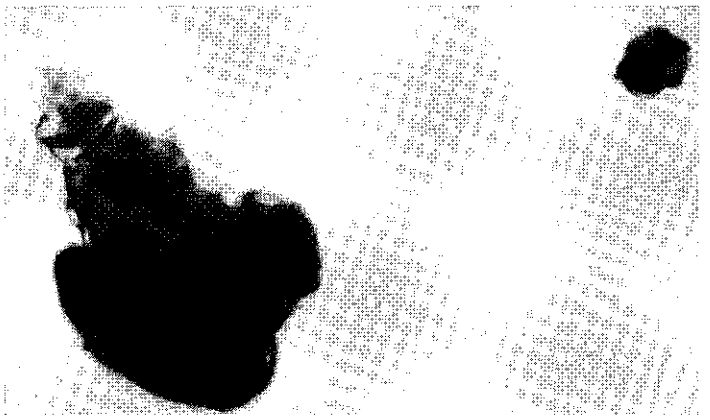


Plate 5. An embryo-like structure found in the shed contents of anthers showing the undivided microsporocyte type of male sterility. At the right a sterile pollen grain.

populations segregated into 7 fertile : 8 sterile and 3 fertile : 10 sterile, respectively. Although there was an excess of sterile plants in the second population, the total number of plants, 10 fertile : 19 sterile, did not contradict the ratio 1:1 ($\chi^2 = 2.79$, $P = 0.10$–0.05) suggesting a monogenic inheritance of this character. Plants 4 and 5 of PI 255544 then should be recessive (*um um*) and Plant 1 heterozygous (*Um um*). The fact that in 1968 in an inbred population from Haw 1542 (Plant 15) only one plant out of six showed this type of sterility points also to the inheritance of this sterility type in a recessive fashion.

5.2 Self-compatibility of S. verrucosum

During the growing seasons of 1966, 1967 and 1968, selfing was always continued on *S. verrucosum* until berry set was observed. A few of the plants produced no berries. These, and others which did not flower or which produced only seedless berries, were raised from tubers and checked the next season. All were then successfully selfed and the berries contained seeds. Out of several hundred plants checked, only one exception was found (Plant 86 from the inbred CPC 2247) which failed to produce seed. This exception appeared to be a triploid and will be dealt with in 5.4.

Thus it may be concluded that *all the diploid S. verrucosum plants studied are self-compatible.*

5.3 Relation between spontaneous berry set and flower structure in S. verrucosum

In most of *S. verrucosum* populations grown in the insect-free glasshouses no berry set was observed without manual pollination. This was found to be largely due to flower structure and pollen shedding.

Table 15. Spontaneous berry set (data of 1967 and 1968) in relation to distance between anther tips and stigma (data of 1968) in 21 introductions of *Solanum verrucosum*.

Introduction	Spontaneous berry set			Length of pistil above anther tip	
	number of plants checked	percent. of plants with one or more berries	maximum number of berries per plant	number of plants checked	average per plant (mm)
PI 160228	50	0	0	6	5.1
CPC 2623a	15	0	0	13	5.0
CPC 2514	86	0	0	10	4.6
PI 310966	15	7	1	14	4.4
PI 275259	15	27	3	13	4.2
Haw 756	34	0	0	10	4.1
PI 161173	10	0	0	10	4.1
PI 275256	46	0	0	11	4.0
CPC 2644	96	5	2	10	4.0
Haw 1350	42	0	0	4	3.7
Haw 1532	8	0	0	7	3.6
CPC 2247	96	2	2		
PI 275255	15	0	0	11	3.5
PI 275258	99	8	1	9	3.5
PI 255544	15	13	1	14	3.1
PI 195172	245	43	10	20	3.0
EBS 2632	15	0	0	3	2.6
CPC 1339	100	17	4	20	2.5
Haw 2246	15	27	5	12	2.1
PI 195171	15	80	20	14	2.0
PI 275260	15	53	26	14	1.8

Five flowers per plant were measured.

In *S. verrucosum* pollen starts shedding after anthesis and in nature it is mainly transferred by insects. Pistils are longer than stamens (Plate 6) and this favours cross-pollination.[3]

The length of the pistil part above the tips of the stamens was found to vary from one population to another (Table 15). The length of the pistil part above the anther tips (l) was distinctly negatively correlated with both the percentage of plants showing spontaneous berry set (b) and with the maximum number of berries set per plant (m): $r_{lb} = -0.68$; $r_{lm} = -0.68$; $r_{bm} = +0.88$ (all the correlation coefficient values are significant at the 1 % level).

The length of the pistil part above anther tips varied from 1.8–5.1 mm, 3.5 mm being more or less the critical length: spontaneous berry set generally does not take

3. This structure will be called 'flowers with supra-staminal pistils'. Some authors (see Bukovac & Honma, 1967) call such flowers heterostylous which seems less adequate (see Elliott, 1958).

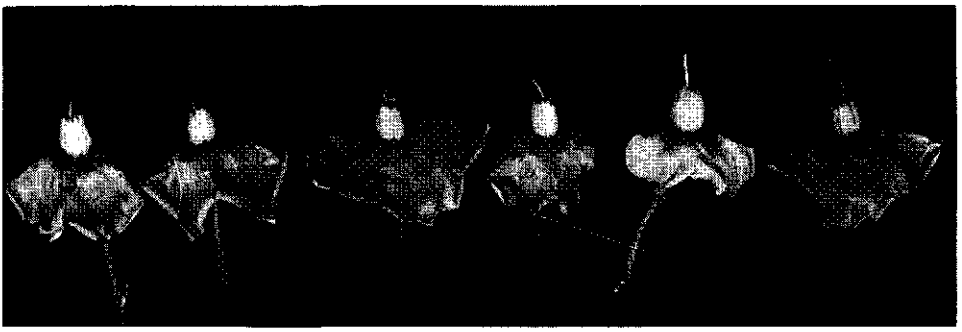


Plate 6. Flowers with supra-staminal pistils from *S. verrucosum* introductions showing the variation in this characteristic (cf. Table 15).

place when the length of the pistil part above the stamen tips exceeds 3.5 mm. Exceptions to both sides are very few (see EBS 2632 and PI 275259).

Spontaneous berry set showed a practically negligible variation from year to year. In addition, inbred populations from different introductions did not differ much from their 'mother' populations, especially in case of low or no spontaneous berry set. Examples were: I_1 and I_2 of CPC 2247 (both 2.1%), CPC 2644 (6.3 and 4.2%) and CPC 2514 (both 0%). In the populations with a higher rate of spontaneous berry set, the variability from one year or inbred generation to another was larger, as demon-

Table 16. Data on berry set and seeds after artificial selfing and spontaneous set on the same plants in insect-free glasshouse.

	Spontaneous berry set		Av. number of seeds per berry after		Av. seed size, in 0.01 cm, after			
					artificial selfing		spontaneous set	
	number of plants checked	plants with berries (%)	artificial selfing	spontaneous set	length	width	length	width
Parent ♀ PI 275258	99	8	130	70	16.5	12.0	16.6	11.7
Parent ♂ PI 195172	245	43	122	74				
F_1	200	49	131	68	16.2	11.8	17.2	12.3
F_2	200	44	113	55	17.1	12.7	18.4	13.5
Parent ♀ CPC 1339 I_2–36	100	17	128	53	16.1	11.8	15.6	11.5
Parent ♂ PI 195172–237–9–13	50	58	122	99	17.5	12.8	17.2	12.8
F_1	48	83	182	84				
F_2	125	92						

For seed set 3–24 plants were checked; average seed sizes calculated for 10 seeds of 5–10 plants in each population. Data from 1967, last line from 1968.

strated in PI 195172:I_0 and I_3 comprised 22% and 58% plants with spontaneous berries while I_3 produced 58% and 74% plants with spontaneous berries in 1967 and 1968, respectively.

The relative consistency in different populations suggested a genetic control of the spontaneous berry set, but the study of parent and hybrid populations revealed no simple segregation ratios.

Spontaneous berry set improved in F_1 and F_2 populations compared with parents (Table 16, third column). This table also gives the number of seeds per berry after artificial selfing and after spontaneous setting. The 'selfed' berries contained nearly twice as many seeds.

Small differences in seed size occurred, but no definite conclusion could be drawn from the available data.

5.4 A natural triploid found in S. verrucosum CPC 2247

Plant 86 from CPC 2247 (see 5.2), when selfed in 1966, produced small parthenocarpic berries. Its pollen grains looked larger than those of its 'sister' individuals. In staining some grains did not react, others stained irregularly or incompletely and showed a breakdown of the cytoplasm; only 2–3% stained well. A chromosome count revealed that the plant is a triploid (2n = 36).

The study of meiosis in 50 pollen mother cells of this plant (Plate 7) showed a high frequency of trivalents at metaphase I (Table 17). The average chromosome pairing in metaphase I was 7.8 trivalents, 4.3 bivalents and 3.9 univalents.

It is supposed that this triploid plant has developed from the function of an unreduced gamete during fertilization.

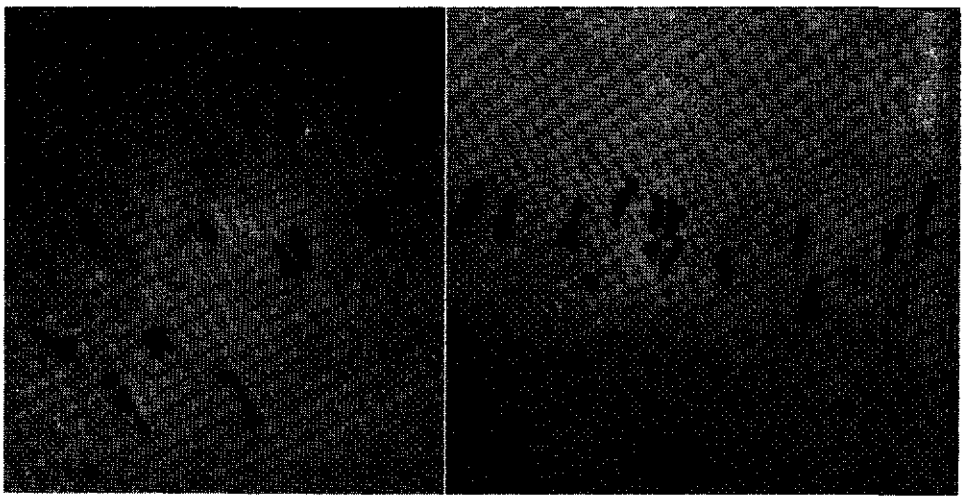


Plate 7. Meiosis in natural triploid plant no. 86 of CPC 2247. On the left $6^{III} + 6^{II} + 6^{I}$, on the right $9^{III} + 3^{II} + 3^{I}$ (cf. Table 17).

Table 17. Chromosome pairing in metaphase I in the triploid plant 86 of *Solanum verrucosum* CPC 2247.

Number of cells	Frequency per cell of			Number of cells	Frequency per cell of		
	trivalents	bivalents	univalents		trivalents	bivalents	univalents
1	5	5	11	1	8	3	6
1	5	7	7	8	8	4	4
1	5	8	5	1	8	5	2
4	6	6	6	1	9	2	5
4	6	7	4	10	9	3	3
1	6	8	2	2	9	4	1
7	7	5	5	6	10	2	2
1	7	6	3	1	10	3	0

5.5 **Inheritance of stem height in two hybrids involving the short stemmed S. verrucosum CPC 1339**

Compared with the other introductions of *S. verrucosum* investigated in 1966, the plants of CPC 1339 were characterized by shorter stems and later flowering (see Plate 1, 4.1.1, 4.2.2).

To study the inheritance of short stem, I_2 plants were crossed with the two long-stemmed verrucosums, CPC 2247 (I_1) and PI 195172–237–9–13 (I_3). The F_1 was raised in 1967 and showed different degrees of heterosis for several characters (see 4.2.2). F_1 plants were selfed to obtain F_2 seed.

Two experiments were carried out in 1968:

(1) ten F_2 populations from CPC 1339 I_2–36 × PI 195172–237–9–13 were grown, together with F_1 plants and plants from both selfed parents,

(2) eleven F_2 populations from CPC 2247 × CPC 1339 were raised but unfortunately neither parental nor F_1 hybrid plants were available for comparison.

The stem height of the plants was measured four times (when seedlings were still in pans, when they were in small pots in cold frames and twice in the field at advanced stages of growth) to minimize the effects of different transplantations and growth under different conditions.

5.5.1 Inheritance of stem height in CPC 1339 I_2–36 × PI 195172–237–9–13

The female parent Plant 36 of CPC 1339–I_2, raised in 1966 (final stem height 33 cm) was pollinated with pollen from Plant 13 of the selfed PI 195172–237–9 (final stem height 65 cm). The selfed parents, the F_1 and ten F_2 populations were measured 42, 87, 146 and 193 days after sowing.

The average stem height of F_1 exceeded that of both parents (Table 18). The F_1 hybrids showed conspicuous growth in the seedling stage and heterosis was most striking in the early growth stages.

Table 18. Average stem height of CPC 1339 I_2–36, PI 195172–237–9–13, their F_1, and ten F_2[1] populations (field experiment 1968).

	Stem height in cm after			
	42 days	87 days	146 days	193 days
♀ CPC 1339–I_2 pl. 36, selfed	0.69	6.62	28.48	39.80
♂ PI 195712–237–9 pl. 13, selfed	1.32	11.99	40.37	59.25
F_1	2.19	16.95	41.00	69.04
F_2 population no. 5	1.18	9.80	44.18	65.16
14	1.62	11.23	41.20	69.78
23	1.27	11.78	37.82	68.13
39	1.19	10.53	37.71	66.28
46	1.22	12.04	37.64	63.93
average height	1.29	11.07	39.71	66.65
F_2 population no. 31	1.10	10.13	35.74	63.02
35	1.30	9.44	36.88	61.76
37	1.14	8.71	35.29	61.46
40	1.42	8.30	38.32	60.52
48	1.09	9.05	35.39	61.85
average height	1.21	9.12	36.32	61.72

1. The reason why two groups were distinguished will be apparent from text and Table 19.

An interesting observation was the positive relation between degree of heterosis in F_1 and degree of inbreeding depression in F_2. The stages of growth where the F_1 plants expressed the highest heterosis percentages (first and second measurements) were the same stages where F_2 populations showed the highest inbreeding depression. The percentages of heterosis were 118%, 73%, 19% and 39% in the first, second, third and fourth measurements, respectively, whereas the corresponding percentages of inbreeding depression in F_2 were 43%, 40%, 7% and 8%.

The recessive short stem characteristic of CPC 1339 appeared to be consistent in all experiments. Therefore, range of stem height in CPC 1339 was used as a criterion to divide plants of other populations into short and tall ones for each of the four measurements. In other words, plants showing stem height within the range of parent CPC 1339 were considered to be short-stemmed. The numbers of plants of the four short class groups were averaged. The same was done with the four tall class groups. The numbers thus obtained were considered to be the best estimate for the phenotypical numbers of short and tall plants in each of the populations studied.

From Table 19 it is apparent that the ratios found in this way fit a hypothesis of two dominant complementary genes T_1 and T_2 (T for tall) determining tall stem [4]. Hence, the genotype of the short parent, CPC 1339 – I_2 pl. 36, is assumed to be $t_1t_1T_2T_2$,

4. On the basis of this segregation two F_2 groups were distinguished in Table 18.

Table 19. Segregation ratios for stem height in selfed parents, F_1 and ten F_2 populations from the hybrid CPC 1339 I_2–36 × PI 195172–237–9–13 and assumed genotypes.

	Number of observed plants		Expected ratios tall : short	P from χ^2 test	Assumed genotypes
	tall	short			
♀ CPC 1339–I_2 pl. 36, selfed	1	127	0:1		$t_1t_1T_2T_2$
♂ PI 195172–237–9 pl. 13, selfed	112	25	3:1	0.10–0.05	$T_1T_1T_2t_2$
F_1	71	5	1:0		$T_1t_1T_2T_2, T_1t_1T_2t_2$
F_2 population 5	75	34	3:1	0.20–0.10	$T_1t_1T_2T_2$
14	93	24	3:1	0.30–0.20	
23	92	28	3:1	0.70–0.50	
39	84	37	3:1	0.20–0.10	
46	114	34	3:1	0.90–0.80	
total	458	157	3:1	0.70–0.50	
F_2 population 31	68	49	9:7	0.70–0.50	$T_1t_1T_2t_2$
35	74	40	9:7	0.10–0.05	
37	64	60	9:7	0.50–0.30	
40	73	41	9:7	0.10–0.05	
48	61	59	9:7	0.30–0.20	
total	340	249	9:7	0.50–0.30	

that of the tall parent, PI 195172–237–9 pl. 13, to be $T_1T_1T_2t_2$. The equal numbers of F_2 populations segregating into the ratios of 3:1 and 9:7 are in agreement with the hypothesis.

Figure 27 presents the stem height distribution in the two parents, in the F_1, and in the two F_2 populations 14 and 48. Class A is the short stem class and includes practically all CPC 1339 progeny (upper row of Fig. 27). For parent PI 195172–237–9–13, most of the progeny turns up in class B. There is no interval between the two parent populations and accordingly there are no gaps between the classes in the F_2's.

Figure 27 and the data of Table 19 suggest small effects of minor genes or environmental conditions or both on stem growth. This may account for the variation between the different measurements and the unexpected deviations of some plants. The differences between the histograms of the four measurements of F_2 48 (Fig. 27) may suggest changes of dominance relationship. However, due to the relatively small difference in frequency between the two categories of segregants, a small shift from the tall to the short class (brought in by deep planting for instance) can affect the frequency of short: tall individuals. At the fourth measurement this deviation disappeared. Neither in F_2 populations segregating into 3:1, nor in all populations of the hybrid CPC 2247 × CPC 1339 (see 5.5.2), was such behaviour observed, which would point to its accidental cause.

In Fig. 28, comprising the pooled data of the four measurements in the populations of Fig. 27, the F_1 shows a relatively wide distribution due to the measurements at the early growth stages (see the third row of histograms in Fig. 27).

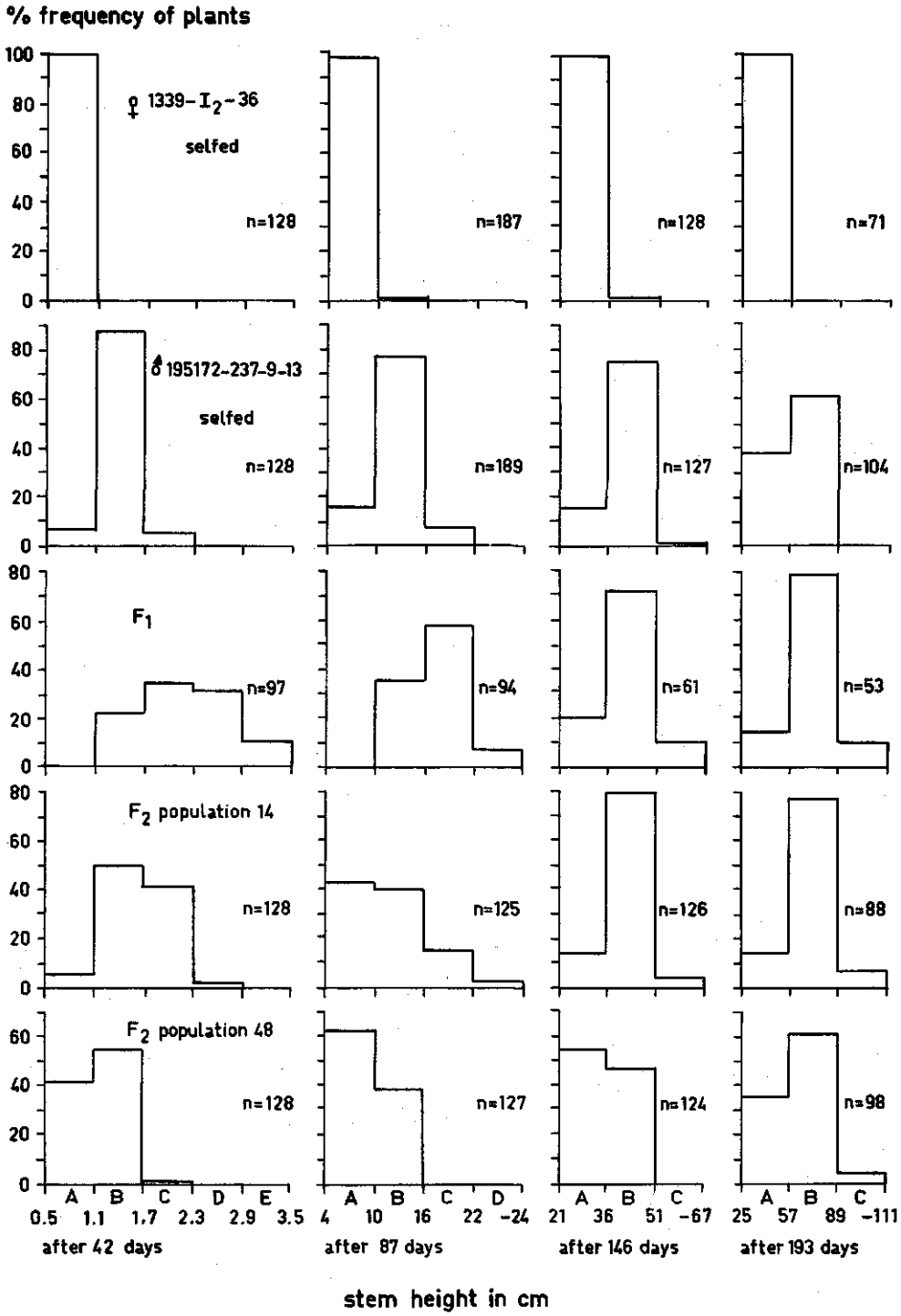


Fig. 27. Frequency distributions for stem height in indicated populations. Number of plants = n.

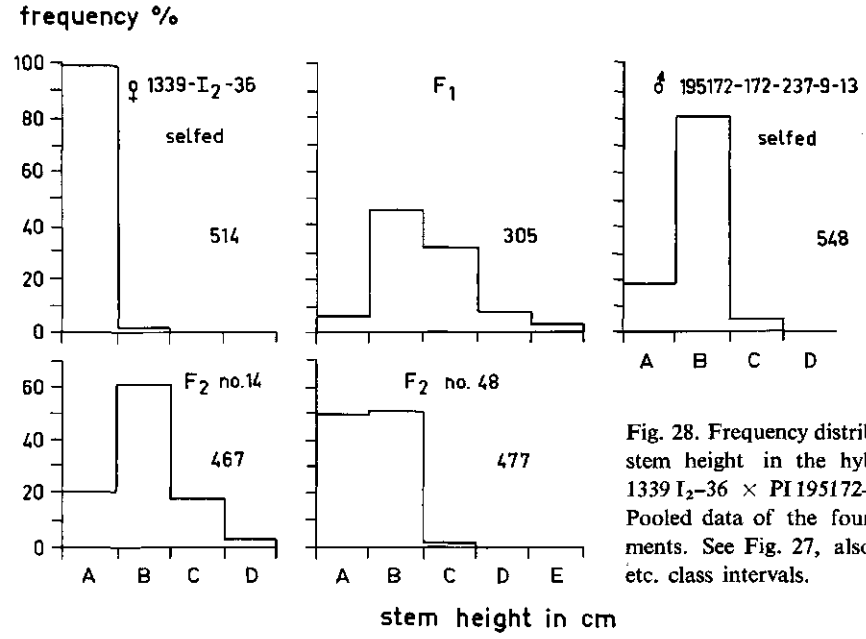


Fig. 28. Frequency distributions for stem height in the hybrid CPC 1339 I_2–36 × PI 195172–237–9–13. Pooled data of the four measurements. See Fig. 27, also for A, B etc. class intervals.

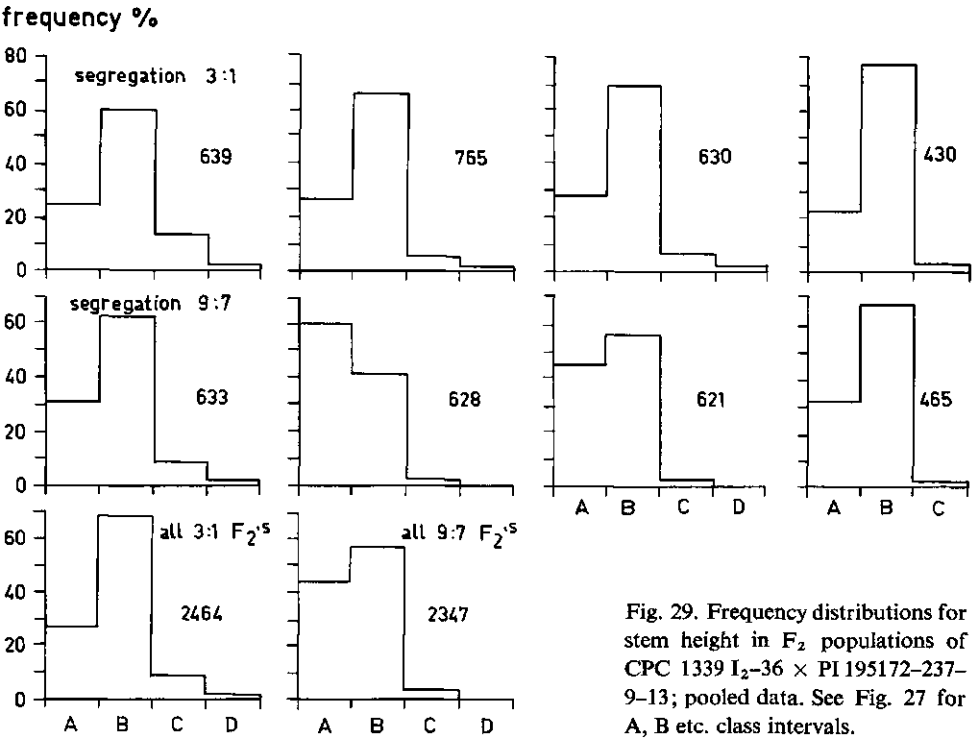


Fig. 29. Frequency distributions for stem height in F_2 populations of CPC 1339 I_2–36 × PI 195172–237–9–13; pooled data. See Fig. 27 for A, B etc. class intervals.

The difference between F_2–14 (3:1) and F_2–48 (9:7) is quite clear, as is the agreement between F_2–14 and the parent PI 195172–237–9–13 (3:1).

Figure 29 shows the four measurements of the pooled F_2's segregating 3:1 in the upper row, those of the 9:7 F_2's in the middle row, whereas in the bottom histograms the data of all pooled 3:1 F_2's and 9:7 F_2's are given. The difference between the two categories of segregating F_2 populations is manifest in the four measurements; this supports the validity of the way these data have been handled. Furthermore it is noteworthy that pooling the data of the four measurements in the five 9:7 populations has adjusted what some may consider to be a change of dominance relationship occurring in some F_2 populations, especially at the second measurement.

5.5.2 Inheritance of stem height in CPC 2247 × CPC 1339

The eleven F_2 populations from CPC 2247 × CPC 1339 were handled in the same way as those in 5.5.1, i.e. using the range of stem height in selfed CPC 1339 as a scaling criterion to differentiate between short and tall plants.

The average stem heights of the F_2 populations in the four measurements (Table 20) were higher than those reported for the F_2's in the former hybrid. A corresponding behaviour was observed both for F_1 plants (see Table 10) and for the tall parent populations (see Tables 9 and 12).

Table 21 shows that three segregation ratios tall: short occur in the eleven F_2 populations, viz. 9:7, 54:10 and 15:1. As expected, the average stem height in the F_2's increased from the 9:7, via the 54:10 to the 15:1–F_2 populations in each of the four measurements (Table 20).

The segregation ratios in Table 21 suggest three *T* loci for stem height (in the

Table 20. Average stem height at four stages in eleven F_2 populations from CPC 2247 × CPC 1339.

F_2 population number	Stem height in cm after			
	43 days	87 days	147 days	199 days
4	1.31	10.67	33.47	65.37
1	1.89	10.72	42.04	69.21
2	2.27	15.18	39.44	69.06
7	1.96	12.83	41.79	66.08
19	1.95	11.65	40.40	66.95
33	1.96	14.62	42.45	63.07
average	2.00	13.00	41.22	66.87
3	1.98	14.56	42.46	72.25
8	2.05	13.25	43.28	71.24
13	2.15	15.12	42.80	71.77
25	2.22	14.40	41.40	73.95
40	2.36	16.62	46.30	70.82
average	2.15	14.79	43.24	72.00

Table 21. Segregation ratios for stem height and assumed genotypes in eleven F_2 populations of CPC 2247 × CPC 1339.

F_2 population number	Number of observed plants		Expected ratios tall: short	P from χ^2-test	Assumed genotypes of F_1
	tall	short			
4	75	46	9: 7	0.30–0.20	$T_a t_a T_b t_b t_c t_c$
1	103	18	54:10	0.90–0.80	
2	104	18	54:10	0.80–0.70	
7	102	18	54:10	0.90–0.80	$T_a t_a T_b t_b T_c t_c$
19	104	18	54:10	0.80–0.70	
33	104	12	54:10	0.20–0.10	
total	517	84	54:10	0.30–0.20	
3	112	11	15: 1	0.30–0.20	
8	140	13	15: 1	0.30–0.20	
13	108	12	15: 1	0.10–0.05	$T_a t_a T_b T_b T_c t_c$
25	112	11	15: 1	0.30–0.20	
40	101	6	15: 1	0.80–0.70	
total	573	53	15: 1	0.05–0.02	

former hybrid two loci were reported). The presence of at least two of them in dominant condition causes tallness. One *T* locus may be a duplication of one of the other two loci.

Thus the genetic basis of tallness of stem is not essentially different in both hybrids studied. Indicating the three *T* loci with the subscripts a, b and c, the genotypes assumed for the short and tall parents are $t_a t_a T_b T_b t_c t_c$ and $T_a T_a T_b t_b T_c t_c$ respectively. Four genotypes of F_1 individuals then are expected in equal frequencies:

$T_a t_a T_b T_b T_c t_c$, producing a 15:1 ratio in F_2
$T_a t_a T_b t_b T_c t_c$, producing a 54:10 ratio in F_2
$T_a t_a T_b T_b t_c t_c$, producing a 3:1 ratio in F_2
$T_a t_a T_b t_b t_c t_c$, producing a 9:7 ratio in F_2

As shown in Table 21, these expected ratios did not all occur; 3:1 was missing and 9:7 was found only once. The 15:1 segregation was in accordance with expectations, but the pooled data of the five populations showed a shortage of tall plants. No definite explanation for these deviations can be presented.

5.6 **Inheritance of flowering time in CPC 1339 I_2-36 × PI 195172-237-9-13**

Flowering time was expressed by the number of days from sowing till opening of the first flower on a plant. Flowering, once started, continued without noticeable breaks.

Compared with other introductions of *S. verrucosum* checked in 1966 (see 4.1.1) CPC 1339 is a late flowering population. This character was studied in the hybrid between the I_2 Plant 36 of CPC 1339 and the relatively early flowering I_3 Plant 13 of

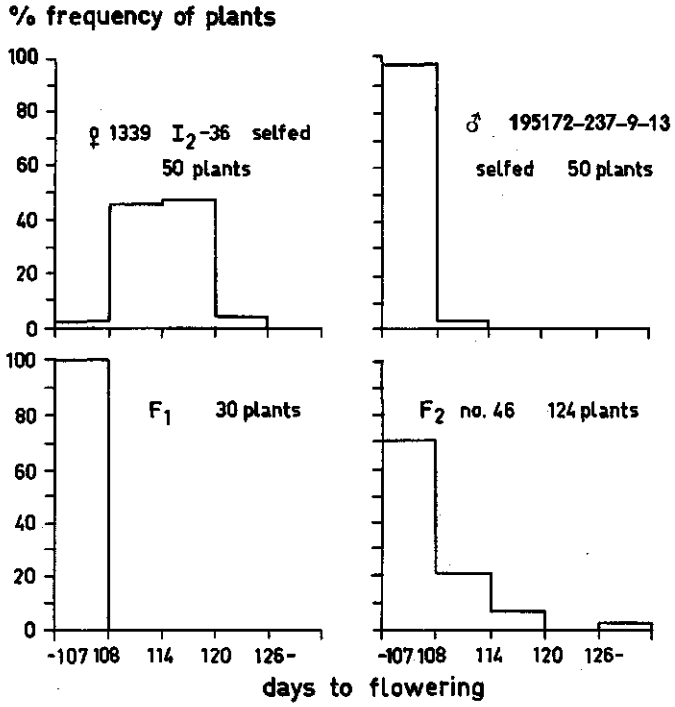


Fig. 30. Days from sowing till opening of the first flower in the selfed progenies of the parents CPC 1339 I_2–36 and PI 195172–237–9–13, their F_1 and F_2 population 46 (glasshouse 1968).

PI 195172 (this hybrid was raised to study inheritance of stem height, see 5.5.1). The F_1 plants grown in 1967 flowered after 89 days whereas the I_2 population of CPC 1339 flowered after 99 days and the I_3 population of PI 195172 after 91 days. Therefore early flowering showed dominance over late flowering.

Flowering time was studied in the selfed parents, F_1 and F_2 populations, all grown in 1968 in both the glasshouse and the field[5]. Both in the field and glasshouse, all plants flowering within 107 days after sowing were considered early flowering.

The data on flowering time are given in Figs. 30 and 31 and Table 22. The field results (Fig. 31) were similar to those in the glasshouse (Fig. 30). Only 8% of the late parent progeny was early, against 90% in the early parent progeny and 97% in the F_1. The corresponding percentages from the glasshouse material were 2%, 98% and 100%. Thus dominance of early flowering was obvious.

A somewhat larger variability was observed in the field (Fig. 31). This may be

5. The assessment of flowering time could not be started until 102 days after sowing. By that date, some plants had already shown some flowers, especially among the F_1. However, as in 1967 (see 4.2.2), the F_1 hybrid population, on an average, flowered earlier in 1968 than the early flowering parent population PI 195172–237–9–13.

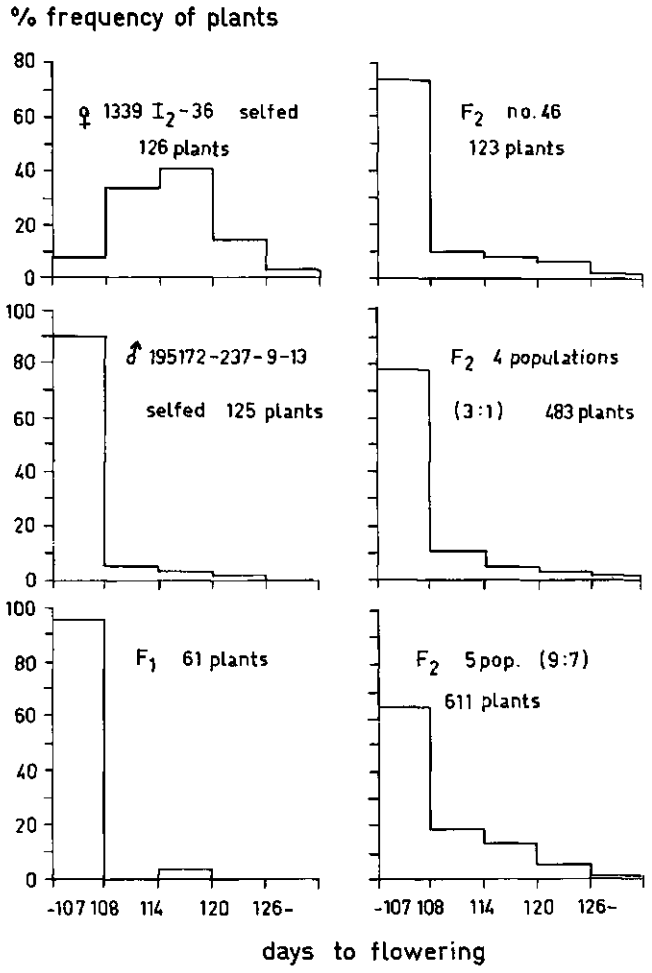


Fig. 31. Days from sowing till opening of the first flower in the selfed progenies of the parents CPC 1339 I_2–36 and PI 195172–237–9–13, their F_1 and F_2 populations (field 1968).

ascribed to the greater variation in environmental conditions in the field. The only F_2 population grown in the glasshouse (46) consisted of 87 early and 37 late flowering plants, which fits a ratio of 3:1. Plants from the same population raised in the field showed 91 early and 32 late plants. This close agreement between field and glasshouse data for F_2 46 indicates that the F_2 ratios from the field may be considered reliable.

Of the ten field-grown F_2 populations, five (including 46) segregated 3:1, the other five 9:7. All ratios fit the expectation, but the pooled data of the 9:7 populations showed an excess of early flowering plants. Hence one might wonder whether the conditions in the field in some way favoured earliness (see also 5.7 and the frequencies of tall and short plants in Table 19).

Table 22. Segregation ratios for flowering time in selfed parents, in F_1 and in ten F_2 populations from CPC 1339 I_2–36 × PI 195172–237–9–13, with assumed genotypes.

	Observed numbers		Expected ratios *Ef*:*ef*	P from χ^2-test	Assumed genotypes
	early flowering (*Ef*)	late flowering (*ef*)			
Glasshouse plants					
CPC 1339–I_2 pl. 36, selfed	1	49	0:1		$Ef_1ef_1ef_2ef_2$
PI 195172–237–9 pl.13, selfed	49	1	1:0		$Ef_1Ef_1Ef_2Ef_2$
F_1	30	0	1:0		$Ef_1Ef_1Ef_2ef_2$, $Ef_1ef_1Ef_2ef_2$
F_2 population 46	87	37	3:1	0.30–0.20	$Ef_1Ef_1Ef_2ef_2$
Field plants					
CPC 1339–I_2 pl.36, selfed	10	116	0:1		
PI 195172-237–9 pl.13, selfed	113	12	1:0		
F_1	59	2	1:0		
F_2 population 5	101	25	3:1	0.20–0.10	$Ef_1Ef_1Ef_2ef_2$
23	93	26	3:1	0.50–0.30	
31	91	31	3:1	0.95–0.90	
39	86	32	3:1	0.70–0.50	
46	91	32	3:1	0.90–0.80	
total (*Ef*:*ef* = 3:1)	462	146	3:1	0.70–0.50	
F_2 population 14	77	49	9:7	0.30–0.20	$Ef_1ef_1Ef_2ef_2$
35	76	42	9:7	0.10–0.05	
37	78	47	9:7	0.20–0.10	
40	78	43	9:7	0.10–0.05	
48	78	43	9:7	0.10–0.05	
total (*Ef*:*ef* = 9:7)	387	224	9:7	small	

On the basis of the ratios obtained (Table 22), early flowering is assumed to be determined by two complementary dominant genes Ef_1 and Ef_2. If the late and the early parent have the genotypes $Ef_1ef_1ef_2ef_2$ and $Ef_1Ef_1Ef_2Ef_2$, respectively, the F_1 is early, but it consists of two genotypes giving rise to equal numbers of 3:1 and 9:7 F_2's. This was indeed found.

5.7 Relation between stem height and flowering time

S. verrucosum CPC 1339 is a short-stemmed and late flowering introduction, whereas inbred PI 195172–237–9 has tall stems and flowers early. According to the assumed genotypes of the parents (see 5.5.1 and 5.6), the hybrid plants should be distributed over four genotypes with equal frequencies.

In the F_2 these four genotypes should show a segregation of tall plants: short

plants, and early flowering : late flowering respectively in the ratios: 3:1, 3:1; 9:7, 9:7; 3:1, 9:7 and 9:7, 3:1. The F_2 populations, however, showed a higher frequency segregating into 3:1 or 9:7 for both characters (4 populations each) whereas the other two expected types of segregation were represented each with one population. The observed frequencies in the F_2 populations fitted the expected one.

From the observations recorded for parents, as well as for the F_2 plants grown in the field, it looked as if flowering time was more or less associated with stem height: later flowering plants of F_2 populations had short stems, but not all short-stemmed plants flowered later. The correlation coefficient for stem height in the third measurement[6] and flowering time in F_2 populations 31, 39 and 48 were –0.22, –0.47, and –.034 (average –0.34). The negative correlation, though low, is statistically significant.

This suggests that the method applied in analysing stem height data was correct, as the picture of flowering time was more clear, with less variability than that of stem height.

5.8 Other characters investigated in CPC 1339 I_2-36 × PI 195172-237-9-13

Table 23 supplies some additional data on the populations raised in the glasshouse. The F_1 hybrids gave better tuber yields, showed more plants producing spontaneous berries, larger numbers of such berries per plant and a higher percentage of tuberiferous plants. Distinct heterosis in the F_1 population occurred in all characters studied.

Table 23. Mean values for various characters in selfed parental populations, in F_1 and in F_2, with percentages heterosis and inbreeding depression for CPC 1339 I_2–36 × PI 195172–237–9–13 (glasshouse 1968).

	Average values					
	CPC 1339 I_2 –36 selfed	PI 195172–237–9–13 selfed	F_1	F_2 population 46	Heterosis in F_1 (%)	Inbreeding in F_2 (%)
Stainable pollen (%)	50.9	48.5	83.6	79.8	+ 68.2	− 4.5
Number of seeds per berry	108	72	184	157	+104	−14
Tuberiferous plants (%)	76	68	83	42	+ 16	−50
Number of tubers per plant	4.0	2.4	5.2	2.8	+ 62.5	−46.2
Weight of tubers per plant (g)	2.8	2.5	4.7	2.9	+ 74.1	−38.3
Plants set spontaneous berries (%)	62.0	74.0	100.0	92.0	+ 47.1	− 8.0
Number of spont. berries per plant	3.2	5.4	16.4	11.8	+281.4	−28.0

The average values were calculated from 50 plants of each selfed parent progeny, from 30 F_1 plants, and from 125 F_2 plants exept male and female fertility. −: depression; +: gain.

6. The third measurement was taken because the plants started flowering between the second and the third measurement and because they were labelled in the field when measured for the third time.

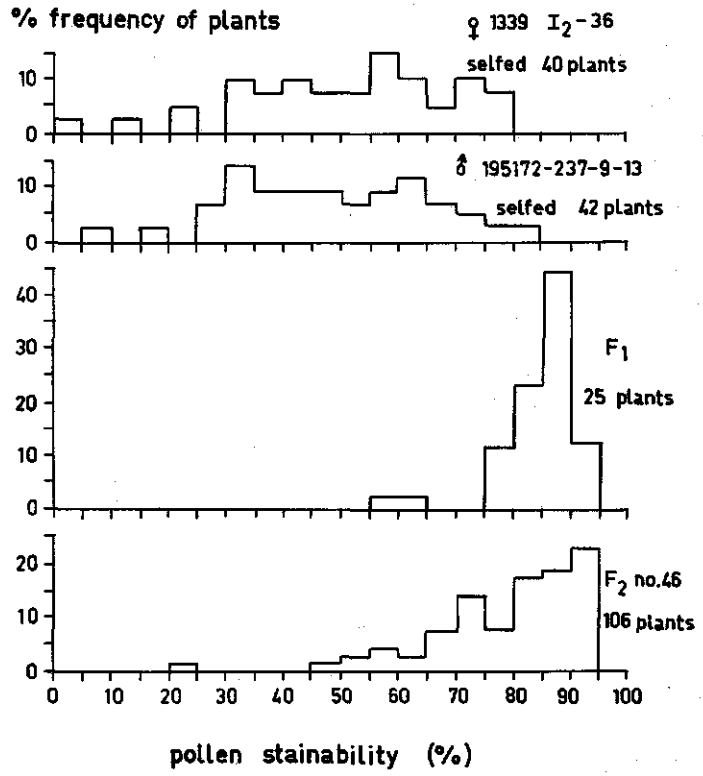


Fig. 32. Frequency distributions of pollen stainability in the selfed progenies of the parents CPC 1339 I_2–36 and PI 195172–237–9–13, in their F_1 and in F_2 population 46 (glasshouse 1968).

In addition the F_2 population showed various degrees of inbreeding depression.

Frequency distributions are presented for percentage pollen stainability in Fig. 32 and seeds per berry in Fig. 33. It is clear from both figures, as well as from Table 23, that the F_1 hybrid population showed a strong heterosis and, at least for pollen stainability, a much lower variability than the parents. F_2 population 46 showed better male and female fertility than the original parents and a slight inbreeding depression as compared with F_1.

The wide variation of pollen stainability in the selfed progenies of the parents was striking and might, together with the low means, be due to inbreeding. The picture given by Figs. 32 and 33 and the strong heterosis observed in the hybrids suggest a different (polygenic) basis for male and female fertility in the parents.

It is noteworthy that in *S. verrucosum* pollen stainability and seed set hardly show any association. A reasonable number of seeds per berry after selfing is nearly always found, irrespective of pollen stainability. This is understandable, as this species usually sheds large quantities of pollen grains. Consequently there will always be sufficient pollen to fertilize the relatively few ovules.

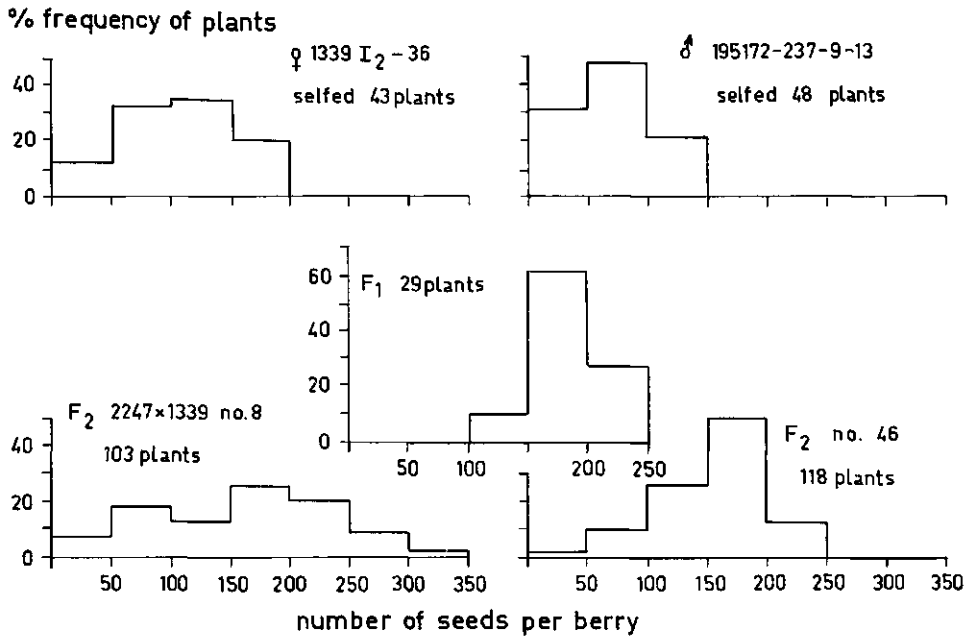


Fig. 33. Frequency distributions of seed set in the selfed progenies of parents CPC 1339 I_2–36 and PI 195172–237–9–13, their F_1 and F_2 population 46. F_2 population 8 of the hybrid CPC 2247 × CPC 1339 is also presented (glasshouse 1968).

Figure 33 also shows the data of F_2 population 8 from CPC 2247 × CPC 1339 (grown in the same glasshouse) for a comparison with data of F_2 population 46 of CPC 1339 I_2–36 × PI 195172–237–9–13. Population 8 showed a wider range of variation in seed set than any other population in Fig. 33. This is due to the influence of the parent CPC 2247, characterized by a higher seed set than parent PI 195172–237–9–13. This supports the genetical control of seed set in *S. verrucosum*.

The study of male and female fertility in different populations of *S. verrucosum*, discussed in Chapter 4, also indicates that these characters are polygenically controlled.

6 Barriers to gene exchange and interspecific crosses in diploid Solanum

In this chapter data are presented on the crossability of self-compatible and self-incompatible species. Certain interspecific hybrids between *S. verrucosum* and self-incompatible species and haploids will be treated in some detail.

6.1 Selfing and intercrossing self-compatible diploid species

Three species were used in these studies. The first two are the tuber-bearing Mexican species *S. verrucosum* (various introductions) and *S. polyadenium* (Haw 1569). The third species used is the nontuber-bearing South American *S. etuberosum* (PI 245939).

6.1.1 General observations and selfing

Plants of *S. etuberosum* showed good flowering when grown in the soil or grafted onto tomato, but those grown in pots hardly flowered. *S. polyadenium* always showed shy flowering, whether raised in pots or grafted.

All three species flowered within the same time and all proved to be self-compatible.

S. polyadenium when selfed, gave 50% berry set and 20–40 seeds per berry, whereas *S. etuberosum* showed 100% berry set and about 280 seeds per berry. Berry set in *S. verrucosum* was intermediate (about 70%) and seed set varied in different introductions; details on seed set were given in Chapters 4 and 5.

The self-compatibility of *S. polyadenium* has been questioned by some authors. Hawkes reported it in his 1956 revision, but not in his 1963 revision. Propach (1940) and Malheiros-Gardé (1959a) reported self-incompatibility, but Pushkarnath (1959) and Pandey (1962c) considered the species to be self-compatible. During the present investigations, the introduction was checked in 1966 and 1969 and proved to be self-compatible.

The controversy about self-compatibility of this species may be related to its 'inertia' in setting berries and its low female fertility. Compared with the other self-compatible species used, *S. polyadenium* needed almost twice as much time before its berries became visible after selfing. Its berries were also smaller than those from *S. verrucosum* and *S. etuberosum* and contained much fewer seeds. The mechanisms controlling seed set in this species may be sensitive to different environments. Whatever the cause, no spontaneous berry set was ever found on this species, although such berry set can occur on the other two self-compatible species as well as on some self-incompatible species, especially near the end of the flowering time.

Unfortunately the fertility of the species could not be tested by interspecific crosses due to its almost complete crossability barrier with other species. Therefore this test could only be carried out within the species.

6.1.2 Intercrossing

Crossing after careful emasculation gave the following results:
(1) No berry set: *S. verrucosum* × *S. polyadenium* and reciprocal; *S. verrucosum* × *S. etuberosum* and *S. etuberosum* × *S. polyadenium*, for *S. verrucosum* both PI 275258 and PI 195172 were used.
(2) Seedless berries: *S. polyadenium* × *S. etuberosum* and *S. etuberosum* × *S. verrucosum* PI 275258.
(3) One small berry: four pollinations of *S. verrucosum* PI 160228 × *S. etuberosum* gave only one small berry with five seeds of which one germinated. This seed produced a plant resembling *S. verrucosum*, which was successfully selfed. The selfed progeny is of great interest and therefore will be discussed in Section 6.2.

6.2 **Parthenogenesis in S. verrucosum**

The plant obtained from the single germinated seed mentioned above proved to possess 24 chromosomes in its root cells. It showed pure *S. verrucosum* characters

Table 24. Comparison of I_1 from selfed and I_1 from parthenogenetic *Solanum verrucosum* PI 160228 (glasshouse 1968).

	Average values	
	selfed	parthenogenetic
Percentage of emergence	79.2	33.2
Stem height after 42 days (mm)	27.4	6.3
87 days (cm)	16.3	5.2
150 days (cm)	93.1	83.7
195 days (cm)	128.1	104.3
Simple leaf length (mm)	46.4	31.7
width (mm)	30.2	20.4
Compound leaf length (mm)	26.7	20.6
width (mm)	11.8	9.2
D value (mm)	72.4	53.8
Days to opening first flower	105.4	108.6
Percentage of stainable pollen	84	66
Seeds per berry	118	172
Weight of 1000 seeds (mg)	209.0	198.0
Percentage of tuberiferous plants	14	80
Number of tubers per plant	2.1	6.3
Weight of tubers per plant (g)	2.6	11.1

Number of plants per population: 50 (pollen stainability and seed set 3–29).

with reduced growth. The plant produced only two inflorescences. Four of its flowers were selfed and the four berries set enclosed 1129 seeds (about 282 per berry). The tuber yield was higher, both in number and in weight, than the average in PI 160228. In addition its seeds were small compared with those of the selfed PI 160228 plants (average length 1.49 mm, average breadth 1.08 mm, as against 1.84 and 1.32 mm in the selfed parent plant).

For comparison in the next season, some of the 1129 seeds were sown together with seeds resulting from artificial self-pollination on the parent verrucosum plant. All ensuing plants proved to be pure *S. verrucosum*. The original seed obtained from the pollination of verrucosum by etuberosum pollen is thought to originate through parthenogenesis and not as a result of fertilization of the egg cell. (More proof will be supplied later).

The I_1 population obtained by selfing the original parthenogenetic plant will be

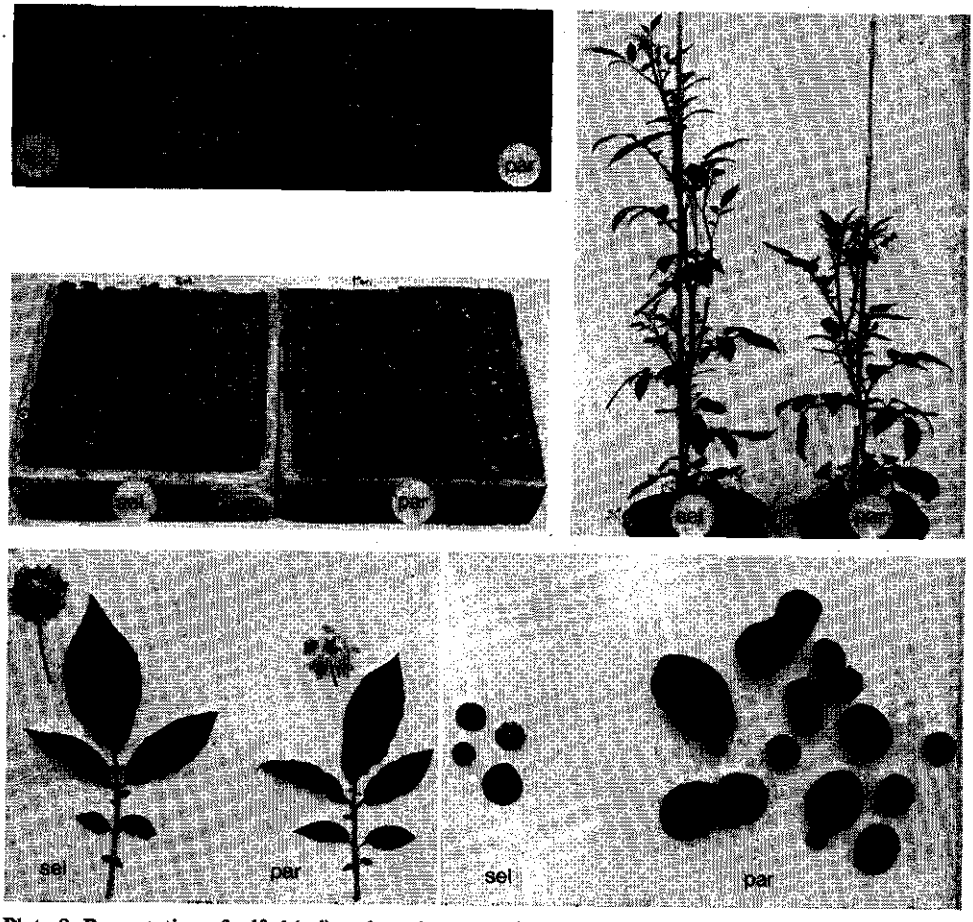


Plate 8. Presentation of selfed (sel) and parthenogenetic (par) populations of *S. verrucosum* PI 160228 in some characters.

referred to as parthenogenetic, whereas the offspring obtained by selfing the original female verrucosum parent will be called selfed.

In 1968, the parthenogenetic and the selfed populations were compared (both in the I_1). As apparent from Table 24 (see also Plate 8), the seed of the selfed population germinated better, gave more vigorous seedlings and plants with taller stems and larger leaflets. Also plants showed darker coloured stems and flowers, and darker green leaves. The tuber yield of the parthenogenetic plants was higher (three times or more) than that in the selfed plants. Stainability of pollen grains in the parthenogenetic population was about 20% lower but seed set per berry was about 50% higher. Seed size and weight were smaller in the parthenogenetic plants. Eight of the parthenogenetic plants died very early in the season; four did not reach a height of more than 2 cm. The parthenogenetic plants were highly uniform; genetic evidence for this uniformity will be presented in Chapter 8.

6.3 The growth of pollen tubes in styles after attempted crossing of self-compatible species

To trace the cause of the failure of the crosses attempted between the self-compatible species, a study was made of the pollen tube growth in styles.

In both cases of *S. verrucosum* pollen in *S. etuberosum* styles and the reciprocal, good germination of pollen grains occurred and pollen tubes were observed penetrating the upper part of the styles. They stopped at about one third of the style, however, in the case of etuberosum × verrucosum, and about the middle of the style in verrucosum × etuberosum. It seemed that verrucosum pollen in etuberosum styles was more strongly inhibited than etuberosum pollen in verrucosum styles. In both cases, a slight thickening could generally be observed near the tips of the tubes.

It is striking that this inhibition reaction is the same as has always been observed in the failure crossings between self-incompatible and self-compatible species (see 6.5.1).

Pollinations between *S. polyadenium* and *S. verrucosum* were checked for pollen germination. It was observed that when polyadenium was the female partner, hardly any pollen germinated. Only a few pollen tubes of verrucosum were observed in polyadenium styles, and they never penetrated deeper than the upper sixth part of the styles. On the other hand nearly all polyadenium pollen germinated, but all were inhibited in the stigmatic region or in the upper sixth or fifth part of verrucosum styles. Again thickening of pollen tube ends was observed.

6.4 Self-compatible (SC) × self-incompatible (SI) and reciprocals

The three self-compatible species *S. polyadenium*, *S. etuberosum* and *S. verrucosum* were used in reciprocal crossing attempts with some self-incompatible species. *S. polyadenium* and *S. etuberosum* have been used in limited pollinations with the self-incompatible species *S. phureja*, whereas *S. verrucosum* was used extensively with

several self-incompatible species. The results of the trials with *S. polyadenium* and *S. etuberosum*, together with those verrucosum investigations which are directly connected will be discussed in 6.4.1. Most data on interspecific crosses involving verrucosum will be treated separately in following sections.

6.4.1 Crossing attempts with self-incompatible S. phureja leading to selfing

S. polyadenium when pollinated with pollen of *S. phureja* PI 225698, produced berries in 8% of the flowers, but all were seedless. The reciprocal gave no berry set.

In 1966, *S. etuberosum* was used as female partner to *S. phureja* PI 225698. About 55% berry set was obtained, but all berries were empty. A second attempt (in 1968) with *S. phureja* PI 225682–22 resulted in 79% berry set: ten empty berries + five berries containing a total of 298 seeds. These seeds were germinated in 1969 and the resulting plants completely resembled *S. etuberosum* with a uniform appearance, so that a parthenogenetic origin would seem probable.

Using etuberosum as male on non-emasculated phureja PI 225698 resulted in 17% berry set (three berries) with an average of 100 seeds per berry. Pollen of verrucosum PI 275258 tried on the same non-emasculated phureja gave about 4% berry set and the only berry obtained enclosed 58 seeds. Both lots of seeds were germinated the following season and the seedlings showed pure phureja characteristics but were not uniform. It seems that the male parents could not fertilize the female ovules and the seed set was a result of selfing.

The failure of the crossings *phu* × *etb*, *phu* × *ver*, *phu* × *pld*, *etb* × *phu* and *pld* × *phu* (see Table 1 for abbreviations of species names) was found to be due either to inhibition of pollen tube growth in the style of *phu*, to inhibition of pollen germination on the stigma of *pld*, or to some barrier(s) preventing seed formation after pollinating *etb* with *phu* pollen. In the *phu* × *ver* pollinations most of the pollen grains germinated, and many of their tubes penetrated the *phu* style for about a third of its length and then stopped. However, a few pollen tubes could be traced deeply in the lower half of the style, though none entered the ovaries. Again many pollen tubes were characterized by swollen ends.

In *phu* × *etb* most pollen grains germinated, but their tubes were inhibited in the upper 1/5–1/4 of the styles. The pollen tubes were thickened, some of them were spine-shaped and nearly all had clearly swollen ends larger than in case of *phu* × *ver*.

The *etb* × *phu* showed normal pollen grain germination and pollen tube growth throughout the styles. Most probably, some mechanism other than inhibition of pollen tube growth was involved in the failure of this pollination, perhaps operating in *etb* ovaries preventing fertilization or preventing the development of the fertilized ovules.

The pollinations between *pld* and *phu* showed results similar to those between *ver* and *pld* (see 6.3). When *phu* was used as a female, many pollen grains of *pld* germinated, but most of them were inhibited in the stigmatic region. Occasionally a tube penetrated 1/8 of the style, but nearly all tubes were short (about one third of those

observed in *ver* × *pld*) and showed spine thickening, also at their ends. In the *pld* × *phu* pollinations very few grains germinated; all were arrested near the stigmatic region.

These observations demonstrate that polyadenium has stronger barriers than etuberosum and that the strongest inhibiting mechanism operates on the female side of polyadenium. This mechanism even prevents pollen grain germination of both SI and SC species.

6.4.2 Studies of some characters in the selfed populations from S. phureja PI 225698

As both lots of seeds set on phureja proved to be selfed phureja whether the pollinator was *etb* or *ver*, it was possible to study the effect of selfing on the self-incompatible *phu*. The seeds of the selfed *phu* obtained when *etb* was the pollinator will be referred to as population E, and the seeds set when *ver* pollen was applied will be named population V (see 6.4.1).

6.4.2.1 Inbreeding effects

In 1967 the self-set seed of populations E and V were germinated together with seed of *phu* PI 225698 (the remnant sib-mated seed obtained from Wisconsin) to compare the effect of selfing. The selfed plants varied considerably in their characteristics and some differed in appearance from the parent *phu*. The I_1 plants showed different degrees of inbreeding depression in almost all characters studied (Table 25): generally the plants were smaller, had shorter stems and smaller leaves, flowered poorly or not

Table 25. Mean values for some characters in *Solanum phureja* PI 225698 compared with two I_1 inbred populations obtained by using *S. etuberosum* (E) and *S. verrucosum* (V) as pollinators (1967).

	PI 225698	I_1–E		I_1–V	
		average value	inbreeding (%)	average value	inbreeding (%)
Percentage of emergence	63.3	43.1		65.5	
Stem height after one month (mm)	25.0	24.1	− 4	25.2	+ 1
after 70 days (cm)	18.6	8.7	−52**	9.3	−50**
at end of season (cm)	143.9	83.8	−42**	110.3	−23**
Compound leaf length (mm)	40.4	25.0	−38**	30.2	−25**
width (mm)	22.5	13.4	−41**	16.8	−25*
T value (mm)	59.7	42.6	−29**	50.9	−15
Percentage stainable pollen	84	32	−62**	14	−83**
Number of tubers per plant	4.4	4.2	− 4	1.8	−59**
Weight of tubers per plant (g)	14.0	5.7	−59**	2.4	−83**
Percentage of tuberiferous plants	50	23	−54	24	−53

*, **: significant differences at 0.05 and 0.01 level (t-test); −: depression; +: gain.

at all, most showed a clear decrease in pollen stainability, and tuber yield was reduced to about half or less compared with the parent sib-mated plants.

6.4.2.2 Classification for growth habit and its genetic basis

Early in the seedling stage, it was easy to classify the *phu* inbred plants visually into three Types according to their growth habit (Plate 9).

The first Type (I) included plants resembling the *phu* parent in their growth with less smooth stems and leaves and with richly coloured flowers. Of this class, 22 plants belonged to population V and 74 to E.

In the second Type (II) the plants were shorter and thinner and had more tender stems and reduced light green leaves; flowering was poor and the flowers were reduced in size with less rich colouring. A total of 29 plants belonged to this group, 9 to V and 20 to E.

The third Type (III) comprised small short plants, with many stems and very small leaves, mostly with dark brown edges; some of the plants produced only simple leaves. The plants of Type III died earlier in the season than those of the other two Types; they neither flowered nor produced any tubers. Some plants of this Type were grafted onto tomato, but here again failed to flower. Chromosome counts in root tips showed the plants to have 24 chromosomes. The number of plants of this Type was 7 in population V and 30 in E.

In this classification, Types I, II and III contained 96, 29 and 37 plants, respectively.

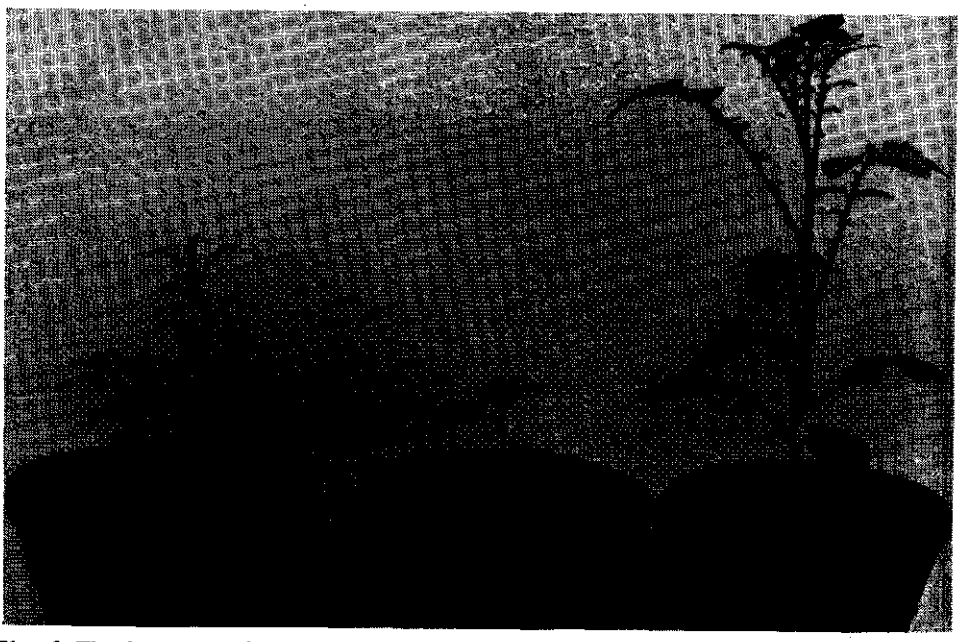


Plate 9. The three types of plant growth observed in selfed *S. phureja* PI 225698. From left to right: Types III, II and I.

These numbers fit the ratio 9:3:4 assuming the plant growth habit of *phu* to be controlled by two complementary dominant genes for which the parent plant is double heterozygous. These genes for normal growth will be referred to as Ng_1 and Ng_2. Thus the *S. phureja* parent plant will be of the genotype $Ng_1ng_1\,Ng_2ng_2$, leading to normal plant growth. Recessivity of one of these two loci (Ng_1–ng_2ng_2) results in the relatively reduced growth observed in Type II, whereas the recessivity at the other locus ($ng_1ng_1\,Ng_2$–) and at both loci ($ng_1ng_1\,ng_2ng_2$) will lead to the stunted growth of Type III. The observed numbers of plants fit well to the expected ratios (with $\chi^2 = 0.62$, P=0.80–0.70).

6.4.2.3 Genetic basis of compound v. simple leaves

As mentioned before, some plants of Type III only produced simple leaves (21 plants out of the total of 162 inbred plants in both populations). Assuming that 'compound leaf' is genetically controlled by the complementary action of two dominant genes, (symbolized by *Cl* = compound leaf) the segregation 141 compound: 21 simple can be explained by the presence of three of such dominant genes in heterozygous condition in parent *S. phureja*: ($Cl_1cl_1\,Cl_2cl_2\,Cl_3cl_3$). This leads to segregation of 54 'compound' to 10 'simple'. The observed numbers 141:21 fit the expected ratio (χ^2 value of 0.86 and P=0.50–0.30).

6.4.2.4 Tentative explanation of flowering v. non-flowering

All plants in Types I and II flowered whereas those of Type III did not flower. The parent phureja plant flowered well. Whether non-flowering is caused by lack of vigour of Type III or is inherited through a special gene linked with the ng_1 gene cannot be decided from these data.

In some crosses between different introductions of *S. phureja*, plants with a growth habit similar to Type III have occasionally been found. These plants showed poor flowering, suggesting the presence of specific genes controlling non-flowering.

So, for the moment, it may be assumed that non-flowering is a recessive character inherited monogenically through the gene *nf* (non-flowering). Thus the *S. phureja* parent would be assumed to have the genotype *Nfnf*. Selfing this plant would result in a segregation ratio of 3 flowering: 1 non-flowering. The observed numbers of 125 flowering to 37 non-flowering fit into the expected ratio of 3:1 ($\chi^2 = 0.12$, P = 0.80–0.70).

6.4.2.5 Possible genetic basis of pollen shedding v. non-shedding

Some of the flowering plants were checked for pollen shedding and stainability. Of the 65 plants checked in the two selfed populations 59 shed pollen, 6 did not. Assuming that non-shedding is controlled by the complementary action of the two recessive genes ns_x and ns_y, and that the parent *S. phureja* plant was double heterozygous for both loci ($Ns_xns_x\,Ns_yns_y$), the expected ratio in the selfed progeny is 15:1. The observed numbers 59:6 fit the expectation ($\chi^2 = 1.66$, P = 0.20–0.10). (See also 6.6.5.2, where data are presented on the inheritance of this character in an interspecific hybrid).

6.4.2.6 Pollen stainability

Pollen from shedding anthers of the two selfed populations were checked for stainability.

As can be observed from Fig. 34, the selfed phureja suffered much from inbreeding. The parent *phu* plant (checked in 1966) showed pollen stainability of 73% whereas in its selfed progeny it varied from 0–80% (average of 32% in population E and 14% in V). The *phu* sister plants checked in the same year as the inbreds (1967) showed stainability ranging from 73%–97% (mean value 84%).

The drastic inbreeding depression in male fertility accompanying selfing *phu* was thus obvious.

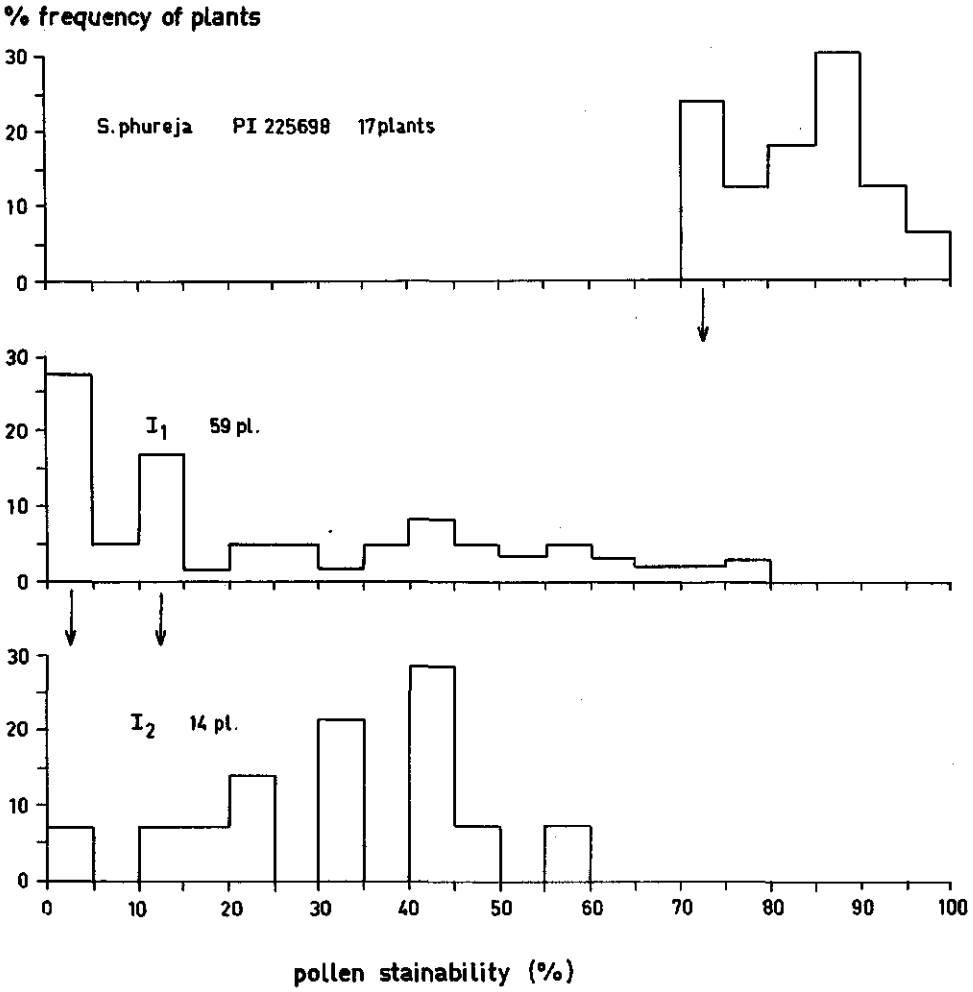


Fig. 34. Frequency distributions for pollen stainability in *S. phureja* PI 225698 and its selfed I_1 and I_2 progenies. The arrows refer to the classes of the pollen stainability of the I_1 and I_2 parents. *S. phureja* and I_1 were checked in 1967 whereas I_2 in 1968.

6.4.2.7 Pollen stainability in relation to chromosome pairing during meiosis

Chromosome pairing in meiosis was checked in anthers from phureja and some inbred plants. Chromosome pairing was normal in phureja (12 bivalents; Plate 10). In the inbreds, plants with good stainability showed regular meiosis and complete chromosome pairing, whereas plants with reduced pollen stainability gave irregular chromosome pairing at metaphase I. In some of the latter plants, half of the cells checked showed 12 bivalents at metaphase I, whereas the other half gave generally 11 bivalents and 2 univalents (Plate 10) and only few cells with 10 bivalents and 4 univalents. In other inbred plants, few cells occurred with complete chromosome pairing, the majority of cells showing configuration varying from 11 bivalents and 2 univalents to 8 bivalents and 8 univalents.

Plant 108 in population E shed some pollen grains with 2% stainability. When checked cytologically, one of its flowers showed only tapetal cells with few PMC's (though the anthers were big), whereas two others showed only the presence of pollen mother cells, all in the first telophase stage. This synchronous behaviour is most astonishing as in general several stages of division can be observed simultaneously in each preparation. It is quite probable that this behaviour represents a distinct type of sterility, with the pollen mother cell division being blocked at first telophase in the majority of the flowers.

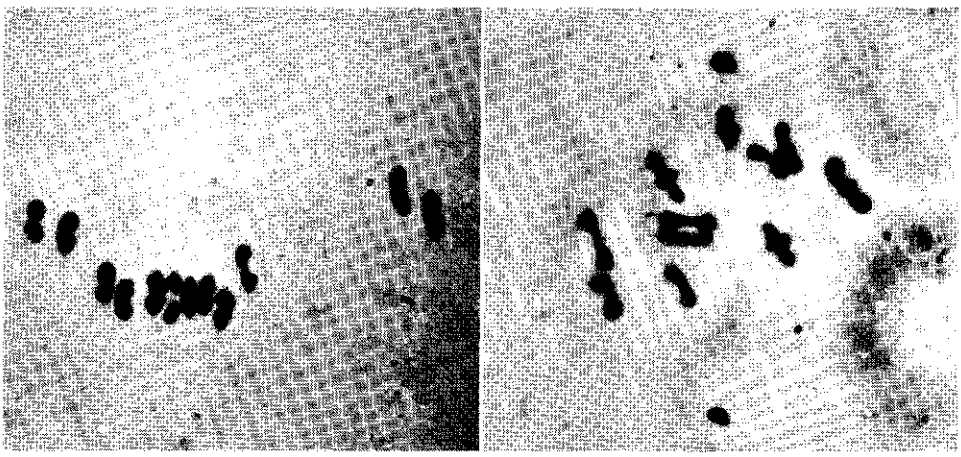


Plate 10. Effect of inbreeding on chromosome pairing at MI. Left: complete pairing (12^{II}) in *S. phureja* PI 225698; right $11^{II} + 2^{I}$ in an I_1-plant.

6.4.2.8 I_2 generation of S. phureja

Selfing was attempted on all the I_1 *phu* plants but was unsuccessful, with three exceptions. The three plants that set four berries upon selfing showed $< 15\%$ stainable pollen (see arrows in Fig. 34).

The four berries enclosed from 8 to 12 unhealthy and irregularly shaped seeds. This low seed set could not solely be attributed to reduced male fertility as in selfed

S. verrucosum there was no relation between pollen stainability and seed set. Thus female fertility of inbred *phu* might have been affected by inbreeding. Furthermore, incomplete breakdown of self-incompatibility in *phu* by inbreeding might have contributed to this low seed set.

The seeds set on the three I_1 plants were sown the following season (1968). Germination was 44%. The seedlings could be grouped into the two growth Types I and II (the three parents all belonged to Type I) in a frequency of 12 and 4 respectively. This suggests that parent plants were homozygous at the locus Ng_1 and heterozygous at the other locus ($Ng_1Ng_1Ng_2ng_2$), but the numbers were too low to be decisive.

Male fertility of the I_2 plants was checked in 1968 (Fig. 34). Though fertility was still low (33%, range 1 to 56%), it showed an improvement over pollen stainability of the parents.

6.4.2.9 Inbreeding effects in growth Types I, II and III separately

The three growth Types detected in inbred phureja populations and the hypothesis presented on their genetic basis made it worthwhile to check, whether the inbreeding depression is associated with the homozygosity of the *Ng* loci controlling plant growth. Table 26 presents the inbreeding depression for various characters in each of the three growth Types of the I_1 populations E and V.

Obviously inbreeding depression increases from Types I to III for nearly all characters. Recessivity at the Ng_1 locus resulted in a stronger depression than at the Ng_2 locus. The inbreeding depression in Type I, which at both loci is heterozygous like the original phureja, indicates that more loci may be involved in determining plant growth. Such loci have only minor effects. So it can be assumed that inbreeding cross fertilized populations leads to two kinds of depression: firstly the obvious

Table 26. Inbreeding depression for various characters in the three Types of plant growth in the inbred populations E and V of *Solanum phureja* PI 225698, in percentages of those found for the original *S. phureja* (compare Table 25).

		Inbreeding %					
		Type of plant growth in I_1–E			Type of plant growth in I_1–V		
		I	II	III	I	II	III
Stem height after 70 days	(cm)	−18.8	−73.7	−67.7	− 4.3	−75.3	−69.9
at end of season	(cm)	−23.1	−49.4	−81.7	− 2.3	−20.7	−83.0
Compound leaf length	(mm)	−14.6	−47.0	−77.0	− 6.7	−17.3	−90.1
width	(mm)	−19.6	−47.6	−71.6	− 5.8	−21.3	−85.3
T value	(mm)	−10.2	−23.6	−71.4	+ 0.5	+ 3.5	−86.9
Percentage stainable pollen		−61	−77	no flowers	−86	−91	no flowers
Number of tubers per plant		− 4.5	− 4.5	no tubers	−50.0	−77.3	no tubers
Weight of tubers per plant	(g)	−62.1	−50.0		−77.1	−87.9	

−: depression; +: gain.

depression expected due to bringing the heterozygous loci into deleterious homozygous recessive condition (this depression as has been presented in Ng_1 and Ng_2 loci differs from one locus to another) and secondly a less pronounced depression due to the change of breeding system (this may be conditioned by minor genes). Both kinds may be of great interest in the hypotheses accounting for the heterosis phenomenon.

Some of the differences observed between the populations E and V were, unexpectedly statistically significant. This may be due to the small size of V which might not be a random sample from the very heterozygous parent.

6.5 **Crossing of S. verrucosum with self-incompatible diploids**

As our knowledge of the problems arising from interspecific crosses involving *S. verrucosum* was rather limited, and such knowledge was of great importance for the transfer of desirable verrucosum genes to other species, rather extensive investigations were carried out on this subject.

Because the problems in using verrucosum as male partner were different from those involved in its use as a female partner, a separate treatment was made.

6.5.1 S. verrucosum as pollinator on haploids and diploid species

The populations of verrucosum used as pollinators were:

Inbreds	Hybrids
CPC 1339, 2247, 2644	275258–121–1 × 195172–237–10
PI 160228	275258–121–6 × 195172–237–10
PI 195172–237–8 and –237–9	195172–237–8 × 275258–121–5
PI 275258–119–3 and –121–1	

The female partners were 165 primary haploids[7] from ssp. *tuberosum*, ssp. *andigena* and haploids from colchicine doubled *S. chacoense* as well as some secondary haploids (see Table 1).

In 1966 on each female plant, 1–87 pollinations were carried out, making a total of 939 pollinations on haploids. In the same year the following material was used:

♂ *S. verrucosum*	♀ *S. phureja*	
CPC 2247	PI 225682	88 flowers pollinated
PI 195172	PI 225698	
PI 275258	PI 225702	
	PI 243461	

7. As mentioned before, haploids are plants with half the chromosome number (2n = 24) extracted from tetraploid (2n = 48) varieties and clones. These haploids have the same chromosome number as the diploid species.

The next year (1967), 197 pollinations using pollen of verrucosum PI 195172 and its inbred progeny were made on primary and secondary haploids. In addition, in all the cases where verrucosum was used as female parent in the crosses with diploid species, the reciprocal crosses (*ver* as male) were attempted, making 5 to 21 pollinations per attempted cross.

The results of the 1400 pollinations with *ver* as male can be summarized as follows:
(1) The majority of the pollinations gave no berry set.
(2) Only seedless berries were obtained from pollinating the female haploids US-W166(2/7) (in which the numerator is the number of berries, the denominator the number of pollinations),
TH 62–15–3 (4/4), TH 66–29–3(1/3), TH 66–30–35 (1/3),
TH 63–78–77(1/2), TH 66–36–2(1/8), TH 66–31a (1/2).
Some of these haploids were pollinated by more than one *ver* population.
(3) In three cases one berry was obtained, but the seeds failed to germinate. From 2 to 7 *ver* populations were used as pollinators. Haploid females were of different origin. Results:
TH 63–71–38 (from Dr. Mac Intosh): 10 pollinations, 1 berry, 17 seeds;
TH 66–1–25 (from Amaryl) : 7 pollinations, 1 berry, 19 seeds;
H 141 (origin unknown) : 26 pollinations, 1 berry, 2 seeds.
(4) Berry and seed set were observed but the expected hybrid seed proved to be the result of selfing.

The first case in which this happened was the self-incompatible non-emasculated *S. phureja* PI 225698, which also after pollination with *etb* produced self set seeds (see 6.4.1).

The second case occurred when the non-emasculated haploid AH 65–41–2 spontaneous was pollinated with different verrucosums. Here three berries set (from 27 pollinations) enclosing 96, 172 and 172 seeds. All plants raised from these seeds closely resembled the haploid female and no single hybrid was detected. Data of stem height, leaf measurements and pollen stainability did not reveal any significant differences between either the inbred populations or between them and their mother plants. However, slight inbreeding depression has been observed particularly in pollen stainability.

The failure of *S. verrucosum* as male partner on self-incompatible species (see 6.16.2 where *ver* succeeded as male) was further investigated by determining the germination of its pollen on stigmata of the self-incompatible species *S. kurtzianum*, *S. chacoense*, and *S. phureja*.

In all three cases, germination was good and the pollen tubes penetrated about 1/10 of the style (in *ktz*), 1/5 to 1/4 (in *chc*) and 1/4 to 1/3 (in *phu*) after which the growth stopped (see Plate 13–7). Some of the pollen tubes showed slight thickening of their ends. In the selfed *ver* plants checked at the same time pollen tubes penetrated the ovary (Plate 13–6).

In the selfed flowers of *phu* some of the pollen grains germinated; few of them

reached the lower third of the style, but none penetrated further. Again some of the pollen tubes showed slightly swollen ends.

6.5.2 S. verrucosum as a female partner with haploids and diploid species

All *ver* introductions mentioned in Table 1 were used as females with one or more male diploids and haploids. As female, *ver* crossed well with several species, and the results can be summarized as follows:

(1) No berry set. The two *ver* introductions PI 160228 and PI 195172 were pollinated with pollen of haploids and diploid species. The haploids were chosen from those known to have reasonable percentages of good pollen.

Many haploids from tuberosum, andigena and doubled chacoense were used as males on *ver* PI 160228 without success (10 or less pollinations were attempted with each haploid except with US – W 253 spontaneous, 49 pollinations).

Both *S. trifidum* and *S. bulbocastanum* failed as male partners on verrucosums PI 160228 and PI 195172.

The behaviour of *pld* and *etb* pollen in *ver* styles was described in 6.3. A similar study on the growth of *blb* pollen in *ver* styles showed that nearly all pollen grains germinated and tubes grew in *ver* styles. Many pollen tubes could be traced from the top to the base of the style, but few entered the ovaries and most of these showed relatively big swollen ends. It seems that the site of inhibition in *ver* × *blb* pollinations lies mainly in the ovary. However, it is not known whether pollen tubes eventually penetrate the ovule and result in fertilization or whether fertilization followed by abortion of zygotes takes place or whether both mechanisms are involved.

(2) Parthenocarpic berry set. Small seedless berries resulted from *ver* 195172 when crossed with *jam*, *cph* and *blb*.

(3) Berry and seed set, but failure of seed to germinate. In two cases seed was formed but failed to germinate: in *ver* PI 275258–119–3 × *phu* PI 225698, with four berries enclosing 201 seeds from four pollinations and in *ver* PI 195172 × *spl* with 3 berries containing 80 seeds from four pollinations.

(4) Berry set enclosing parthenogenetic seed of *S. verrucosum*. Parthenogenetic *ver* seed was obtained from *ver* PI 160228 pollinated by *etb* (see 6.2 for details).

(5) Seed set and hybrid plants obtained. With the exception of the cases mentioned above all attempts to cross female *ver* with male haploids and diploid species were successful. Some of the seeds were sown the following season(s) and all plants raised proved to be hybrids except in two cases where as well as hybrids, few individuals were found which most probably had originated from androgenesis (see below).

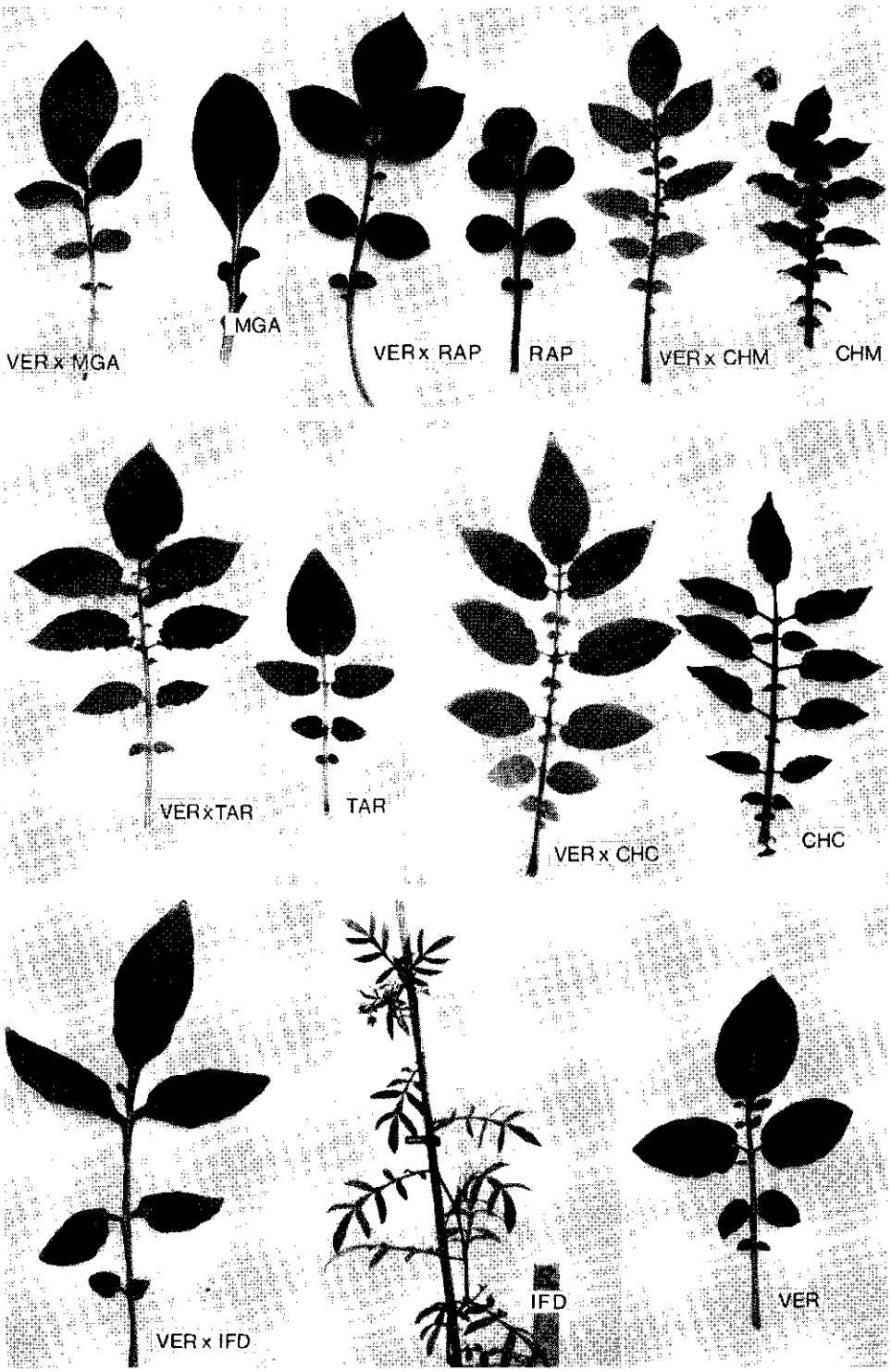
The data of berry and seed set and seed germination of the crosses between verrucosum females and different males are presented in Table 27; some of these interspecific hybrids are shown in Plate 11.

Some of the hybrid seeds looked unhealthy and showed a brownish colour, e.g. those with *can* and *lph* as male parents. The hybrid seed with *tar* as a male contained several empty-looking dark brown seeds. These three seed stocks showed very low

Table 27. Crossability and hybrid seed germination of three *Solanum verrucosum* introductions used as females to some diploid species and haploids.

S. verrucosum ♀	♂ partner	Year	Number of		Av. number of seeds per berry	Germination %
			pollinations	berries		
PI 160228 parthenogenetic	AH 66–94–28	1968	7	1	22	73
PI 160228	AH 66–88–14	1966	4	1	21	33
	H 114	1966	9	2	76	90
	US–W 42	1966	4	2	35	64
	SH 66–110–2	1966	3	2	34	66
	SH 66–114–2, self-compatible	1966	4	2	34	71
	SH 66–117–2	1966	2	2	136	66
	S. phureja PI 225682–22	1966	3	2	15	73
PI 195172	*S. tarijense* Haw 58	1967	7	6	44	1
	S. chacoense CPC 1153	1967	5	5	87	19
	S. chomatophilum CPC 3515	1967	6	1	42	88
	S. infundibuliforme PI 283077	1967	7	7	96	42
	S. raphanifolium Haw 694	1967	4	3	19	89
	S. megistacrolobum PI 210034	1967	5	2	104	87
	S. canasense PI 265864	1967	4	3	7	5
	S. kurtzianum Haw 4003	1967	5	4	77	72
	S. leptophyes PI 210056	1967	6	6	2	23
	S. gigantophyllum Haw 42	1967	5	3	68	80
	S. multidissectum PI 210052	1967	7	3	30	40
	S. vernei PI 230468	1967	5	5	57	94
	S. phureja PI 225682–22	1968	1	1	22	100
	S. stenotomum WAC 780[1]	1962	39	24	42	61
	S. goniocalyx WAC 204 × 257[1]	1963	15	15	130	68
	US–W 1[1]	1962	3	2	48	74
	US–W 4[1]	1962	5	1	19	21
	US–W 42[1]	1962	199	41	23	41
PI 275258	*S. goniocalyx* WAC 204 × 257[1]	1963	7	3	63	37
	US–W 1[1]	1962	2	1	88	48
	US–W 42[1]	1962	51	4	55	71

1. Seed made available by J. G. Th. Hermsen, Plant Breeding Institute, Wageningen.


VER x MGA
MGA
VER x RAP
RAP
VER x CHM
CHM
VER x TAR
TAR
VER x CHC
CHC
VER x IFD
IFD
VER

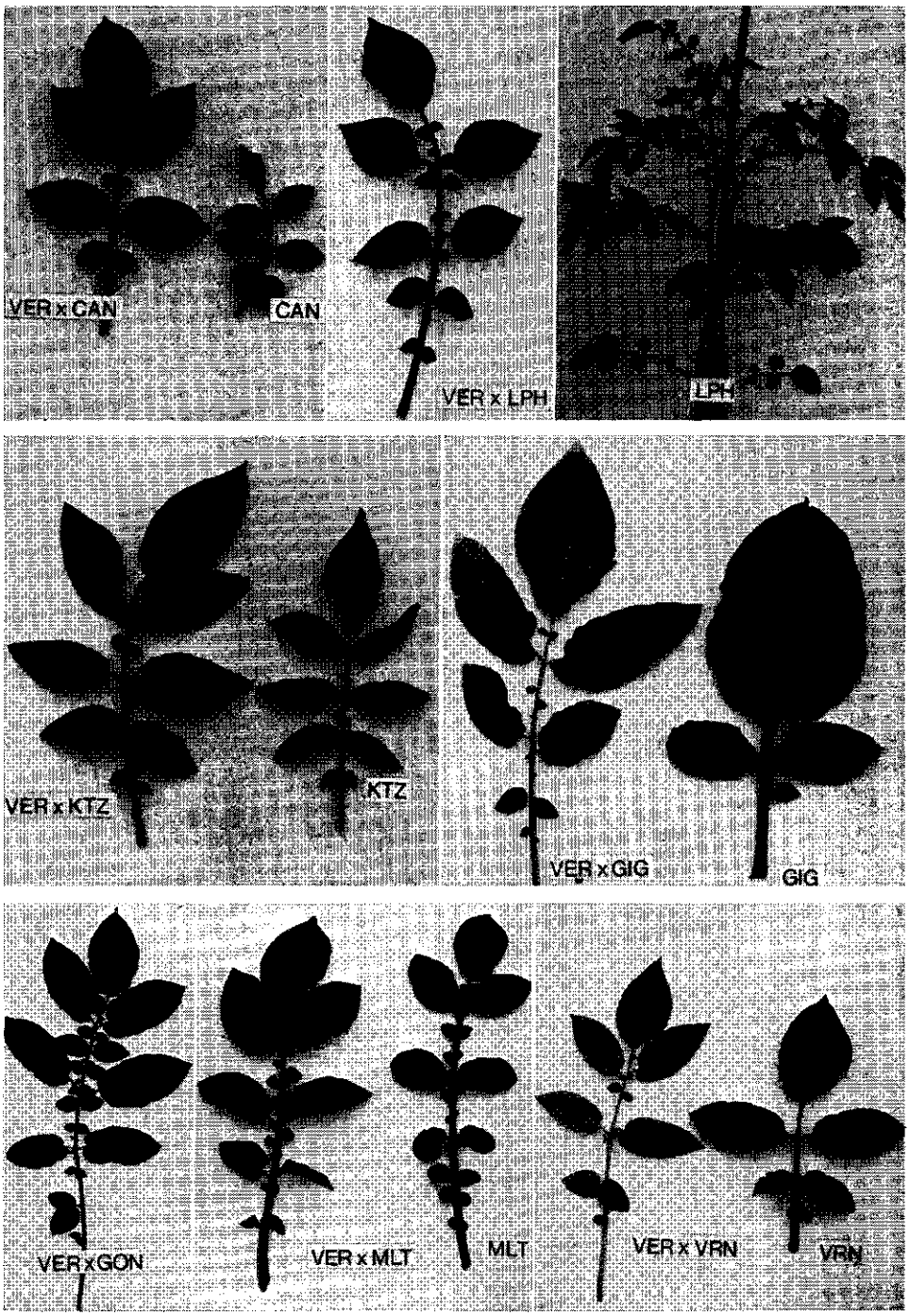


Plate 11. Interspecific hybrids between female *S. verrucosum* PI 195172 and some male diploid species. See Table 1 for abbreviations of species names.

germination.

(6) Androgenetic plants within hybrid populations. In two of the interspecific crosses few plants of the hybrid populations possessed the growth habit of the male parents.

In the hybrid parthenogenetic *ver* × AH 66–94–28 one plant out of the eleven (tested in 1969) had the very pale violet flower of the male haploid parent, contrary to the light purple flower of the normal hybrid plants (the *ver* parent had strong purple flowers). This plant was male sterile. It showed good vegetative growth, but its leaf shape closely resembled that of the male parent. Tuber colour of that plant was the same as the male haploid parent.

All attempts to cross this plant to the haploid parent failed whereas hybrid plants were successfully backcrossed to the male haploid. This points to the possibility that this plant has the same *S* alleles as the parent haploid. This observation together with its morphology supports the view that the plant developed through androgenesis and originated from the functioning of an unreduced pollen grain.

The other expected androgenetic plants were found within the hybrid *ver* PI 310966

Plate 12. Interspecific hybrid, *S. verrucosum* PI 310966 × *S. phureja* PI 225682–22 (right) and expected androgenetic plant (left).

× *phu* PI 225682–22. Seven plants from the 20 raised in 1969 were found to have a peculiar, poor growth with *phu*-type leaves, but whose size was about one fourth to one third the leaf size of *phu* (see Plate 12). Such plants showed either poor flowering or no flowering at all. They looked like intermediates between growth Types III and II in the selfed *phu* (see 6.4.2.2). One of the androgenetic plants could be checked at the flowering stage. It showed relatively reduced flowers and expressed the same type of sterility (which will be discussed later) as observed in the normal hybrid plants. The chromosome numbers in some of these plants were found to be 24.

With the exception of the two cases of probable androgenesis mentioned, all the hybrid populations with female verrucosums proved to be real hybrids.

6.5.3 Crossability and barriers between female S. verrucosum and other species

Generally speaking, under suitable conditions the interspecific crosses between female *ver* and many diploids from different series can be easily obtained. However, when the data for the crossing and hybrids between *ver* and other species were compared with those on selfing or sib-crossing *ver*, or with those on sib-mating in the male partners, barriers to gene exchange between *ver* and other species were observed, irrespective of the success of the crossings. Such barriers either influence berry and seed set and germination of the hybrid seed, or they affect male fertility in the hybrids.

As is apparent from Table 27, in some interspecific crosses with female *ver*, low percentages of berry set occurred and they differed according to male parents (see e.g. *ver* PI 195172 × *chm*, *mga*, *ktz*, and *lph*). Differences also occurred between the introductions of female *ver*, (male US-W42 caused 50% berry set on *ver* PI 160228 but 21% on PI 195172 and 8% on PI 275258). Comparing these percentages with the average berry set (70%) found in the selfed populations of *ver*, berry set in such interspecific crosses seems to be greatly reduced.

Some of such differences may be attributed to the fact that crosses were made in different years. Others may be due to crossability barriers.

The number of seeds per berry also varied from one cross to another (Table 27). Clear interserial differences with female *ver* were not found. In both species of series *Commersoniana*, berry set with *ver* was good, but the cross with *chc* gave about twice as many seeds per berry as that with *tar*. A similar behaviour was observed with *rap* seed set on *ver* as compared with seed set of *mga* on the same *ver* (19 *v.* 104 seeds per berry). Both *rap* and *mga* belong to series *Megistacroloba*. In the species of series *Tuberosa*, *can* and *lph* showed low seed set on *ver* as compared with those of *ktz*, *gig*, *mlt* and *vrn*, and all values were lower than that for the hybrid *ver* × *gon* on the same *ver* (PI 195172). Also in crosses between ♀ *ver* and haploids with similar male fertility, SH 66–117–2 gave a seed set per berry about twice that of SH 66–110–2 and SH 66–114–2. US – W1 crossed to *ver* PI 195172 and PI 275258, showed higher berry and seed set than US – W 42 on the same females.

The seed set of the interspecific crosses was lower than that obtained in selfing *ver* or sib-mating within the male partners. The average seed set per berry in interspecific

crosses involving females *ver* PI 160228, PI 195172 and PI 275258 was 50, 50 and 63 respectively, whereas selfing these three *ver* introductions gave 118, 124 and 180 seeds per berry in 1967. *S. phureja* showed 15 and 22 seeds per berry in the crosses with *ver* PI 160228 and PI 275258, respectively, but 120 to 600 seeds per berry in sib-crosses with other phureja introductions. *S. goniocalyx* which set 346 seeds per berry in sib-mating, showed 63 seeds per berry when used as male partner with female *ver* PI 275258 and 130 seeds per berry on *ver* PI 195172.

All these observations demonstrated the reduced seed set in the interspecific crosses as compared with selfing *ver* and intraspecific crosses in the male parents.

The germination of the hybrid seed varied from 1–100% (Table 27). Hybrid seed of *tar* and *can* showed the lowest percentages of seed germination followed by *chc*, US – W4 and *lph*. As mentioned before, seeds from *ver* × *spl* and *ver* × *phu* PI 225698 did not germinate at all.

The selfed and sib-crossed *ver* introductions showed higher percentages of seed germination than the interspecific hybrids.

In several interspecific crosses a lower seed set per berry was associated with lower germination percentages.

From the foregoing it seems that, apart from other factors that prevent the success of the crosses between *S. verrucosum* and other species, reduced berry and seed set and reduced hybrid seed germination are components of the barriers to gene exchange. The barriers affecting the fertility of the interspecific hybrids will be dealt with in detail in the following sections.

6.5.4 General features of the interspecific hybrids with S. verrucosum

In general the hybrid plants showed good vegetative growth and rich flowering.

Flower colour of the hybrids was intermediate between that of the parents. However, different shades from purple to lilac, and sometimes whitish corolla lobes were found in hybrids between *ver* and whitish flowering parents like *chc* and US – W 42 (*S. verrucosum* had strong purple flowers more richly coloured in PI 275258 than in PI 195172).

Flower size was intermediate between the small-flowered *S. verrucosum* and the larger-flowered male parents, but a shift towards the larger parent was sometimes observed.

Growth was more vigorous in hybrids between *ver* and diploid species than *ver*-haploid hybrids.

The hybrid plants showed higher tuber yield than female *ver*. Haploid parents contributed more to tuber yield of their progenies with *ver* than cultivated diploids, whereas *ver* × wild species showed the lowest yields.

Variation in the hybrid populations was observed for several characters (stem height and colour, number of stems, the expression of the wavy wings of stems, leaf measurements and tuber yield).

Some distinctive characters were observed in a few hybrids. Hybrids with *stn* produced more stems and stolons.

Hybrids with *gon* showed some stunted, unhealthy growing plants and plants with brown edged leaflets.

Hybrids with *ifd* showed nearly complete dominance of the *ver* type of leaves over the linear lanceolate leaflets of *ifd* (Plate 11). The leaves of the hybrid with *gig* were also more *ver*-like, whereas the hybrids with *mlt* showed the presence of the numerous interjected leaflets of the male parent (Plate 11).

The smell of male *tar* was clearly expressed in *ver* × *tar* hybrids.

Male sterility was the characteristic expressed by all the hybrids irrespective of their male parents. Different types of male sterility were discovered in the hybrids.

Some of the hybrid populations were extensively studied. Certain characters, especially the sterility types will be described and hypotheses on their genetic basis will be presented.

6.6 **Hybrid 1: S. verrucosum PI 195172-237-6 × haploid US-W 42**

The hybrid plants of *ver* PI 195172–237–6 × US – W 42, investigated in 1966, grew more vigorously than their parents and the plants flowered well.

The flowers of the hybrid plants looked somewhat abnormal and were slightly reduced in size. The anthers were slightly shorter and thinner; nearly all were poorly coloured and some were yellowish. Some of the anthers had brownish spots on their sides (see Plate 21).

The hybrid plants proved to be highly male sterile. Anthers shed smaller quantities of pollen than *ver* parent; this pollen did not stain or stained only faintly. The pollen was characterized by lobed appearance. Each individual pollen showed a maximum of 3 lobes. Generally the grains were clustered in groups, mostly in tetrads (Plate 13–2). Sometimes a few of these tetrads stuck together forming larger clusters and occasionally individual pollen was observed. Some of the hybrid plants also gave some 'well' stained pollen (up to 30%), but stainability was very irregular in such plants.

Because of the cluster of pollen in tetrads, this sterility will be called 'tetrad' sterility, proposed symbol *Tr*.

The male sterility of the hybrids between female *ver* and male diploid species were reported by Buck (1960) and Grun et al. (1962). Grun et al. (1962) named it 'lobed sterility' (*Lb*). The present author avoided the use of this term because (as we shall see in 6.6.3.4) lobedness of pollen grains is a distinct character and not a sterility expression.

The reciprocal cross of Hybrid 1 (US–W 42 as female) did not succeed in spite of the many attempts made.

6.6.1 Selfing some F_1 plants of Hybrid 1

Although F_1 plants were highly male sterile, not 100% of their pollen were sterile. Selfing was tried on 55 F_1 plants and 645 pollinations were made. Eleven of the F_1 plants showed some berry set in 4–33% of the flowers pollinated. Number of pollin-

ations on these plants varied from 13–34.

These eleven plants were characterized by an open posture and they shed reasonable quantities of pollen grains, some of which stained well.

Only one of the eleven plants gave seedless berries; in the others number of seeds varied between 3–27 per berry (average 12 seeds). These seeds were sown the next year (1967) and proved to originate from selfing as shown by the segregation for several characters in the offspring.

This indicates that few of the pollen (most probably the 'well' stained ones) of F_1 plants succeeded in fertilization.

6.6.2 Backcrossing Hybrid 1

Backcrossing some F_1 plants as females with both parents, *ver* PI 195172–237–6 and US–W 42, succeeded. The only plants which set seed after selfing were those which were also successfully backcrossed with *ver* parent. The backcross plants were similar to their male parents in many characters.

6.6.3 Studies on parents, backcrosses and F_1 through F_3 of Hybrid 1

6.6.3.1 Male sterility of F_1 plants

As observed from Fig. 35, a female fertile plant of *ver*, PI 195172–237–6 with good pollen stainability (average in selfed population 81%) crossed with haploid US–W 42 (52% stainability), produced a highly male sterile F_1 (over 80% of the F_1 plants gave no stainable pollen at all).

The backcross to parent US–W 42, was also highly male sterile, whereas that to *ver* parent and the F_2 population showed segregation for pollen stainability. As all these populations carry *ver* cytoplasm, this behaviour indicates that the sterility of the F_1 plants is due to the interaction between *S. verrucosum* cytoplasm and one or more dominant genes from US–W 42.

The ten F_1 plants which produced some seeds after selfing (see 6.6.1), really have the major gene(s) for male sterility, but it seems that they have weak modifiers of male sterility (associated with open posture) which led to the success of selfing. Their pollen showed very low stainability which was irregular when tested on different days. The plants gave low berry set and reduced seed set per berry. Together with the segregation in the F_2 populations, these facts indicate the presence of male sterility genes in these ten F_1 plants.

The success of selfing of some F_1 plants with modifiers for male sterility means that these plants are self-compatible. This self-compatibility was confirmed by the fact that nearly all F_2 plants and F_3 plants showing good pollen stainability gave good seed set after selfing.

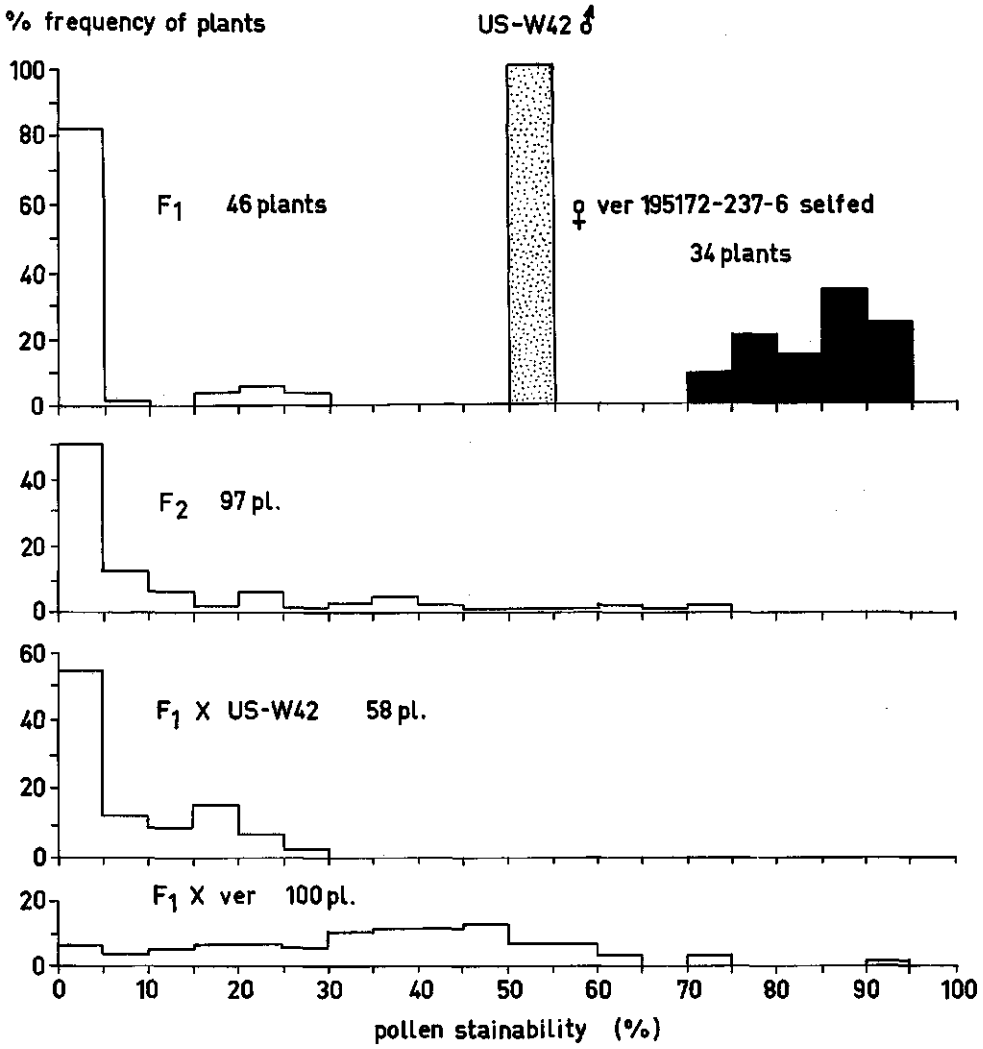


Fig. 35. Frequency distributions for pollen stainability in selfed ♀ *S. verrucosum* PI 195172–237–6, ♂ haploid clone US–W 42, their F_1 and F_2 and the two backcross populations. Parents and F_1 checked in 1966, F_2 and backcrosses in 1967.

6.6.3.2 Pollen stainability and pollen function

To overcome the difficulty of separating male fertile and male sterile plants in the segregating populations on the basis of pollen stainability, berry set and seed set after selfing were checked and related to pollen germination *in vitro*. Germination of pollen grains gave considerably lower values than pollen stainability; whereas the highest percentages of pollen stainability was 94%, pollen germination was never above 54%.

The results showed that plants with pollen stainability over 35% could be considered as fully or partially male fertile. Below 35% stainability, as a rule pollen

germination *in vitro* did not exceed 1%. Berry and seed set were also lower in plants with low stainability. In the backcross to *ver*, the average number of seeds per berry after selfing was 35 in plants showing up to 35% pollen stainability against 75 for plants producing over 35% stainable pollen.

In a backcross population of another hybrid (*ver* × *gon*) no berry set was observed in plants with less than 35% pollen stainability.

In addition, the variation in pollen stainability was greater in populations with relatively low stainability than in plants with higher stainability percentages.

On the basis of the arguments in this thesis 35% pollen stainability in lactophenol acid fuchsine is being used as the percentage discriminating male fertile and sterile plants, fertile plants having stainability above 35%. According to the limited comparisons made, it was found that pollen stainability in lactophenol acid fuchsine is lower by about 10% compared with pollen stainability in acetocarmine.

The discriminating percentage of pollen stainability may be considered by some research workers to be too high, but it should be emphasized that minor genes can raise the stainability up to 30% in some plants as has been observed in F_1 and in BC to US–W 42. Also plants with 35% or less pollen stainability gave low pollen germination (less than 1%). According to Mortenson et al. (1964) only pollen with at least 2% germinability was functional in crosses.

The need for a discriminating percentage of stainability was felt when more than one type of sterility was discovered in the segregating generations.

6.6.3.3 The inheritance of tetrad sterility and ordinary type in populations of Hybrid 1

The data on fertility-sterility segregation are presented in Table 28. Using the 35% pollen stainability as the discriminating percentage between male fertile and male sterile plants (see 6.6.3.2), all F_1 and all plants resulting from the backcross with US–W 42 have to be considered male sterile.

The backcross to *ver*, made on the same plant used for backcrossing to US–W 42, segregated into 55 male fertile and 45 male sterile plants. This agrees with the assumption of one dominant gene from US–W 42 leading to male sterility in plants with *ver* cytoplasm ($\chi^2 = 1.00$, $P = 0.50$–0.30).

Two types of cytoplasm were discovered. The first is the sterilizing or sensitive cytoplasm of *S. verrucosum* and the second is the non-sterilizing or resistant cytoplasm of haploid US–W 42.

The indication of the plasmons by the same symbols as those for the genes interacting with them (Grun et al., 1962) was used. Accordingly the plasmon of *ver* will be referred to as [Tr^s] plasmon: sensitive (^{s}) plasmon to the dominant gene *Tr* leading to tetrad sterility; plasmon symbol will be kept between brackets. The plasmon of US–W 42 will thus be [tr^r]: resistant (^{r}) plasmon, the symbol of the recessive allele *tr* was used here because the sensitive cytoplasm interacts only with the dominant allele *Tr*. Consequently, plants with [tr^r] plasmon never show tetrad sterility, whereas plants with the sensitive plasmon [Tr^s] will cause tetrad sterility only in association with *Tr* gene.

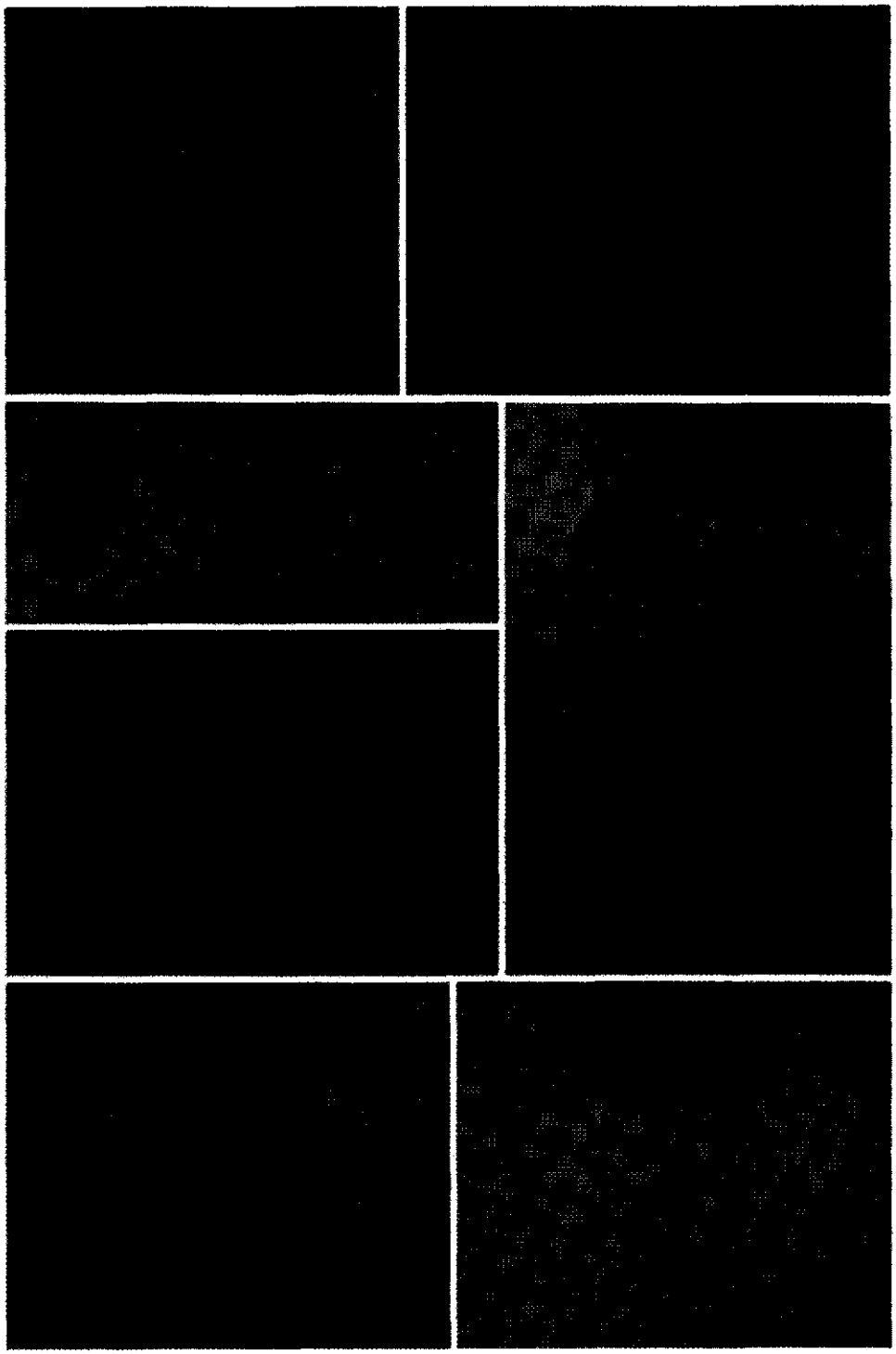


Plate 13. 1–5 stainability in lactophenol acid fuchsine: 13–1 = normal pollen grains of *S. verrucosum*, 13–2 = tetrad (*Tr*) sterility, 13–3 = striped vacuolar (*Sv*) sterility, 13–4 = partly stained (*Ps*) sterility, 13–5 = undivided microsporocyte (*um*) sterility; 6–7, pollen tube growth in styles after 48 hrs from pollination using acid fuchsine light green dye: 13–6 = pollen tubes reaching the ovary in selfed *S. verrucosum*, 13–7 = pollen tube inhibition in *S. kurtzianum* (SI) × *S. verrucosum* (SC) pollinations.

Table 28. Segregation for male sterility of the tetrad (*Tr*) and 'ordinary' (*ms*) types in Hybrid 1, and assumed idiotypes of parents, F_1, F_2, F_3, both backcrosses, and some selfed progenies of backcross to *Solanum verrucosum* (*ver*).

	Pollen stainability % in parent	Observed numbers		Expected ratios fertile : sterile	P from χ^2-test	Assumed idiotypes (cytoplasm in brackets)
		fertile	sterile			
♀ *ver* 195172–237–6 selfed		36	0	1 : 0		[Tr^s]*trtr*/$Ms_1Ms_1\ Ms_2Ms_2\ Ms_3Ms_3$
♂ US–W 42		1	0	1 : 0		[tr^r]*TrTr*/$Ms_1ms_1\ Ms_2ms_2\ Ms_3ms_3$
F_1		0	46	0 : 1		[Tr^s]*Trtr*/... (8 genotypes)
F_1 × US–W 42		0	56	0 : 1		[Tr^s]*TrTr*/... and [tr^s]*Trtr*/...
F_1 × *S. verrucosum*		55	45	1 : 1	.50–.30	[Tr^s]*Trtr*/... and [tr^s]*trtr*/...
F_2 1(1:3), 3(3:13), 3(9:55), 1(27:229)		14	83	343 : 1705 (pooled)	.70–.50	several idiotypes
F_3 M 18 pl. 6	72	72	10	segregation of polygenes		[Tr^s] *trtr*/$Ms_1Ms_1\ Ms_2Ms_2\ Ms_3Ms_3$
M 34 pl. 11	63	29	4	segregation of polygenes		Do.
M 18 pl. 1	67	28	10	3 : 1	.90–.80	[Tr^s]*trtr*/*Msms MsMs MsMs*[2]
M 34 pl. 5	44	5	6	9 : 7	.50–.30	[Tr^s]*trtr*/*Msms Msms MsMs*[2]
M 20 pl. 8	40	8	16	27 : 37	.50–.30	[Tr^s]*trtr*/$Ms_1ms_1\ Ms_2ms_2\ Ms_3ms_3$
M 2 pl. 9	30	0	8	0 : 1		[Tr^s]*TrTr*/...
M 18 pl. 8	10	0	23	0 : 1		Do.
M 2 pl. 14	11	3	19	3 : 13	.70–.50	[Tr^s]*Trtr*/*Msms MsMs MsMs*[2]
M 21 pl. 8	24	1	25	3 : 13	.10–.05	Do.
M 21 pl. 11	29	16	25	1 : 3	small	[Tr^s]*Trtr*/$Ms_1Ms_1\ Ms_2Ms_2\ Ms_3Ms_3$
M 4 pl. 8	39[1]	4	17	1 : 3	.70–.50	[Tr^s]*Trtr*/$Ms_1Ms_1\ Ms_2Ms_2\ Ms_3Ms_3$
M 24 pl. 5	54[1]	6	40	9 : 55	.90–.80	[Tr^s]*Trtr*/*Msms Msms MsMs*[2]
F_1 × *ver* selfed, pl. 1	93	43	5	segregation of polygenes		[Tr^s]*trtr*/$Ms_1Ms_1\ Ms_2Ms_2\ Ms_3Ms_3$
pl. 33	75	40	18	3 : 1	.30–.20	[Tr^s]*trtr*/*Msms MsMs MsMs*[2]
pl. 4	50	18	37	27 : 37	.20–.10	[Tr^s]*trtr*/$Ms_1ms_1\ Ms_2ms_2\ Ms_3ms_3$
pl. 70	33	17	36	1 : 3	.30–.20	[Tr^s]*Trtr*/$Ms_1Ms_1\ Ms_2Ms_2\ Ms_3Ms_3$
pl. 46	17	0	41			

[1] The parent plants showed tetrad sterility but several pollen grains stained.
[2] Subscripts left out to avoid equivalent combinations.

As tetrad sterility proved to be controlled by one dominant gene from US–W 42 in *ver* plasmon, the idiotype (genotype + plasmotype; see Rieger et al., 1968) of *ver* will be [Tr^s] *trtr*, having the sensitive plasmon but lacking the plasmon-sensitive genes. The idiotype of US–W 42 will be indicated by [tr^r] *TrTr*, with resistant plasmon, and the dominant plasmon-sensitive gene in homozygous condition.

The F_2, F_3 and selfed progenies of the backcross to *ver* also showed the male sterility type, earlier referred to as 'ordinary' type of male sterility (see 5.1.1). This ordinary type was found to be determined by recessive male sterility genes (*ms*) and minor genes reducing pollen stainability. The *msms* genotype will lead to male sterility. Three *ms* genes were supposed to enter the hybrid population from parent US–W 42 (Table 28). *S. verrucosum* is assumed to be homozygous dominant, US–W 42 heterozygous at each of the *Ms* loci.

In the indications of the idiotype the *Ms* genes were separated from the *Tr* gene by a solidus line to distinguish plasmon-sensitive and plasmon non-sensitive genes. So the *ver* idiotype is [Tr^s] $trtr/Ms_1Ms_1\ Ms_2Ms_2\ Ms_3Ms_3$, that of US–W 42 is [tr^r] $TrTr/Ms_1ms_1\ Ms_2ms_2\ Ms_3ms_3$. On the basis of these idiotypes all expected ratios have been calculated. The F_1 plants will be heterozygous for the *Tr* locus and eight genotypes are expected within F_1 plants with respect to the presence of the three *Ms* genes.

Heterozygous *Ms* genes are expected to cause the reduced fertility of male fertile backcross plants to *ver* (Fig. 35). In that population an inbreeding effect on stainability might also be involved. The segregation of both *Ms* loci and polygenes is expected to find expression both in the F_2 and F_3 populations and in the selfed BC populations. Because of these complications, all plants with reduced male fertility but with pollen stainability above 35%, were considered in the analysis as male fertiles.

The F_2 populations were expected to show the ratios 1:3, 3:13, 9:55 and 27:229 with frequencies of 1, 3, 3 and 1, respectively, leading to a pooled expected ratio of 343:1705. The segregation 14:83 fitted this pooled ratio.

As the male fertile backcross plants to *ver* and male fertile F_2 segregants showed decreased pollen stainability as compared with male fertile *ver* plants (Fig. 35) it was decided to raise F_3 plants and selfed progenies of BC.

All F_2 plants and all BC individuals that showed stainability of pollen grains over 35% and that were selfed a reasonable number of pollinations showed berry and seed set. Even some plants with stainability under 35% were successfully selfed on repeated pollinations and selfed progenies of these were checked for comparison.

The populations of the selfed plants used in 1968 were chosen in accordance with the different degrees of male fertility in the parent plants studied in 1967. The segregations in these populations, as well as the assumed idiotypes of the parent(s) are also presented in Table 28.

The segregation in F_3 populations of male fertile F_2 plants showed the expected segregation, if the parent plants were heterozygous for *Ms* genes and/or segregation of polygenes leading to male sterility took place. Similar results were found in selfed BC plants.

Segregation in the progenies originating from supposed male sterile parents showed

ratios of the segregations expected where parents heterozygous at *Tr* and *Ms* loci were involved.

Figure 36 shows the different types of segregation in F_3 and that the stainability in the F_3 plants is associated with the stainability of their F_2 parents. Populations M18 pl. 8 (F_2 parent, *TrTr*, stainability 10%) had all plants concentrated in the lower stainability classes and over 75% of the plants belonged in the 0–5% class. Also M2 pl. 14 (F_2 parent *Trtr*, stainability 11%) showed 50% of its progeny in stainability class 0–5%. The segregation of *Ms* genes was manifested in M20 pl. 8 (F_2 parent *trtr*/Ms_1ms_1-Ms_2ms_2 Ms_3ms_3, stainability 40%). Here the male sterile segregants showed a wider distribution for stainability. The segregation of polygenes represented by population M18 pl. 6 (stainability in the F_2 parent 72%) resulted in a stainability distribution clearly different from that of the other categories, and resembling the segregation observed before in the inbred plants of pure *ver* origin.

Attention has to be drawn to the behaviour of the F_3 populations M4 pl. 8 and M24 pl. 5. Here the F_2 parent plants had shown the tetrad sterility but they gave higher percentages of stainable pollen grains (over 35%). The selfed progenies of these F_2 plants (F_3) showed segregations which were expected assuming the F_2 parent plants were *Tr*-carriers. Hence the stainability observed in the parent F_2 plants must have been affected by weak modifiers, as was observed in the F_1.

The segregation in the selfed progeny of the backcross plants pointed to the use of an F_1 plant triple heterozygous for the three *Ms* genes in the pollinations made to obtain BC seeds.

So far the data in Table 28 fit the hypothesis for the inheritance of male sterility in this cross. However, few deviations occurred such as the high χ^2 value in the F_3 population M21 pl. 11 and the behaviour of the selfed progeny of plant 46 of the backcross population. It may be possible that the progeny of F_3 M21 pl. 11 were not a random sample of the expected genotypes, or that modifiers promoting stainability of pollen grains had been at work (there was an excess of fertile plants). The behaviour of the selfed progeny of the BC plant 46 cannot be explained. All the progeny was male sterile, showed irregularity in pollen shedding and several plants had unreduced pollen mother cells among their shed pollen grains, whereas other plants showed irregularly shaped pollen grains with granuled structures and no cell walls. It seems that some other genes were operating here causing this complex behaviour.

A male sterile plant (No. 21) from the BC (F_1 × *ver*) was crossed as a female with *ver* PI 195172–237–9 (this may be considered a second backcross). In most of the characters the progeny was quite similar to pure *ver* plants. The pollen stainability test resulted in 35 steriles and 25 male fertile plants indicating the ratio 1:1 ($\chi^2 = 1.67$, $P = 0.20–0.10$) expected with the assumption that the female plant (BC_1 No. 21) had the idiotype [Tr^s] *Trtr* and the *ver* used in the backcrossing was [Tr^s] $trtr$[8]. Such segregation in the second backcross plants support the data found in the first back-

8. It is expected that plants PI 195172–237–6 and PI 195172–237–9 of *ver* will have the same idiotype for this character.

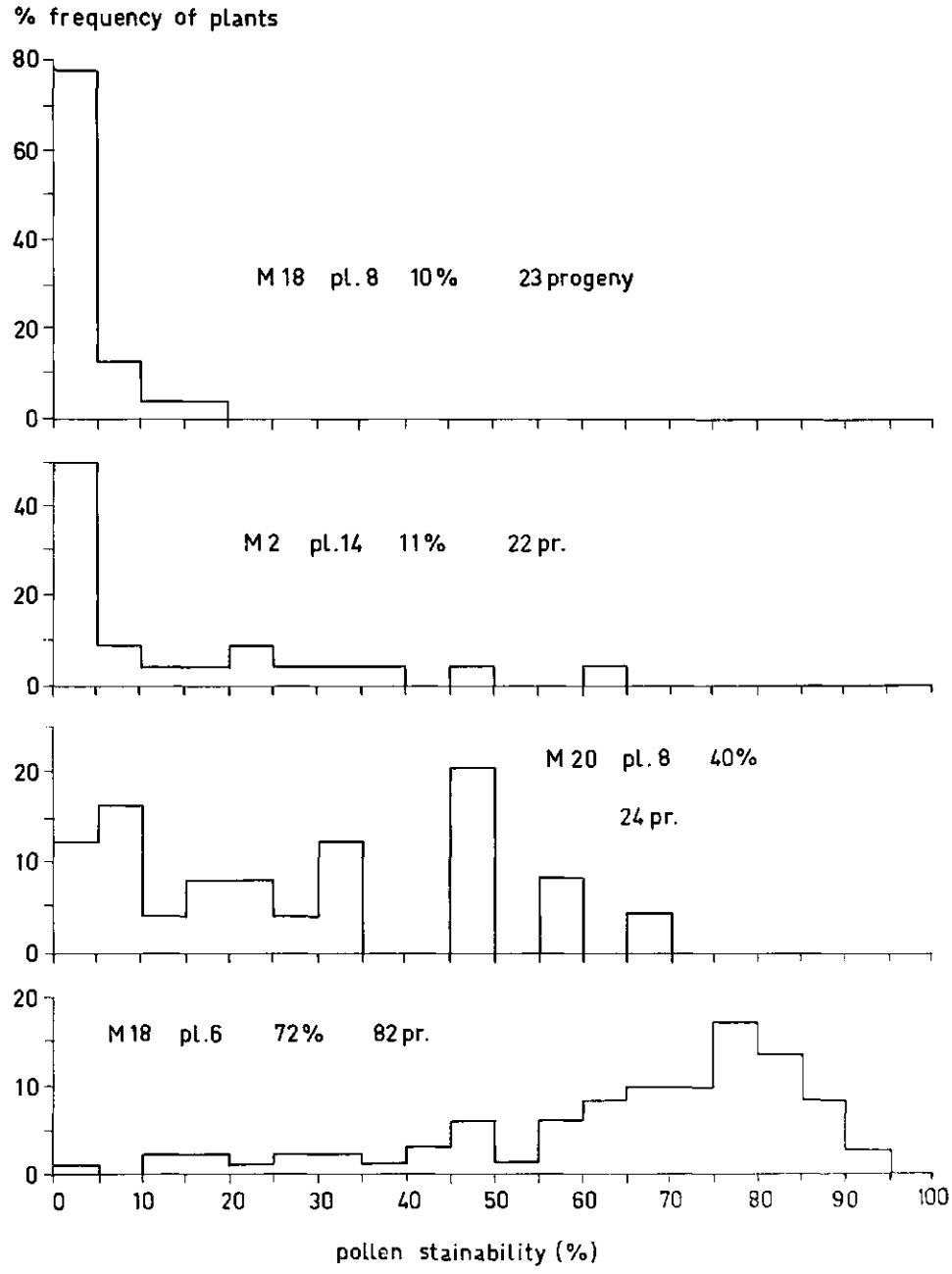


Fig. 36. Frequency distributions for pollen stainability in four F_3 populations derived from Hybrid 1 checked in 1968. The upper histogram represents the population homozygous for the *Tr* locus, the second shows the segregation of a selfed genotype *Trtr/Msms*, the third gives the progeny of the selfed genotype $trtr/Ms_1ms_1Ms_2ms_2Ms_3ms_3$, the last shows segregation due to polygenes reducing pollen stainability. Percentages indicate stainability in F_2 parents tested in 1967 (see also Table 28).

cross and the hypothesis that one dominant gene (*Tr*) leads to the male sterility of the hybrid plants having *ver* plasmon.

6.6.3.4 The genetic independence of lobed pollen and tetrad sterility in Hybrid 1

As mentioned in 6.6, the F_1 plants of Hybrid 1 (*ver* PI 195172–237–6 × US–W 42) showed tetrad sterility, originally named lobed sterility by Grun et al. (1962) and their pollen were lobed. When the present author was checking the backcross plants of Hybrid 1 (F_1 × *ver*), he observed the presence of male fertile plants with well stainable pollen showing the lobed appearance (Plate 14). Their pollen gave better germination *in vitro* than sister plants with spheroidal pollen.

The obvious conclusion thus reached was: pollen lobedness is a distinct character not inherent to nor an expression of male sterility. That is why the term tetrad sterility is used here instead of lobed sterility adopted by Grun et al. (1962).

Lobedness is a characteristic of individual pollen grains (see also Plate 16 and 6.6.4). Under the microscope, a pollen grain shows a maximum of 3 lobes[9]. Only tetrads may show more than three-lobed appearance. When only one lobe of each pollen is visible, the tetrad shows a 4-lobed appearance. When more lobes per pollen grain can be observed, the tetrad shows more than 4-lobed appearance.

When the BC plants of Hybrid 1 (F_1 × *ver*) showed a segregation of male fertile and male sterile plants and also segregation for lobedness of pollen grains, an attempt was made to elucidate the relation – if any – between lobedness of pollen grains and tetrad sterility (tetrad sterility is controlled by one dominant gene in *ver* plasmon, see 6.6.3.3).

Tubers from the backcrosses to both parents and from all F_2 populations were planted the following season (1968) to re-check the results of 1967. Again pollen stainability and lobedness were determined and the results agreed quite well with those obtained from the seedlings in 1967.

The numbers lobed *v.* non-lobed observed in BC to *ver*, in BC to US–W 42 and in F_2

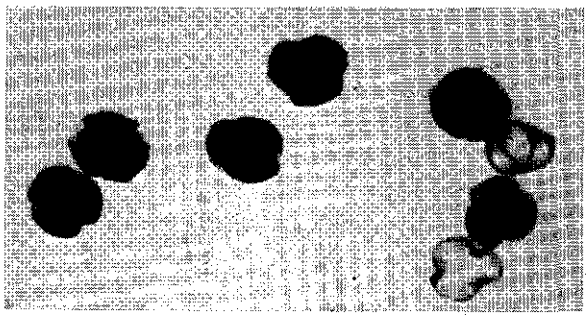


Plate 14. Fertile lobed pollen grains of some plants from backcrossed and segregating generations of Hybrid 1.

9. According to Grun et al. (1962) and Buck (1967, pers. comm.) 'microspores' have a diagnostic four lobed appearance. The present author never observed a single microspore with four lobes.

were 34:43, 35:10 and 68 :29 respectively. The analysis of BC to *ver* for both lobedness and tetrad sterility gave the following data:

Male fertile plants with non-lobed pollen	23
Male fertile plants with lobed pollen	12
Male sterile plants with non-lobed pollen	20
Male sterile plants with lobed pollen	22

The observed numbers fit the ratio 1:1:1:1, indicating independent inheritance for each of the two characters studied ($\chi^2 = 3.88$, P = 0.30–0.20) (see 6.6.3.5, 6.6.4 for further analysis). This suggests that tetrad sterility and lobed pollen are different and genetically independent characters.[10]

Several plants with lobed pollen grains were successfully selfed, and male fertile self-compatible plants with lobed pollen have been grown till F_3 and F_4 generations.

6.6.3.5 The inheritance of the lobedness of pollen grains

As has been mentioned, in the F_1 plants of Hybrid 1 (*ver* PI 195172–237–6 × US–W 42) pollen grains had three lobes (Plate 13–2). Both parents, *S. verrucosum* (Plate 13–1) and US–W42 had spheroidal pollen.

As discussed in 6.6.3.4 there was no linkage between lobedness and tetrad sterility in this hybrid, and male fertile plants with lobed pollen (Plate 14) were observed in the segregating populations and BC plants. The backcross plants, the F_1, F_2 and F_3, and the selfed populations of the BC to *ver* were studied for this character (Table 29).

The lobed characteristic of pollen grains will be symbolized by *Ld* to distinguish it from the *Lb*, used by Grun et al. (1962) for 'lobed sterility'. Lobedness has been found to be controlled by the interaction of genes and cytoplasm. Two dominant genes (Ld_1 and Ld_2) and one recessive (ld_3) are assumed to condition it in such a complementary way that the presence of any two of them in *ver* cytoplasm leads to lobed pollen grains. The idiotype [Ld^s] $ld_1ld_1Ld_2Ld_2Ld_3Ld_3$ is assumed for the parent *ver*, Ld_2 being the only gene for lobedness. The other parent, US–W 42, is supposed to have the idiotype [ld^r] $Ld_1Ld_1\,ld_2ld_2\,ld_3ld_3$, with two genes for lobedness; Ld_1 and ld_3. Both parents will have non-lobed pollen, US–W 42 owing to its resistant plasmon and *ver* because it carries only one gene for lobedness. The F_1 plants will have lobed pollen grains due to the presence of the two complementary dominant genes in *ver* sensitive plasmon.

All data presented in Table 29 fit the hypothesis assumed for the inheritance of this character. Only one F_3 population, M 18 pl. 1, showed an excess of plants with lobed pollen.

The gene Ld_3 probably causes an inhibition of the action of the other two genes if they are present separately. The presence of both Ld_1 and Ld_2 together overcomes the inhibiting action of Ld_3 (see 6.11, 9.4.3.3).

10. See 6.11 where linkage between the two characters was detected in the Hybrid *ver* PI 160228 × AH 66–88–14.

Table 29. Segregation for lobedness (*Ld*) of pollen grains and assumed idiotypes in the Hybrid 1 populations of Column 1.

	Parent pollen	Observed numbers		Expected ratios	P from	Assumed idiotypes (cytoplasm in brackets)
		non-lobed	lobed	non-lobed: lobed	χ^2-test	
♀ *S. verrucosum* PI 195172–237–6 selfed	non-lobed	34	0	1: 0		[Ld^s]$ld_1ld_1Ld_2Ld_2Ld_3Ld_3$
♂ US–W 42	non-lobed	1	0	1: 0		[ld^r]$Ld_1Ld_1ld_2ld_2ld_3ld_3$
F_1		0	46	0: 1		[Ld^s]$Ld_1ld_1Ld_2ld_2Ld_3ld_3$
F_1 × *S. verrucosum*		43	34	1: 1	.50–.30	different
F_1 × US–W 42		10	35	1: 3	.70–.50	different
F_2	lobed	29	68	22:42	.50–.30	different
F_3 M 2 pl. 9	lobed	2	6	1: 3	1.00	one of the 4 idiotypes
M 2 pl. 14	lobed	5	17	1: 3	.90–.80	[Ld^s]$Ld_1Ld_1Ld_2ld_2Ld_3Ld_3$
M 18 pl. 8	lobed	6	17	1: 3	.95–.90	[Ld^s]$Ld_1ld_1Ld_2Ld_2Ld_3Ld_3$
M 21 pl. 8	lobed	7	19	1: 3	.90–.80	[Ld^s]$Ld_1ld_1ld_2ld_2ld_3ld_3$
M 21 pl. 11	lobed	11	30	1: 3	.80–.70	[Ld^s]$ld_1ld_1Ld_2ld_2ld_3ld_3$
M 4 pl. 8	non-lobed	19	2	3: 1	.20–.10	
M 20 pl. 8	non-lobed	21	3	3: 1	.20–.10	one of the 2 idiotypes
M 34 pl. 5	non-lobed	9	2	3: 1	.70–.50	[Ld^s]$Ld_1Ld_1ld_2ld_2Ld_3ld_3$
M 34 pl. 11	non-lobed	28	5	3: 1	.20–.10	[Ld^s]$ld_1ld_1Ld_2Ld_2Ld_3ld_3$
M 18 pl. 1	non-lobed	23	15	3: 1	small	
M 18 pl. 6	lobed	4	78	1:15	.70–.50	[Ld^s]$Ld_1ld_1Ld_2ld_2ld_3ld_3$
M 24 pl. 5	lobed	3	43	1:15	.95–.90	
F_1 × *S. verrucosum*, selfed, pl. 1	non-lobed	48	0	1: 0		one of the 2 idiotypes
pl. 4	non-lobed	55	0	1: 0		[Ld^s]$ld_1ld_1Ld_2Ld_2Ld_3Ld_3$
pl. 33	non-lobed	58	0	1: 0		[Ld^s]$ld_1ld_1Ld_2ld_2Ld_3Ld_3$
pl. 46	lobed	5	36	1: 3	.10–.05	[Ld^s]$Ld_1ld_1Ld_2Ld_2Ld_3Ld_3$
pl. 70	lobed	15	38	1: 3	.70–.50	

6.6.4 Cytological observations on tetrad sterility and lobedness of pollen grains

Chromosome pairing in the male sterile F_1 plants of Hybrid 1 was normal, similar to pure *ver* material. In nearly all the cells of the F_1 plants, 12 bivalents were observed. In a few cells, some univalents were found, as a rule two, rarely four, and the rest of chromosomes forming bivalents.

Interesting was the observation that in almost all the cells, fragments were observed (also in other interspecific hybrids, with female *ver*). These fragments[11] were observed till the later stages of meiosis (see Plate 15) indicating their regular persistence during cell division.

Cell division was normal and microspores were released from pollen mother cells. The young microspores were characterized by their typical lobedness (see Plate 16). This means that the genes controlling lobedness start operating very early, probably before the young microspores are released from the pollen mother cells. From the morphological point of view, pollen lobedness is an inward bending at the places where the germination pores are located.

The cytoplasm stained well, and darkly stained nuclei were present in the young lobed microspores (Plate 16). Checking lobed pollen grains shed from the male sterile plants showed the absence of any stained nuclei and the failure of the cytoplasm to hold the stain.

Male fertile plants of pure *ver* showed the presence of elongated darkly stained nuclei (Plate 17) in most of the pollen grains. But in 4–7% of the grains, non-elongated less stained nuclei were observed. The sterile shrunken pollen of *ver* failed to show any stained nucleus.

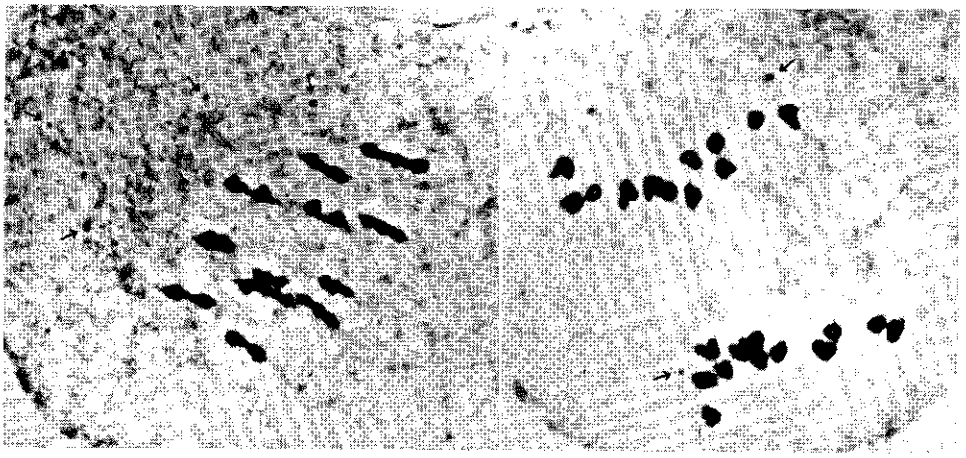


Plate 15. Normal metaphase (left) and anaphase (right) in Hybrid 1. Observe the presence of fragments (arrows).

11. The behaviour and significance of such fragments will be described in another publication.

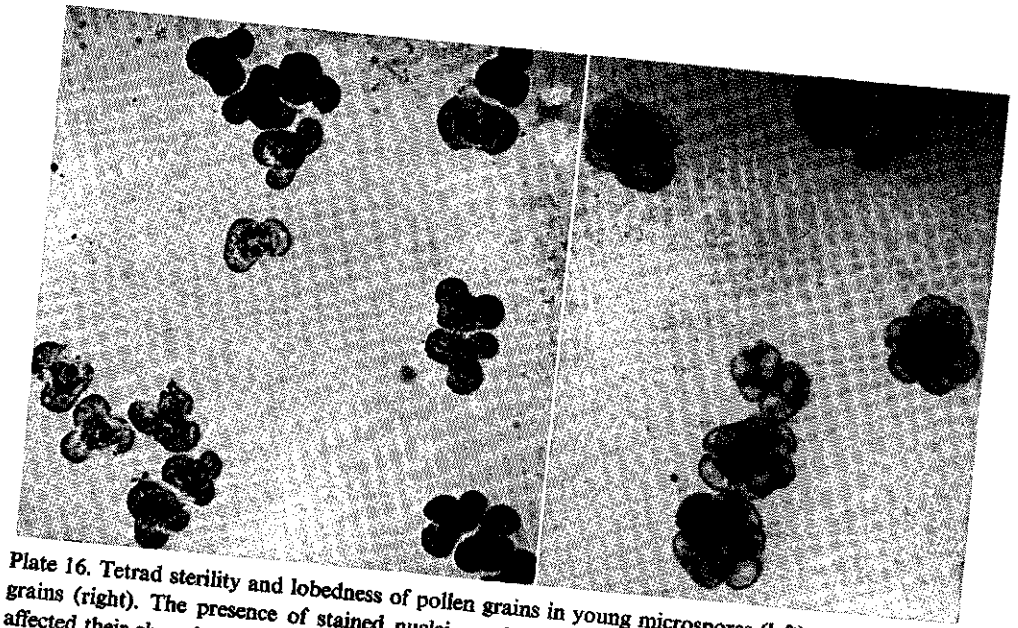


Plate 16. Tetrad sterility and lobedness of pollen grains in young microspores (left) and shed pollen grains (right). The presence of stained nuclei can be observed in young microspores (squashing affected their clumping).

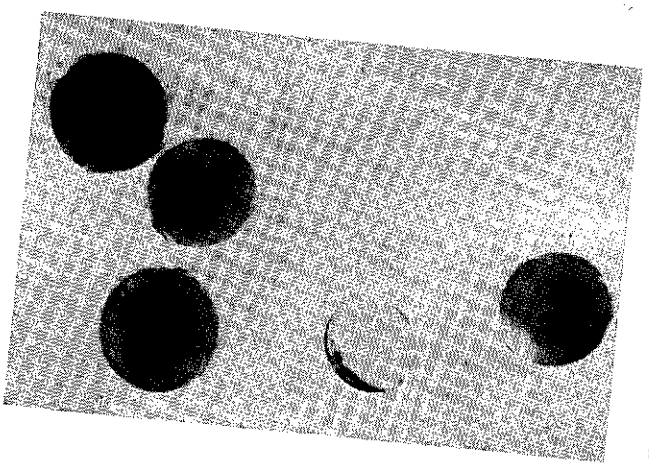


Plate 17. Stainability of *S. verrucosum* pollen nuclei in orcein. The sterile pollen (middle) looks smaller and did not accept the stain.

In their early stages of development, the young microspores have rounded nuclei. The fertile pollen enlarged and showed darkly stained nucleus with an elongated shape. In some of them a second, smaller and less stained nucleus could be observed; this is believed to be the vegetative nucleus.

From all these observations it may be concluded that the young microspores of the sterile F_1 plants, when released from pollen mother cells, have the normal appearance of fertile ones. Their breakdown, resulting in sterility, seems to occur after their release. It is probable that the nuclei stop functioning normally, fail to develop further, and degenerate.

6.6.5 Undivided microsporocyte and non-shedding types of male sterility in populations derived from Hybrid 1

In the backcross of F_1 to US–W 42 and the segregating generations (F_2, F_3 and selfed BC to *ver*), two other types of male sterility were observed.

The first was the one discovered before in pure *ver* plants, characterized by undivided pollen mother cells 'undivided microsporocytes'.

In the second the anthers failed to shed pollen grains 'non-shedding'.

6.6.5.1 Inheritance of the undivided microsporocyte type of male sterility

The character 'undivided microsporocyte' was discussed in 5.1.3. This type of sterility is characterized by pollen mother cells which stop development before the microspores are released. The nucleus of the PMC divides, but not the PMC itself, resulting in shedding of undivided microsporocytes containing four nuclei. The PMC cell wall undergoes some changes (see Plate 4 and Plate 13–5).

The only difference between the pure *ver* plants and the plants in the segregating populations derived from Hybrid 1 showing this type of sterility is that whereas in pure *ver* plants the anthers contain only undivided microsporocytes (100%), few pollen grains could be observed in the contents shed from anthers of the hybrid populations.

The undivided microsporocyte type of male sterility (*um*) proved to be due to the complementary action of two recessive genes in Hybrid 1. Although this character is most probably controlled through the interaction between genes and cytoplasm, the possibility of only genotypic control cannot be ruled out. The available data are not conclusive.

In Table 30 the segregation ratios for this character are presented, together with the proposed genotypes. *S. verrucosum* PI 195172–237–6 is assumed to be homozygous dominant for the two genes Um_1 and Um_2 ($Um_1Um_1\ Um_2Um_2$), whereas US–W 42 is heterozygous for one locus and recessive for the other ($Um_1um_1\ um_2um_2$). On that basis two genotypes are expected in F_1 plants with equal frequency, *viz.* Um_1Um_1–Um_2um_2 and $Um_1um_1\ Um_2um_2$. Only the second genotype produces a segregating progeny (15:1).

As expected the ten F_1 plants of Hybrid 1 which were selfed (see 6.6.1) produced five F_2's which segregated and five which did not. In the backcross plants to *ver* this character was absent, whereas the backcross to US–W 42 segregated. The segregation in this backcross population agreed with the expectation that only one out of eight backcross plants will show this character if a double heterozygous F_1 plant is used in the backcross. The segregation in the selfed BC to *ver* affirms this conclusion.

The 9 non-segregating F_3 populations were believed to come from F_2 parents that were homozygous dominant for at least one of the two *Um* loci, and the 3 segregating F_3 populations must have come from F_2 parents that were double heterozygous ($Um_1um_1\ Um_2um_2$) for this character.

The small numbers of plants checked in pure *ver* material (PI 255544 and Haw 1542) showing segregation for this character (see 5.1.3) suggested that it is determined by

Table 30. Segregation for 'undivided microsporocyte' (*um*) type of male sterility in populations derived from Hybrid 1 and the assumed genotypes.

		Observed numbers		Expected ratios	P from	Assumed genotypes
		Um	*um*	*Um* : *um*	χ^2-test	
♀ *S. verrucosum* 195172–237–6, selfed		34	0	1:0		$Um_1Um_1\,Um_2Um_2$
♂ US–W 42		1	0	1:0		$Um_1um_1\,um_2um_2$
F_1		46	0	1:0		$Um_1Um_1\,Um_2um_2$ and $Um_1um_1\,Um_2um_2$
F_1 × *S. verrucosum*		100	0	1:0		Um_1 – Um_2 –
F_1 × US–W 42		45	7	7:1	.70–.50	different
F_2		62	5	15:1	.70–.50	half of F_2 populations segregated
F_3 M 2 pl. 9		8	0	1:0		
M 2 pl. 14		22	0	1:0		
M 4 pl. 8		21	0	1:0		one of the 5 genotypes
M 18 pl. 1		38	0	1:0		$Um_1Um_1\,Um_2Um_2$
M 18 pl. 6		82	0	1:0		$Um_1Um_1\,Um_2um_2$
M 18 pl. 8		23	0	1:0		$Um_1Um_1\,um_2um_2$
M 21 pl. 11		41	0	1:0		$Um_1um_1\,Um_2Um_2$
M 34 pl. 5		11	0	1:0		$um_1um_1\,Um_2Um_2$
M 34 pl. 11		33	0	1:0		
M 20 pl. 8		24	1	15:1	.70–.50	
M 21 pl. 8		26	2	15:1	.90–.80	$Um_1um_1\,Um_2um_2$
M 24 pl. 5		46	2	15:1	.70–.50	
F_1 × *S. verrucosum*, selfed	pl. 1	48	0	1:0		one of the 3 genotypes
	pl. 4	55	0	1:0		$Um_1Um_1\,Um_2Um_2$
	pl. 33	58	0	1:0		$Um_1Um_1\,Um_2um_2$
	pl. 70	53	0	1:0		$Um_1um_1\,Um_2Um_2$
	pl. 46	41	3	15:1	.90–.80	$Um_1um_1\,Um_2um_2$

one recessive locus, *umum*. The data obtained in pure *ver* plants could also be explained with the hypothesis of two complementary recessive genes. For instance if plant 1 of *ver* PI 255544 had the genotype $Um_1um_1um_2um_2$ and the male sterile sisters plants 4 and 5 were double recessives ($um_1um_1um_2um_2$), the sib-mating between plant 1 and both plants 4 and 5 would have led to the ratio 1:1, as observed in the progenies of the two sib-mated plants.

Therefore it may be concluded that in both pure *ver* and the populations derived from Hybrid 1, the sterility type 'undivided microsporocytes' is based on two complementary recessive genes.

6.6.5.2 Inheritance of the non-shedding type of male sterility

Some of the backcross plants from $F_1 \times$ US–W 42 and some individuals from the segregating generations (F_2, F_3 and selfed BC to *ver*) showed the inability to shed pollen grains from their anthers. Sometimes one or two flowers (from about 10 checked per plant) shed few grains, but others from the same plants did not shed at all. A plant was classified as non-shedding if the majority of its flowers checked shed no pollen.

This character will be referred to as non-shedding and the genes involved will be denominated *ns* (see also 6.4.2.5). Grun et al. (1962) have referred to non-shedding as 'pollenless'. They used this term to differentiate between indehiscence of anthers based on a recessive locus (pollenless) and the same character conditioned by dominant genes (indehiscence). The term non-shedding[12] is preferred here because the observable character is the inability of anthers to shed pollen grains and no investigations have been made yet to check the absence or presence of pollen grains in these anthers.

Non-shedding appeared to be conditioned by interaction of recessive genes and cytoplasm. Four recessive genes were assumed to be present in US–W 42, any three of them acting complementary and in the presence of *ver* plasmon, leading to non-shedding of anthers. The observed segregations are presented in Table 31.

On the basis of the inheritance assumed for the non-shedding[13], *S. verrucosum* parent PI 195172–237–6 is assumed to have the idiotype [ns^s] $Ns_1Ns_1Ns_2Ns_2Ns_3Ns_3Ns_4Ns_4$ (sensitive plasmon and plasmon non-sensitive genes), whereas US–W 42 has the plasmon-sensitive genes in resistant plasmon: [Ns^r] $ns_1ns_1ns_2ns_2ns_3ns_3ns_4ns_4$. The F_1 plants will be heterozygous for the four loci. The backcross to *ver* will show no segregation as all four loci will be in dominant condition, but F_2 and the backcross to US–W 42 (data of 1967 were used in Table 31) will segregate.

12. Grun & Aubertin (1966b) reported the presence of pollen grains in some anthers of the type referred to as indehiscent. As it may appear in the future that so-called pollenless anthers also contain pollen grains, the term 'pollenless' probably is not the right term.

13. It has to be mentioned that plants showing the undivided microsporocyte character have been excluded from the genetic analysis of non-shedding. Likewise, when checking the segregation ratios for undivided microsporocytes, plants with non-shedding anthers were omitted.

Table 31. Segregation for non-shedding (*ns*) in different populations derived form Hybrid 1, and assumed idiotypes.

	Observed numbers		Expected ratios	P from χ^2-test	Assumed idiotypes (cytoplasm in brackets)
	Ns	*ns*	*Ns* : *ns*		
♀ *S. verrucosum* 195172–237–6, selfed	34	0	1: 0		[ns^s]$Ns_1Ns_1\,Ns_2Ns_2\,Ns_3Ns_3\,Ns_4Ns_4$
♂ US–W 42	1	0	1: 0		[Ns^r]$ns_1ns_1\,ns_2ns_2\,ns_3ns_3\,ns_4ns_4$
F_1	46	0	1: 0		[ns^s] $Ns_1ns_1\,Ns_2ns_2\,Ns_3ns_3\,Ns_4ns_4$
F_1 × *S. verrucosum*	100	0	1: 0		[ns^s]$Ns_1 - Ns_2 - Ns_3 - Ns_4 -$
F_1 × US–W 42	56	18	11: 5	.20–.10	5 out of 16 had 3 or 4 recessive loci
F_2	97	4	243:13	.70–.50	13 out of 256 had 3 or 4 recessive loci
F_3 M 18 pl. 1	38	0	1: 0		
M 18 pl. 6	82	0	1: 0		[ns^s]*NsNs NsNs*...[1]
M 34 pl. 11	33	0	1: 0		
M 4 pl. 8	21	7	3: 1	1.00	
M 21 pl. 8	26	6	3: 1	.50–.30	[ns^s]*NsNs Nsns nsns nsns*[1]
M 2 pl. 9	8	9	9: 7	.50–.30	
M 2 pl. 14	22	15	9: 7	.70–.50	
M 20 pl. 8	24	20	9: 7	.90–.80	[ns^s]*Nsns Nsns nsns nsns*[1]
M 34 pl. 5	11	12	9: 7	.50–.30	
M 18 pl. 8	23	1	15: 1	.70–.50	
M 21 pl. 11	41	3	15: 1	.90–.80	[ns^s]*NsNs Nsns Nsns nsns*[1]
M 24 pl. 5	46	7	54:10	.70–.50	[ns^s]*Nsns Nsns Nsns nsns*[1]
F_1 × *S. verrucosum*, selfed pl. 1	48	0	1: 0		
pl. 4	55	0	1: 0		[ns^s]*NsNs NsNs*...[1]
pl. 33	58	0	1: 0		
pl. 46	41	2	63: 1	.20–.10	
pl. 70	53	2	63: 1	.30–.20	[ns^s]*NsNs Nsns Nsns Nsns*[1]

1. Gene subscripts 1, 2, 3 and 4 omitted; only basic genotype is given to avoid mentioning all equivalent genotypes.

6.6.6 Other characters investigated in the parents, hybrid plants and segregating populations of Hybrid 1

During the investigations on populations of Hybrid 1, several characters other than sterility were recorded. Some of these will be briefly mentioned.

6.6.6.1 Variability and cripple segregants

The variability in vegetative growth was clear in F_2 and F_3 plants, and some populations showed distinctive segregants.

Stem height varied in F_2 plants but the within-population variability was less pronounced in F_3 populations though clear differences occurred among the populations themselves (Plate 18).

Segregants differing in size, type, and intensity of the greenish colour of leaves were observed in both F_2 and F_3 populations (Plate 18).

Differences between segregating plants also occurred in stem and flower colour. The whitish colour of flowers was always true breeding, but the F_2 plants with coloured flowers segregated in F_3 either into whitish and various coloured, or they showed segregants with different colour intensities.

Some cripple segregants were observed in F_2 and F_3 populations. Their leaflets rolled upwards. This character is recessive and might be expressed in *ver* plasmon or it might be controlled only by recessive gene(s) without specific cytoplasm. In one of the F_3 populations 20 plants were found to have normal growth and 12 were crippled. The latter 12 plants could be divided into three groups according to the degree in which their leaflets rolled upwards: five showed strong rolling, five were intermediate and two rolled only slightly.

Such behaviour was never met with in pure *ver* plants.

6.6.6.2 The white verrucose characteristic of berries

The white verrucose berries of *S. verrucosum* proved to be a genetically controlled character. Three selfed F_3 populations were checked. One proved to be true breeding for verrucose, the second produced only non-verrucose berries but three plants out of 11 showed from 1 to 3 whitish flecks on some of its berries and the third population gave 15 plants with verrucose berries and 10 with non-verrucose berries; of the latter 10, three plants showed whitish flecks on some of its berries.

Such segregation suggests verrucose (*Vb* = verrucose berries) to be a dominant character, but the data are too scanty for a decisive conclusion on the number of genes involved.

6.6.6.3 Tuber yield

Data on tuber production are presented in Tables 32 and 33. All plants were grown from tubers except those for *ver*. Tuber set in *ver* was very poor compared with US–W 42, and some plants produced no tubers at all under the experimental conditions. The F_1 plants (*ver* 195172–237–6 × US–W 42) had an intermediate yield (in weight), but they outranked both parents in number of tubers per plant.

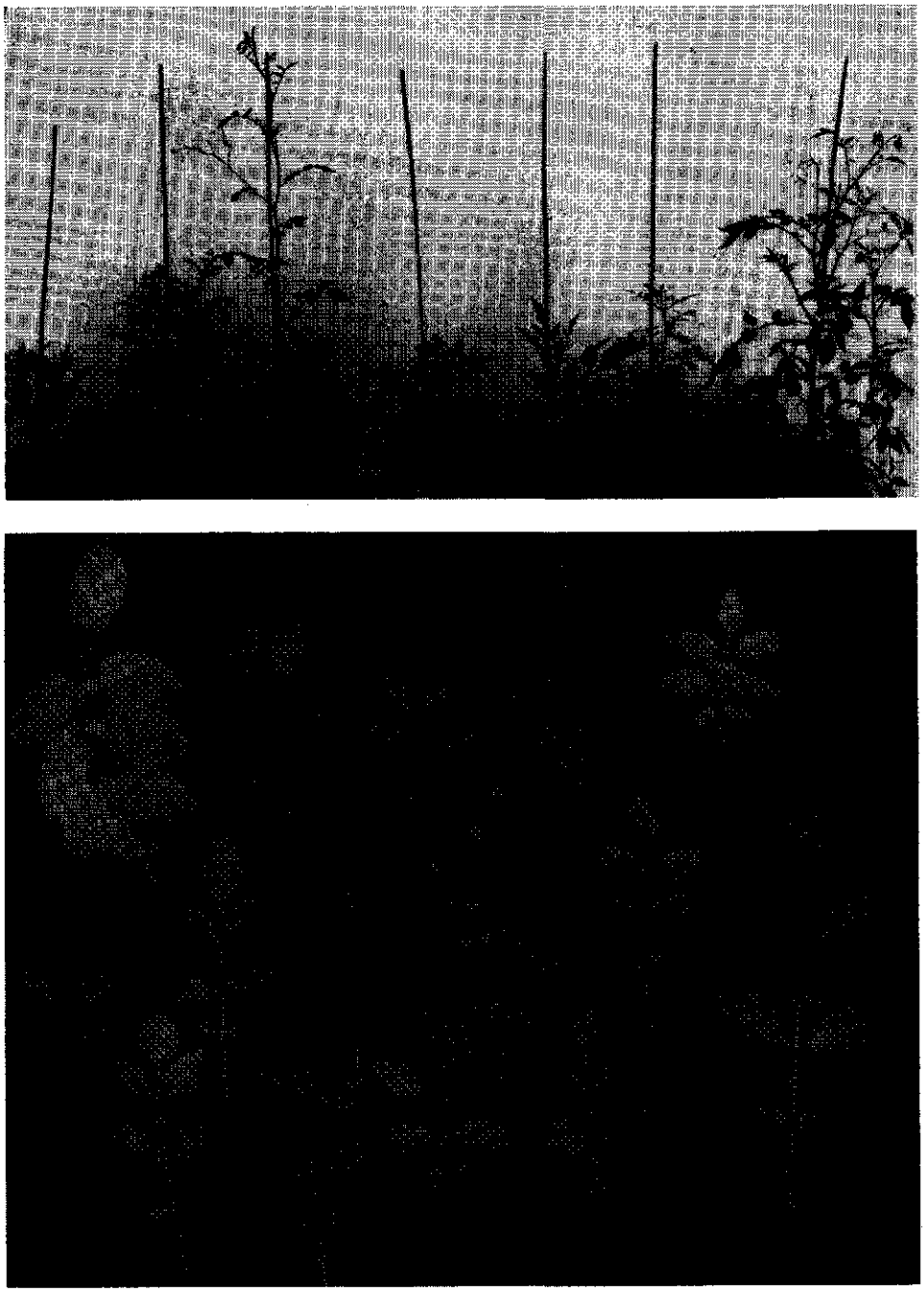


Plate 18. Variation in F_3 plants of Hybrid 1.

Table 32. Number of tubers per plant in parents, F_1, F_2, and backcross populations of the Hybrid *S. verrucosum* (*ver*) PI 195172–237–6 × US–W 42. Some tetraploid *S. tuberosum* varieties are included for comparison.

	Generation	Class intervals for number of tubers												Average number	Plants checked
		0	1–5	6–10	11–15	16–20	21–25	26–30	31–35	36–40	41–45	46–50	51–		
ver PI195172–237–6, selfed	1966 S	22	10	2										3.3	34
US–W 42	1968 T	1	5	2										5.0	8
F_1	1967 T	0	4	6	4	2	1							9.5	17
F_2	1968 T	21	33	22	14	8	7	4	2	3	1	0	3	12.3	118
F_1 × *S. verrucosum*	1968 T	12	39	20	16	3	5	1	1					8.3	97
F_1 × US–W 42	1968 T	1	34	31	12	10	6	3	1	2				10.1	100
Tetraploid varieties	1968 T	0	4	14	5									8.3	23

S: plants raised from seeds; T: plants raised from tubers.

Table 33. Weight of tubers per plant in the populations mentioned in Table 32.

	Class intervals for tuber yield (g)											Average yield per plant	Av. single tuber weight	Plants checked
	–25	26–50	51–75	76–100	101–125	126–150	151–175	176–200	201–225	226–250	251–			
ver PI 195172–237–6, selfed	12											1.3	0.4	12
US–W 42	0	1	0	0	4	1	0	0	0	1		126.4	25.3	7
F_1	10	4	1	1	1							32.5	3.4	17
F_2	62	16	9	7	1	1	1					27.4	2.2	97
F_1 × *S. verrucosum*	68	13	3	0	1							16.4	2.0	85
F_1 × US–W 42	26	10	12	17	14	6	3	5	1	1	4	84.9	8.4	99
Tetraploid varieties	0	0	0	5	3	3	3	3	2	1	3	202.4	24.4	23

Backcrossing the F_1 caused an obvious shift towards the backcross parent. In both backcrosses the tuber number per plant was higher than that of either parent, but tuber weight was intermediate.

Some plants belonging to different commercial varieties (Alpha, Eba, Libertas, Marijke, Prevalent, Radosa and Saturna) raised in pots in the glasshouse were included for a rough comparison. Notwithstanding the limited trials, the possible seasonal effects and the differences in propagation, some general conclusions can be drawn.

Tuber yield is a genetically controlled character, probably based on many genes; it is very sensitive to environmental conditions.

Yield of the haploid US–W 42 is about 62% of that of the tetraploid varieties. However, selection for tuber yield in haploid × haploid populations may lead to haploid clones outyielding even the commercial varieties (see also 9.8).

The F_1 of *ver* × US–W 42 showed a higher number of tubers per plant than either parent, and in F_2 transgressive segregation for tuber number per plant occurred. However, both F_1 and F_2 gave less tuber weight per plant than the haploid parent (only 2.2% of F_2 plants reached the average of the haploid). F_2 plants showed a slight depression in tuber weight compared with F_1 plants.

The backcross to the haploid parent improved the yield than F_2 and F_3. Of the BC progeny 20% yielded more and heavier tubers than the average haploid; 4% gave a higher yield than the maximum found among the haploid plants. It will interest potato breeders that one backcross only can give better yielding segregants than the haploid parent. Within three seasons, plants with good tuber yield and the desired characters of the wild species could be obtained.

Under commercial conditions the yield of selected haploids or haploid-hybrids is not expected to be much lower than that of tetraploid varieties. The number of haploid plants grown per unit area will usually be higher than the number of tetraploids. The relatively small tubers of many haploids and their relatively high numbers as compared with the tetraploids grown nowadays, are favourable characters for canning. An extension of potato canning might lead to an increase of potato breeding at the diploid level.

6.7 Hybrid 2: S. verrucosum PI 195172-237-9 × haploid US-W 1

Fifty plants from the hybrid *ver* PI 195172–237–9 × US–W1 were grown in 1966. Half of them did not flower. Those that flowered showed good growth and vigour. The non-flowering plants had an average stem length of 28 cm, which was less than half that of their flowering sister plants, and leaves (L and W measurements of compound leaves) were smaller. Tuber yield, however, was much higher: the averages per plant were 14.2 tubers and 55.6 g for the non-flowering plants as against 9.9 tubers and 15.2 g for the flowering individuals.

Unlike in Hybrid 1, the flowers of this hybrid looked 'almost' normal, also in the size of their anthers. The shedding of pollen grains was also better but the plants were

male sterile and had lobed pollen.

All 166 attempted self-pollinations on the hybrid plants failed, as did the 12 backcrossings with the hybrid plants as females to *ver*. The backcross to parent US–W 1, however, was successful.

As expected, the BC plants gave higher tuber yield than the F_1 plants. Thirteen out of the 37 did not flower. The non-flowering BC plants showed reduced growth with higher tuber yield. These plants (also the non-flowering F_1's) generally died before the end of the season. A few tubers of the non-flowering plants were planted the following season. The plants grown from these tubers flowered and showed normal growth at day length equal to their seedling generation.

Tetrad sterility and lobed pollen were found in the F_1, whereas in the BC (F_1 × US–W 1) the same three types of male sterility were observed as in Hybrid 1 – derived populations: 'tetrad sterility' in 4 plants, 'undivided microsporocytes' in 3 plants and 'non-shedding' in 3 plants. In Hybrid 2 no genetic analysis was carried out because of the inability to obtain either BC to *ver* or segregating generations. However, sterility and lobedness of F_1 pollen grains could be interpreted on the same basis as in Hybrid 1.

The differences between the amounts of pollen shed in Hybrids 2 and 1 indicate the possibility that plants of Hybrid 1 possessed some genetic mechanism affecting the quantity of pollen production.

6.8 **Hybrid 3: S. verrucosum PI 195172-237-3 × S. stenotomum ssp. stenotomum WAC 780**

The F_1 of Hybrid 3 (*ver* PI 195172–237–3 × *stn* WAC 780), of which 50 plants were grown in pots and 66 grafted onto tomato, grew well and flowered profusely. In general, the plants were intermediate between both parents (Plate 19). The flowers and anthers were somewhat smaller and the anthers looked pale and were sometimes orange-greenish.

As in Hybrids 1 and 2, the F_1 plants of Hybrid 3 showed tetrad sterility and lobedness of the pollen grains.

Grafting resulted in a stronger vegetative growth: stem height, compound leaf length and width and T value in the non-grafted plants were 63%, 56%, 55% and 62% of those of the grafted ones. Grafting did not affect the sterility of the hybrids, although the average pollen stainability of the sterile hybrids was somewhat higher on the grafts.

Selfing 766 flowers on F_1 plants gave no berry set at all, but backcrossing to both parents (F_1 as females) was successful.

The frequency distributions for pollen stainability in the backcross populations, the F_1 and the selfed population of the *ver* parent are presented in Fig. 37. When selfed, the progeny of the parent *ver* plant showed different degrees of pollen stainability. Unfortunately the *stn* parent could not be checked for stainability as it produced only one flower which was needed for making the backcross (the *stn* clone was attacked by

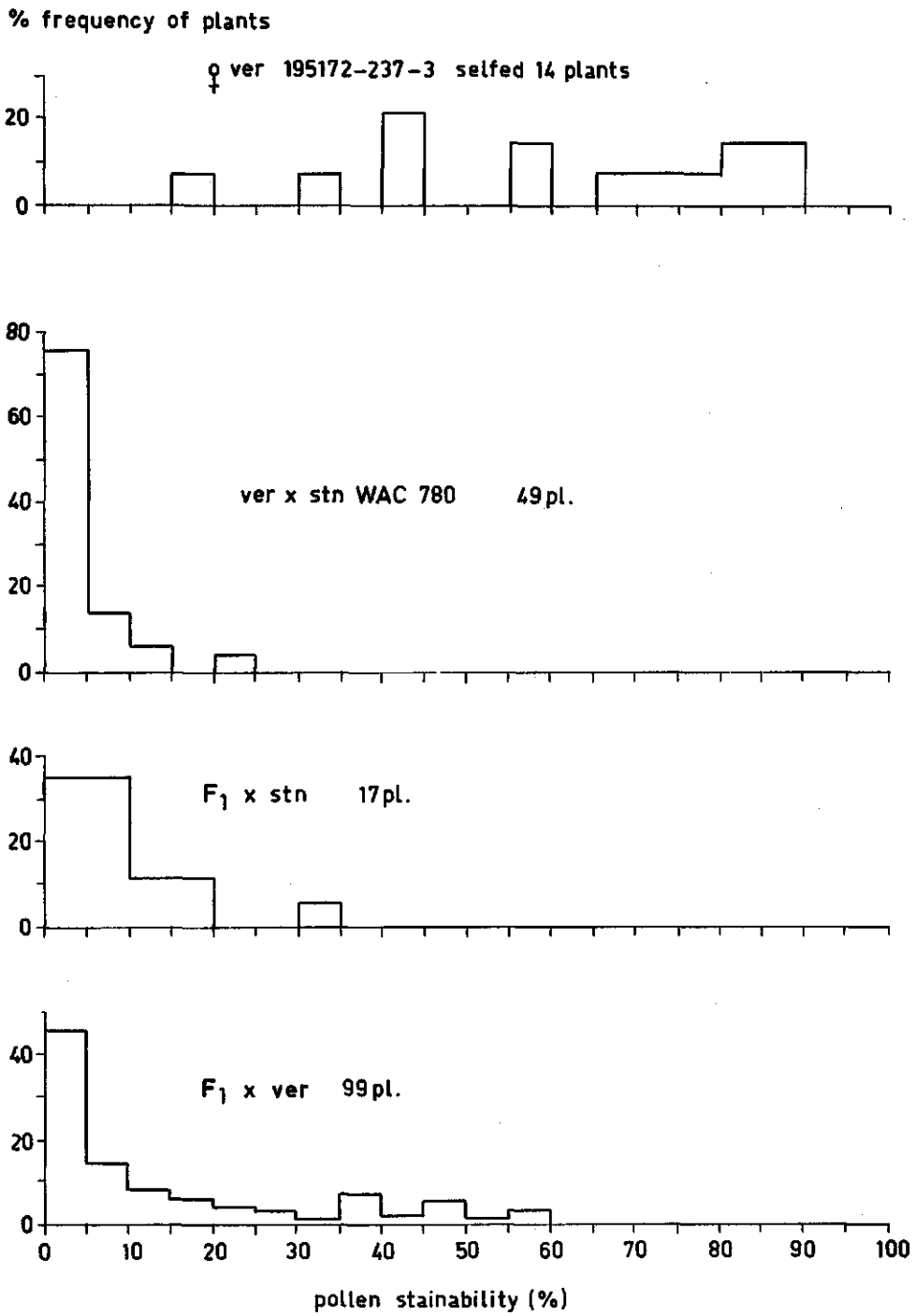


Fig. 37. Frequency distributions for pollen stainability in selfed ♀ *S. verrucosum* PI 195172–237–3, its F_1 hybrid with *S. stenotomum* WAC 780 and the backcrosses to both parents. *S. verrucosum* and F_1 checked in 1966, backcross plants in 1967.

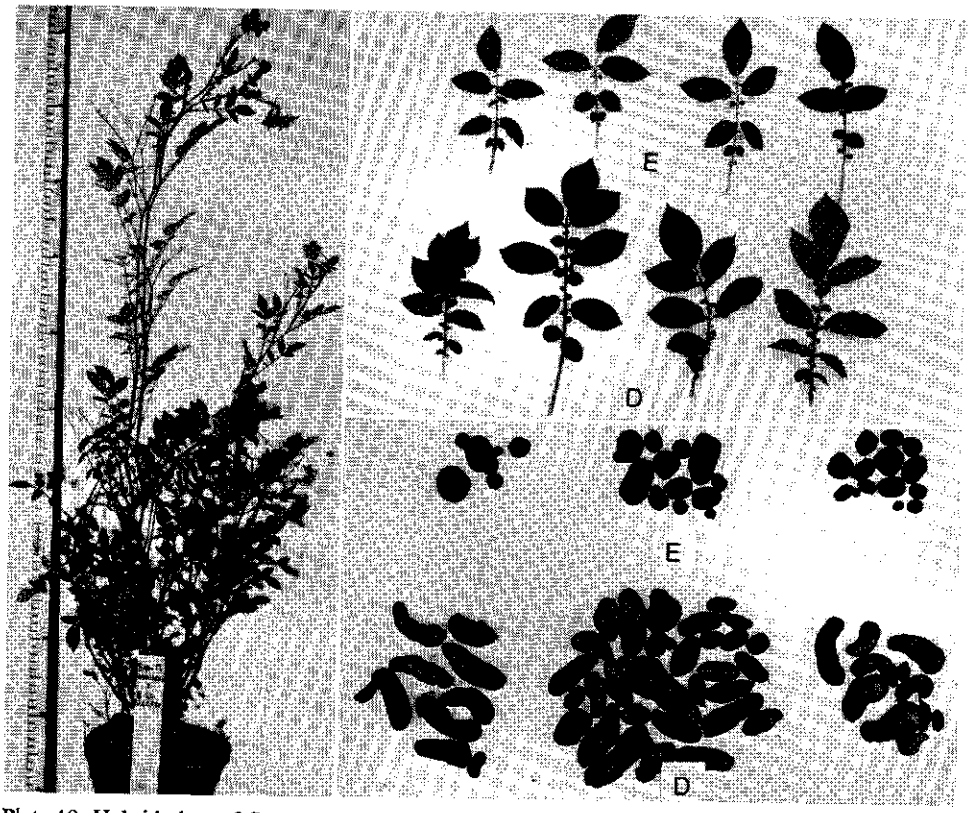


Plate 19. Hybrid plant of *S. verrucosum* PI 195172–237–3 × *S. stenotomum* WAC 780, and leaves and tubers from backcross to *S. verrucosum* (E) and backcross to *S. stenotomum* (D) respectively.

virus). The F_1 population was highly male sterile[14] and although a few individuals gave some stainable pollen (maximum 25%), the majority of the plants were grouped in the lowest stainability class (0-5%).

The backcross plants resembled their male parents in vegetative characters. The backcross to *stn* gave the growth type of the latter and produced more stolons and a higher tuber yield than F_1. Most tubers were elongated or kidney-shaped like those of *stn* (Plate 19). The plants segregated for flower colour. Backcross plants of F_1 × *ver* resembled *ver* in growth habit but showed a higher tuber yield.

The backcross F_1 × *stn* consisted of highly sterile plants only, as did the F_1, but showed a higher level of stainability whereas, the BC to *ver* showed segregation for pollen stainability (Fig. 37).

14. Chromosome pairing was normal i.e. 12 bivalents were frequently observed. Fragments were detected in all the cells (see also 6.6.4).

6.8.1 Tetrad sterility and ordinary sterility

As apparent from Fig. 37, there are differences between pollen stainability in the different plant populations.

S. stenotomum WAC 780 is known to be male fertile. The behaviour observed in the F_1 and BC plants indicated that the male sterility of the hybrids was due to the interaction between *ver* plasmon and dominant genes from *stn*. Again using 35% stainability as the borderline to separate fertile and sterile plants (see 6.6.3.2), the backcross of $F_1 \times$ *ver* showed a segregation of 18 male fertile and 81 male sterile plants. These numbers fitted the ratio of 1 fertile to 3 sterile, indicating the presence of two dominant genes in *stn*, any of which when present in *ver* plasmon leads to the male sterility of the tetrad type. The gene symbols chosen were Tr_b and Tr_c.

Table 34 gives the segregations for male sterility in this hybrid and in derived populations as well as the proposed idiotypes. According to the method applied in Hybrid 1 (see 6.6.3.3), *S. verrucosum* PI 195172–237–3 will be [Tr^s] $tr_btr_btr_ctr_c$ and *S. stenotomum* WAC 780 will have the idiotype [tr^r] $Tr_bTr_bTr_cTr_c$. The F_1 plants [Tr^s] $Tr_btr_bTr_ctr_c$ showed the tetrad sterility due to the presence of the *Tr* genes in *ver* plasmon.

The BC plants to *ver* showed partial male fertility, and stainability never exceeded 60%. This behaviour may be ascribed to the presence of genes from both parents reducing pollen stainability.

In general the BC plants of $F_1 \times$ *ver* with good pollen stainability were successfully selfed and therefore proved to be self-compatible. A few plants with lower stainability also set seed after selfing. Some of the selfed BC progenies were checked in 1968 for pollen stainability. The segregation ratios found in these populations pointed to the presence of three male sterility genes (*ms*), any of which in homozygous condition would lead to reduced pollen stainability and to the male sterility of the type indicated before as 'ordinary' (see 5.1.1 and 6.6.3.3).

The presence of two *Tr* genes and the small numbers of plants checked in each population probably masked the effect of *ms* genes on the ratios in the selfed progenies of the sterile plants.

The genes controlling male sterility of the 'ordinary' type were not included in the idiotypes of *ver* and *stn* in Table 34 because it was not sure whether the *ms* genes came from the *stn* parent only or from both parents. If the *S. verrucosum* parent had no *ms* genes, the segregants with low pollen stainability occurring in its selfed progeny might result from the presence of polygenes leading to this reduced stainability. Such genes were also expected to play a role in reducing the stainability of the backcross to *ver* and in the segregation of the selfed plants of this BC, as these populations are inbreds.

Table 34. Segregation for male sterility of tetrad (*Tr*) and 'ordinary' type (*ms*) for the populations of Hybrid 3, and assumed idiotypes.

		Stainability % of parent	Observed numbers		Expected ratios fertile: sterile	P from χ^2-test	Assumed idiotypes (cytoplasm in brackets)
			fertile	sterile			
♀ *S. verrucosum* PI 195172–237–3, selfed			12	2			[Tr^s]$tr_b tr_b tr_c tr_c$
♂ *S. stenotomum* WAC 780			1	0			[tr^r]$Tr_b Tr_b Tr_c Tr_c$
F_1			0	49	0: 1		[Tr^s]$Tr_b tr_b Tr_c tr_c$
F_1 × *S. stenotomum*			0	17	0: 1		[Tr^s]$Tr_b - Tr_c -$
F_1 × *S. verrucosum*			18	81	1: 3	.20–.10	3 [Tr^s]$Tr -$, 1 [Tr^s]$tr_b tr_b tr_c tr_c$
Do. selfed,	pl. 36	45	27	14	3: 1	.20–.10	[Tr^s]$tr_b tr_b tr_c tr_c$/*Msms MsMs MsMs*[1]
	pl. 21	50	31	26	9: 7	.80–.70	[Tr^s]$tr_b tr_b tr_c tr_c$/*Msms Msms MsMs*[1]
	pl. 89	57	7	15	27:37	.50–.30	[Tr^s]$tr_b tr_b tr_c tr_c$/$Ms_a ms_a Ms_b ms_b Ms_c ms_c$
	pl. 61	28	13	43	1: 3	.80–.70	either [Tr^s]$Tr_b tr_b tr_c tr_c$/$Ms_a Ms_a Ms_b Ms_b Ms_c Ms_c$
	pl. 71	13	7	32	1: 3	.50–.30	or [Tr^s]$tr_b tr_b Tr_c tr_c$/$Ms_a Ms_a Ms_b Ms_b Ms_c Ms_c$
	pl. 3	21	2	38	1:15	.80–.70	[Tr^s]$Tr_b tr_b Tr_c tr_c$/$Ms_a Ms_a Ms_b Ms_b Ms_c Ms_c$
	pl. 80	14	4	39	1:15	.50–.30	

1. Subscripts omitted to avoid equivalent combinations.
Ms genes were not included in parental idiotypes (see text).

6.8.2 Lobedness of pollen grains

As previously mentioned, the F_1 plants of the hybrid *ver* × *stn* showed lobed pollen grains. The *ver* female parent had spheroidal pollen and the *stn* male parent may be assumed to have similar pollen. The backcrosses to both parents showed plants with both lobed and non-lobed pollen grains.

The segregations for lobedness are presented in Table 35 and it is assumed that lobedness is a character controlled by the interaction of *stn* genes and *ver* cytoplasm.

S. verrucosum parent PI 195172–237–3 is assumed to have an idiotype similar to that of its sister plant (PI 195172–237–6) used in Hybrid 1. Consequently *ver* PI 195172–237–3 is assumed to be $[Ld^s]$ $ld_a ld_a Ld_b Ld_b Ld_c Ld_c$[15], whereas *S. stenotomum* WAC 780 is assumed to be $[ld^r]$ $Ld_a Ld_a ld_b ld_b ld_c ld_c$. The expected ratios were based on the hypothesis presented in 6.6.3.5 and the observed results proved to be in accordance with expectation.

The observed segregations in the selfed BC populations 3 and 21 also fit the ratio 13 : 3, while that of 61 fits 3 : 13 and those of the 71 and 80 fit 22 : 42. These theoretical ratios may also be expected on the basis of the hypothesis, but owing to the small size of the populations the observed numbers give no conclusive evidence.

6.8.3 Undivided microsporocyte sterility

The backcross of F_1 plants of Hybrid 3 to *stn* parent included plants with undivided microsporocytes, but neither the BC to *ver* nor its selfed progenies showed any segregants with this type of male sterility. With the limited data available, the mode of inheritance of this character could not be clarified in this cross. Suffice it to report here that *S. stenotomum* carries recessive genes, which in certain combinations (and probably in *ver* plasmon) lead to plants with undivided microsporocytes.

6.8.4 Non -shedding sterility

Segregation for non-shedding was observed in the backcross to *stn* as well as in some of the selfed progenies of BC plants to *ver* (Table 36). As reported for Hybrid 1 (see 6.6.5.2), this character was assumed to be controlled by the complementary action of three recessive *ns* genes in *ver* plasmon.

S. stenotomum WAC 780, like US–W 42, is assumed to have the idiotype $[Ns^r]$-$ns_a ns_a ns_b ns_b ns_c ns_c ns_d ns_d$, and that of *S. verrucosum* PI 195172–237–3 is $[ns^s]$ $Ns_a Ns_a$-$Ns_b Ns_b Ns_c Ns_c Ns_d Ns_d$. The F_1 plants will be $[ns^s]$ $Ns_a ns_a Ns_b ns_b Ns_c ns_c Ns_d ns_d$ and will shed pollen grains. The backcross plants to *ver* are expected to have all the dominant genes and will consequently shed pollen grains, but BC plants to *stn* are expected to segregate for non-shedding. The selfed BC plant populations either showed segregation or not.

15. The genes controlling the lobed appearance in this cross have been given the subscripts a, b and c to distinguish them from those discovered in Hybrid 1, as there is no definite evidence that they belong to the same loci.

6.9 Hybrid 4: S. verrucosum PI 195172-237-9-9 × S. stenotomum ssp. goniocalyx WAC 204 × 257

The plants of Hybrid 4 (*ver* PI 195172–237–9–9 × *gon* WAC 204 × 257) grew normally and vigorously except for few which were stunted and looked unhealthy. All F_1 plants proved to be male sterile of the tetrad type and their pollen grains were lobed.

Unfortunately the parent *gon* was no longer available, but the backcross to *ver* parent readily succeeded.

The backcross progeny showed segregation for pollen stainability. By applying the 35% stainability limit, the BC plants were classified into 46 sterile and 31 fertile plants. These numbers fitted the ratio 1:1 ($\chi^2 = 2.92$, P = 0.10–0.05) expected if one dominant gene in *gon* leading to tetrad sterility of F_1 plants with *ver* plasmon, is present. This ssp. *goniocalyx* was assumed to have the idiotype [tr^r] Tr_dTr_d and *ver* parent [Tr^s] tr_dtr_d.

Hybrid 4 and its backcross progeny were quite interesting in their male sterility and seed set. The 38 F_1 plants grown in pots expressed a stronger sterility than the three hybrids reported before. All F_1 plants belonged to the lowest class of pollen stainability (0–5%), and two-thirds of the male sterile BC plants were grouped in that class.

All the 743 attempted self-pollinations of the F_1 plants failed, whether made on grafted or on non-grafted individuals. When the backcross plants were selfed, only those showing pollen stainability over 35% gave berry and seed set. It seems that such a 'strong' expression of male sterility finds its origin in the absence of any modifiers, contrary to the findings in Hybrid 1[16].

The 59 F_1 plants of this hybrid grafted onto tomato showed stimulated flowering and vegetative growth, but only a slight effect on fertility: about 30% of the grafts showed over 5% stainability, with a maximum of 16%. In compound leaf measurements (L, W and T) and in stem height, the non-grafted plants gave values corresponding to 56%, 51%, 61% and 58%, respectively of that of their grafted sisters.

The BC Plant 17 (0% pollen stainability) was crossed as a female with *ver* PI 195172–237–9. This cross may be considered as a second backcross (BC_2), as PI 195172–237–9 and PI 195172–237–9–9 were expected to have the same genotype with respect to the *Tr* locus. The BC_2 plants closely resembled pure *ver* plants. The check for pollen stainability gave 20 fertiles and 25 male steriles. This segregation fitted the ratio 1:1 ($\chi^2 = 0.55$, P = 0.50–0.30), indicating heterozygosity of Plant 17 at the *Tr* locus and confirming the results obtained in the first backcross.

6.10 Chromosome doubling in S. verrucosum and absence of effect on sterility of interspecific hybrids

Seeds from *S. verrucosum* PI 195172–237–9 were soaked in 0.2% colchicine for 24 hours, after which they were germinated in water and transplanted to seed pans.

16. Modifiers in Hybrid 1 promoted pollen stainability and consequently seed set after selfing (6.6.1).

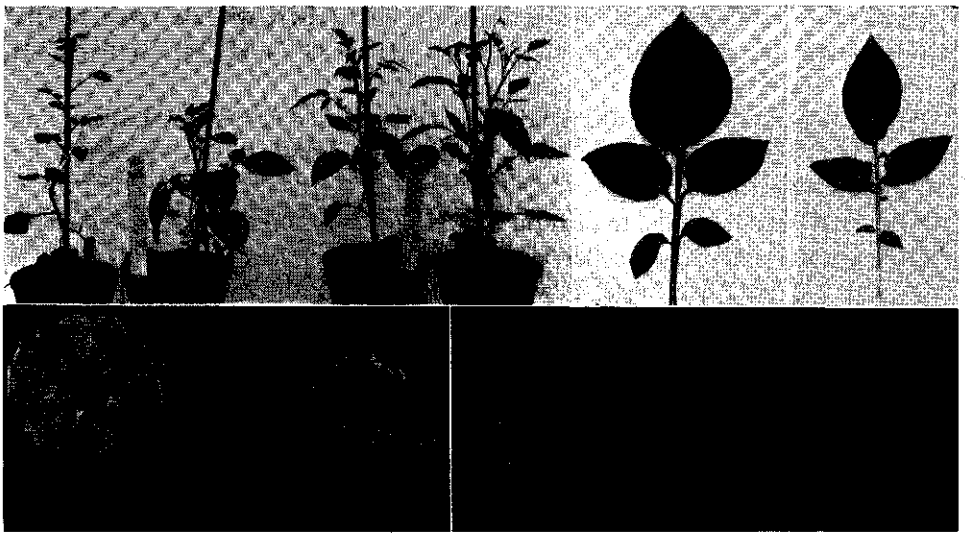


Plate 20. Diploid *S. verrucosum* PI 195172–237–9 (right) compared with its colchicine-induced tetraploid (left).

About one third of the seedlings showed polyploid characteristics (see Plate 20). These plants were checked for chromosome numbers and proved to have 48 chromosomes.

The tetraploid plants grew slower, had darker green leaves with the terminal leaflets more hearted-shaped. They had a more plump appearance and the flower organs were also larger. The stomata of the tetraploids were larger and the average number of chloroplasts was about 20, as compared with 12 in the diploid plants. The doubled plants flowered poorly (only in the first season) and showed reduced male and female fertility. Pollen stainability was 50%, as against 70% in the diploids, and seed set per berry was about two thirds of that in the diploids. Seeds of the tetraploids were larger, and the yield (in number and weight) of the tubers was higher.

Hybrids between tetraploid plants of *ver* PI 195172, and variety Prof. Broekema and MPI 19268 (from seed stocks of the Institute of Plant Breeding) were checked for pollen stainability and appearance. The two tetraploid hybrid populations showed tetrad male sterility and lobed pollen. This indicates that doubling *ver* chromosomes had no effect on the function of the plasmon and also points to the absence of cryptic structural differences (see Stebbins, 1950) at the loci determining tetrad sterility and lobedness.

6.11 Hybrids with S. verrucosum PI 160228 and the presence of two linkage groups between Tr and Ld genes

Table 27 shows that *phu* PI 225682–22 and different haploids of *tbr* and *adg* were successfully crossed as male partners on female *ver* PI 160228.

The hybrid between the parthenogenetic *ver* and the *adg* haploid, AH 66–94–28 was

In both grafted and non-grafted populations about one third of the plants did not flower and showed reduced growth. The values for compound leaf measurements (L, W and T) and stem height of the non-flowering plants were 37%, 45%, 100% and 39%, respectively, of the corresponding values in their flowering sisters.

Some of the non-flowering plants died relatively early but their tuber yield was nearly three times as high as that of the flowering plants (52 g tubers as against 18 g per plant). The higher tuber yield of the non-flowering plants was mainly due to their heavier tubers rather than to a higher number of tubers per plant, which averaged 10.8 in the non-flowering and 9.4 in the flowering plants. Tubers from some of the non-flowering plants were planted the following season and they produced normally flowering plants (see also 6.7).

The non-grafted plants as compared with the grafted ones gave relative values of 58%, 54%, 57%, and 38% for compound L, W, T and stem height, respectively. Flowering was better in the grafted plants and lasted longer.

6.13.2 Partly stained sterility in Hybrid 5 and other hybrids

Contrary to what has been observed in the hybrids with *ver* PI 195172 and PI 160228, the flowers of the F_1 plants of Hybrid 5 (*ver* PI 275258–121–6 × US–W 42) were deeply coloured and showed normal size without any deformity. The anthers looked healthy and plump (Plate 21) and shed normal quantities of pollen.

Stainability of this pollen was sometimes over 60%, but staining was mostly irregular and incomplete. Three types of 'stained' pollen could be distinguished in this and other hybrids with *ver* PI 275258 as the female parent: faintly stained (pollen

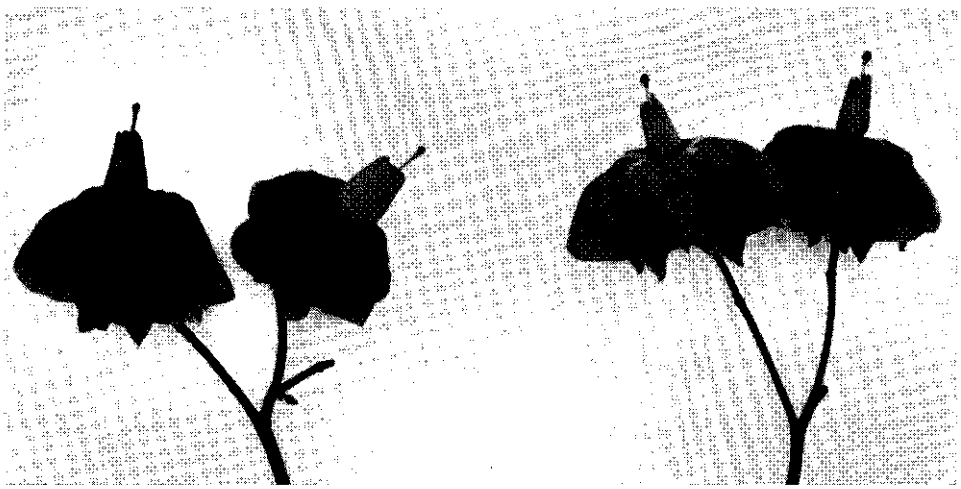


Plate 21. Flowers from hybrids *S. verrucosum* PI 275258 × US–W 42 (left) and *S. verrucosum* PI 195172 × US–W 42 (right). Tetrad sterility is accompanied by reduced size of anthers.

grains sometimes small), partly stained, and well-stained (Plate 13–4). In addition small non-stained irregularly shaped grains occurred. The partly stained grains were spheroidal, generally had the normal size (few were larger or smaller) and the stain never covered the whole grain: a non-stained area could be observed near the pollen wall. Such non-stained area differed in size and sometimes passed into the normally stained area via a faintly stained zone.

Variability was observed within the partly stained pollen of different hybrid plants. Such pollen showed either good, medium or weak colouring, and in all these cases the non-stained areas were either colourless or very poorly coloured. The contrast between the non-stained and stained areas was not always as clear as that in Plate 13–4.

Pollen grains of the hybrids with female *ver* PI 275258 usually show more variation in size than those of pure *ver* plants (apart from the irregularly shrunken grains which were 14–29 % smaller in diameter than the good pollen).

In hybrids of *ver* PI 275258 with haploids US–W 1 and US–W 42 (40 plants tested), the frequency of non-stained pollen grains varied from 9–75 %, with an average of about 40 %, while the partly stained pollen averaged 50 % and varied from 20–79 %, whereas the completely coloured pollen including the well stained ones varied from 2–30 % with an average of less than 10 %. Hybrids between other *ver* introductions and *S. phureja* showing a similar behaviour, also varied in the frequency of the different types of pollen grains mentioned.

The F_1 plants of Hybrid 5 were self-pollinated (637 flowers) and sib-mated (107 flowers) without success. Using pollen of F_1 plants on *ver* parent (20 flowers) gave no berry set. Using the F_1 plants as females in backcrosses to both parents (64 pollinations with *ver* pollen and 28 with US–W 42) hardly showed a better result: only late in the season one berry containing one unhealthy seed was obtained on a grafted F_1 plant pollinated with pollen from US–W 42; all other backcross pollinations failed. The use of other verrucosum introductions as male partners on several F_1 plants of Hybrid 5 also failed, although 115 flowers were pollinated.

The female fertility of the hybrid plants was assessed by using the F_1 plants as females in crosses with other species. *S. phureja* PI 225682–22, *S. chacoense* CPC 1153 and the hybrid *S. stenotomum* WAC 425 × *S. phureja* PI 225682–22 were used as pollinators on these [18] F_1 plants. Berry and seed set were always normal indicating good female fertility of the F_1 plants. The following years, the tri- and tetra-hybrids obtained were checked and no change was observed in the pollen characteristic.

To shed more light on male fertility, pollen of F_1 plants from Hybrid 5 and other hybrids showing similar behaviour was germinated *in vitro*. Out of 41 plants from 6 hybrids, 22 showed not a single germinating grain, and 19 gave less than 1 % germination. The high sterility of these hybrids had already been expected from the staining pattern of their pollen.

The single seed obtained from the backcross of the F_1 plant of Hybrid 5 to US–W

18. Other male sterile hybrids of *ver* PI 195172 and PI 160228 × haploids and diploid species were also tested for female fertility.

42 germinated. The resulting plant showed the same type of male sterility as the F_1.

The male sterility of the type discussed above will be referred to as 'Partly stained sterility' (proposed symbol *Ps*). The above mentioned data indicate that this type of male sterility results from the interaction between one or more dominant genes from the haploid or diploid species with *ver* plasmon. Thus *S. verrucosum* PI 275258–121–6 will be assumed to have the plasmon [Ps^s] and US–W 42 [ps^r]. Unfortunately, due to the unsuccessful attempts to obtain F_2 and BC to parent *ver*, no more data can be reported on the inheritance of the partly stained type of male sterility. Another plant of the same *ver* introduction (PI 275258–121–4) crossed with male US–W 42 gave hybrids showing the same 'partly stained sterility' as did the hybrid *ver* PI 275258–119–1 × US–W 1. The 30 plants checked from the hybrid *S. verrucosum* PI 275258–121–1 × PI 195172–237–10 crossed with *S. phureja* PI 225682–22 and the 44 hybrid plants of *S. verrucosum* PI 275258–121–4–2 × *S. goniocalyx* WAC 204 × 257 showed the same type of male sterility. In the latter hybrid neither selfing (318 pollinations), sib-pollination (114 attempts) nor backcrossing to the *ver* parent (17 trials) had any success.

6.13.3 Cytological observations on Ps-sterile plants

Young anthers checked from F_1 plants with the partly stained male sterility showed normal chromosome pairing and 12 bivalents were generally observed. As soon as the young microspores had separated, they were characterized by being partly stained. In the young microspore one third to one half of the total area was uncoloured or faintly stained, whereas in the shed pollen grains relatively smaller areas were unstained or only faintly stained.

Shed grains showed a low frequency of pollen with stainable nuclei. Many pollen grains from plants of Hybrid 5 lacked them entirely (48–77%), some grains showed round nuclei (9–30%) and only in 14–18% of the plants were stainable elongated nuclei observed. Pure *ver* pollen checked on the same day gave 74% with elongated nuclei, 6% with rounded ones, and 20% showed absence of stainable nuclei.

Hence it appears that the breakdown of the pollen grains takes place soon after nuclear division and before the release of the young microspores from the pollen mother cells. It is possible that this type of male sterility is brought about by the breakdown of the cytoplasm of the microspores accompanied, followed or preceded by mal-functioning or non-functioning of the pollen nuclei.

6.14 **Plasmon differentiation of some S. verrucosum introductions**

The various ways in which male sterility due to the use of different *ver* introductions as females with the same male parent, was expressed in the F_1 plants proved the existence of different plasmon types within *S. verrucosum*.

To study this plasmon differentiation in connection with male sterility in hybrids, 21 *ver* introductions were crossed as females with *S. phureja* PI 225682–22. The choice

of this *phu* clone as 'plasmon tester' was made for the following reasons. It contains at least one homozygous dominant plasmon-sensitive gene; it crosses readily with *ver*; it shows good male fertility and flowers profusely for a long period.

The crosses between *ver* introductions and the *phu* clone were very successful and usually resulted in a high berry set with many seeds per berry (50–150 as a rule). It was generally observed that the higher the female fertility (number of seeds per berry upon selfing) of the *ver* introduction, the higher the number of seeds per berry in crosses with the *phu* clone.

Some of the hybrid seedlings were checked for the number of chloroplasts in the leaf epidermis. The average for the 850 seedlings tested varied from 13–16. Six plants from hybrids involving CPC 2247, CPC 2644 and a *ver* × *ver* hybrid were found to have higher averages (24–25). These were checked for chromosome number and all had 48 chromosomes. In the seedling stage they looked vigorous with broad dark green leaves. The origin of these tetraploid hybrids most probably lies in a functioning of unreduced gametes from both parents. When the plants were checked at flowering time, they showed the same type of male sterility as their diploid counterparts. The relatively large pollen grains of some plants predominantly had four pores, whereas in other plants most pollen grains have three.

The fact that tetraploid hybrids showed the same expression of male sterility as the diploid hybrids ruled out the possibility of the presence of cryptic structural differences involving the loci for male sterility. This confirms the previous observations (see 6.10).

As reported before two types of plasmons were discovered in *ver* introductions with respect to their interaction with the dominant plasmon-sensitive genes from haploids and diploid species. These plasmons were [Tr^s] in *ver* PI 195172 and PI 160228, and [Ps^s] in *ver* PI 275258. When the hybrids between the 21 introductions of *ver* with *phu* PI 225682–22 were checked, all proved to be male sterile. The hybrid plants were grouped according to three types of male sterility. Besides the 'tetrad sterility' and the 'partly stained pollen sterility', a third type was detected occurring only in hybrid plants with female *ver* Haw 1350. The pollen grains of the F_1 plants (*ver* Haw 1350 × *phu*) were smaller than normal, generally spheroidal and sometimes had an irregular or oblong shape. The grains stained only faintly and some of them looked slightly bubbled. The anthers of F_1 plants shed very small quantities of pollen (much less than in the case of tetrad sterility), but the grains shed separately.

The most characteristic feature of that third type of male sterility may be described as 'striped vacuolar' pollen grains where most of the pollen looked as if divided by relatively dark stained stripes separating the somewhat bubbled vacuoles (Plate 13–3). This type of male sterility will be called striped vacuolar pollen (proposed symbol *Sv*).

For the same reason as in the other two types of male sterility, the *Sv* sterility will be assumed to be controlled by dominant gene(s) from *phu* in *ver* plasmon. *S. verrucosum* Haw 1350 was thus assumed to have the plasmon [Sv^s] and *phu* [sv^r].

Based on the male sterility types observed in the F_1 hybrids all available *ver* introductions were classified into the three types of plasmons [Tr^s], [Ps^s] and [Sv^s]

Table 37. Classification of 21 introductions of *Solanum verrucosum* with respect to the [Tr^s], [Ps^s] and [Sv^s] plasmons using *S. phureja* PI 225682–22 as 'plasmon tester'.

Plasmon type [Tr^s]		Plasmon type [Ps^s]			Plasmon type [Sv^s]
PI 160228	PI 195171	PI 161173	PI 275255	PI 275256	Haw 1350
PI 195172	PI 255544	PI 275258	PI 275259	PI 275260	
PI 310966	Haw 2246	Haw 756	Haw 1532	CPC 2247	
CPC 1339	CPC 2623a	CPC 2514	CPC 2644	EBS 2632	

(Table 37). Only *S. verrucosum* Haw 1350 carries [Sv^s] plasmon. Eight introductions proved to have [Tr^s] plasmon and twelve appeared to possess [Ps^s] plasmon.

Some of these *ver* introductions were checked more than once because they were received under different donor numbers (for instance *ver* CPC 2644 had also been checked under Haw 1542 and PI 251756). In the same way *ver* PI 161173, PI 195172 and PI 275256 were checked more than once. In all these tests, however, there was no difference in the type of male sterility.

All the hybrid plants with female *ver* introductions and male *phu* were used for backcrossing, selfing and sib-mating. The only successful pollinations were in the backcross $F_1 \times$ *phu*. The failure of $F_1 \times$ *ver* pollinations was found to result from the inhibition of the germinating pollen tubes of *ver* in the upper third of the style of F_1 plants.

6.15 [Tr^s] and [Ld^s] plasmons

S. verrucosum introductions with [Ps^s] and [Sv^s] plasmons have non-lobed pollen grains, so that they are assumed to have [ld^r] plasmon.

Among the introductions with [Tr^s] plasmon the presence of [Ld^s] plasmon could be clearly demonstrated in PI 160228, PI 195172, PI 310966 and CPC 1339, whereas it could not be ascertained in Haw 2246, CPC 2623a, PI 195171 and PI 255544 owing to the irregular behaviour in various hybrid plants when tested at different dates. It is believed that in the last four *ver* introductions the [Ld^s] plasmon is present, but its effect on pollen behaviour appears to be influenced by the genetic background.

6.16 Male fertile backcross plants as pollinators on haploids and diploid species

As previously mentioned, the backcrosses of $F_1 \times$ *ver* segregated into male fertile and male sterile plants. Male fertile BC plants of the Hybrids 1, 3 and 4 (see 6.6, 6.8, 6.9) were used as pollinators on some diploid species (*ktz*, *jam*, *phu*, *stn* and *gig*) and on several haploids from different origin. The 409 pollinations attempted on the emasculated haploids and diploid species were successful in only two cases: on the *S. tuberosum* haploid TH 67–17–2 (3 pollinations giving 2 berries and 27 seeds) and on the *S. andigena* haploid AH 66–94–28 (5 pollinations giving 2 berries with 28 seeds).

The male parent was in both cases the backcross of Hybrid 1 (*ver* × US–W 42 × *ver*).

The seeds were sown in 1968 and both populations showed good germination (61 % in *S. tuberosum* haploid hybrid and 74 % in *S. andigena* haploid hybrid). The seedlings proved to be of hybrid origin. Some data about these two hybrid populations will be reported.

6.16.1 TH 67–17–2 × backcross (ver × US–W 42) × ver

The female tuberosum haploid had whitish flowers and about 50 % pollen stainability. The male parent population (*ver* × US–W 42 × *ver*) had lilac to purplish flowers and varied in pollen stainability from 36 % to more than 90 %. The pollen grains of the male population were either spheroidal or lobed: a mixture of pollen from different flowers collected from the male fertile BC plants was used for the pollinating.

The hybrid plants grew and flowered well. Six plants showed whitish flowers while in the other eight the intensity of flower colouration varied.

Pollen stainability of the hybrid plants was between 30 and 78 %, with an average of 52 % in the 14 plants checked. All grains were spheroidal. Attempts to self the hybrid plants failed, although in some cases more than 30 pollinations were tried at different dates on each plant.

Pollen tube growth was checked after attempted selfing some of these plants. Many pollen grains germinated and pollen tubes could be observed in the upper half of the styles, but none penetrated further. Thus these F_1 plants are apparently self-incompatible.

When *ver* PI 195172–237–9 was used to pollinate the hybrid plants, pollen tubes penetrated only 1/5 to 1/2 of the styles and showed thickening of tube ends. This reaction was similar to the SI × SC inhibition, indicating that TH 67–17–2 had only been fertilized by pollen carrying self-incompatibility alleles from US–W 42, whereas pollen carrying self-compatibility alleles from *ver* was prevented from fertilization (see 9.4.6.1, 2).

6.16.2 AH 66–94–28 × backcross (ver × US –W 42) × ver

The haploid AH 66–94–28 had very pale violet flowers. Pollen stainability was about 20 %.

The hybrid plants showed flower colours varying from purple to poor lilac, but flowers with whitish lobes and coloured stripes were also observed.

The hybrid plants gave better pollen stainability than the female haploid; it varied in the F_1 plants from 36–83 % (mean value 63 %). All pollen grains were spheroidal.

Twelve out of 15 hybrid plants set berries on selfing (berry set between 7 and 100 %).

Eight plants produced verrucose-berries, whereas four plants showed non-verrucose (pure greenish) berries. It has been mentioned earlier (see 6.6.6.2) that verrucose characteristic of berries of *S. verrucosum* is probably inherited as a dominant charac-

ter; these data confirm that hypothesis. Assuming the verrucose characteristic to be inherited through one dominant gene, the BC plants of Hybrid 1 to *ver* are expected to consist of two genotypes for 'verrucose': *VbVb* and *Vbvb*. As pollen of these two genotypes was used to pollinate AH 66–94–28 (*vbvb*), the ratio 3 verrucose: 1 non-verrucose was expected in the F_1. The observed segregation 8:4 fitted this ratio ($\chi^2 = 0.44$, $P = 0.70$–0.50).

Among the 12 plants which set berries after selfing, one produced a parthenocarpic berry from 15 self-pollinations. The other eleven plants gave low numbers of seeds per berry compared with the selfed *ver*. Seed set was nearly equal to that of the original cross. The number of seeds per berry in the hybrid plants varied from 3 to 61 (average 21).

The plants which set no berries after selfing proved to be self-incompatible: the germinated pollen tubes showed a maximum penetration of two thirds of the style length. These self-incompatible plants must have originated from the fertilization of the female haploid by pollen carrying self-incompatibility alleles, expected to be present among pollen of BC plants (see 9.4.6.1, 2).

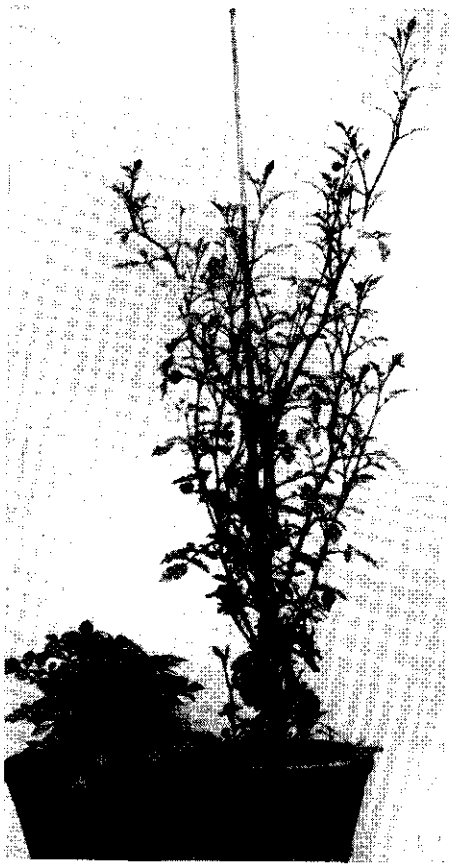


Plate 22. Rosette plant and normal plant from F_2 population of Hybrid AH 66–94–28 × backcross.

When the haploid plant AH 66–94–28 was pollinated with *ver* PI 195172–237–9, normal berry and seed set were obtained indicating the absence of the SI × SC inhibiting mechanism in the self-incompatible haploid.

The seeds set after selfing 11 F_1 plants of AH 66–94–28 × BC were sown in 1969. The resulting F_2 plants grew well except for some individuals with dwarf growth. These dwarfs had small dark green leaves and short internodes resulting in a 'rosette' growth type (Plate 22) which did not flower.

From the 11 F_2 populations checked, only 4 segregated into rosette and normal plants. These 4 segregating F_2 populations showed normal: rosette plants in the numbers of 6:1, 28:1, 37:11 and 3:1. This segregation can be explained by assuming the rosette characteristic to be inherited through the complementary action of two recessive genes (proposed symbols rs_1 and rs_2) with the genotype $rs_1rs_1Rs_2rs_2$ for the female haploid and $Rs_1rs_1Rs_2Rs_2$ for male parent. On that basis half of F_2 populations will not segregate whereas the other half will segregate into either 3:1 or 15:1. The data mentioned above fitted these expectations.

The flowering F_2 plants showed somewhat reduced pollen stainability, segregants with low percentages of stainable pollen and male sterile segregants with non-shedding characteristic (all of these complications could be expected in such an inbred population). The F_2 plants with good pollen grains were successfully selfed (self-compatible).

7 Inheritance of self-incompatibility in S. phureja and S. stenotomum × S. phureja

The two related South American cultivated species *S. phureja* and *S. stenotomum* were used to study incompatibility, one of the major obstacles in crosses between related diploid clones.

The term 'incompatibility' refers here to the prevention of zygote formation due to the presence of identical self-incompatibility (*S*) alleles in male and female gametes. Incompatibility in *Solanum* is mainly expressed as stylar inhibition of pollen tube growth. However, stylar inhibition is not always caused by *S* alleles as it can also occur in pollinations between self-compatible species (see 6.3).

In 1966 crosses were attempted between clones and introductions of *S. phureja* and the hybrids were investigated in 1967 (see 7.1). The hybrid between *stn* and *phu* was obtained from Dr. J. G. Th. Hermsen and was studied in 1966 and 1967 (see 7.2).

Diallel crossings were tried between individuals of each F_1 hybrid population in an attempt to determine the incompatibility groups within each hybrid progeny. Seed set was used as a criterion of cross compatibility. At least 3 flowers of *phu* × *phu* and 5 flowers of *stn* × *phu* were used in pollinations attempted in the diallel crossing.

7.1 Incompatibility within S. phureja

7.1.1 Crossing attempts between clones 1 and 22 of S. phureja PI 225682

The fertile clones 1 and 22 of *S. phureja* PI 225682 (used for extraction of haploids from tetraploids), were reciprocally pollinated. The 42 attempted pollinations were unsuccessful: the plants of the two clones proved to be cross-incompatible as well as self-incompatible. It seems that the two clones had identical alleles, controlling incompatibility.

7.1.2 S. phureja PI 225702–2 × PI 225682–22

In the original cross of *phu* PI 225702–2 × PI 225682–22 six flowers were pollinated and three berries were set enclosing 1799 seeds (about 600 seeds per berry).

The 16 F_1 plants chosen at random were grafted onto tomato and a nearly complete diallel cross was tried between them. The results are presented in Fig. 38. Irregular behaviour, sporadic compatibility and erratic female incompatibility were observed.

Irrespective of the irregularities, it was possible to place the 16 plants in five intra-incompatible groups: Group A was characterized by its female incompatibility with

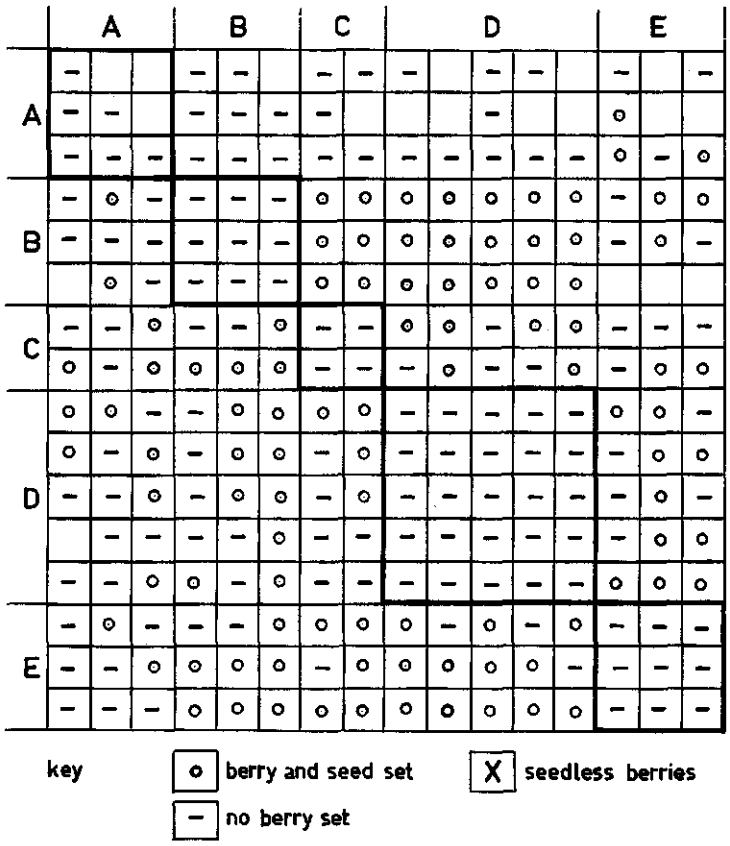


Fig. 38. Attempted selfing and sib-pollination in 16 F_1 plants from the hybrid *S. phureja* PI 225702–2 × PI 225682–22.

Groups B, C and D, but the relation between A and E was not very clear though A showed several female incompatibilities to some plants of E. For the rest the groups were cross compatible but several individual inter group matings gave no berry set.

7.1.3 S. phureja PI 225682–22 × PI 225702–2

In the hybrid *phu* PI 225682–22 × PI 225702–2, the reciprocal of that mentioned under 7.1.2, three flowers were pollinated and three berries set. Seeds per berry varied from 224 to 380 (average 288), which was less than half that of the reciprocal.

Fourteen hybrid plants were grafted onto tomato for the diallel crossing. As apparent from Fig. 39, four incompatibility groups were formed, one of which (A) seemed incompatible as a female with the other three. Erratic behaviour was also observed in this experiment.

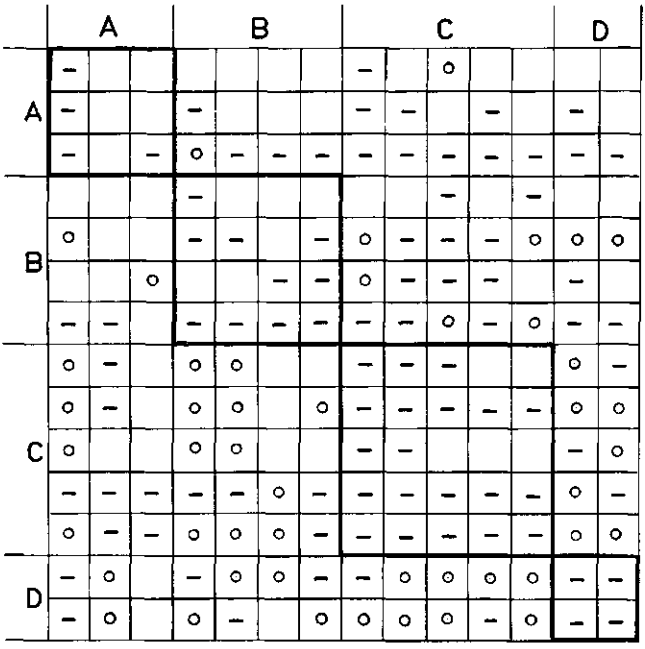


Fig. 39. Results of the attempted pollinations in 14 F_1 plants from the hybrid *S. phureja* PI 225682–22 × PI 225702–2 (see key in Fig. 38).

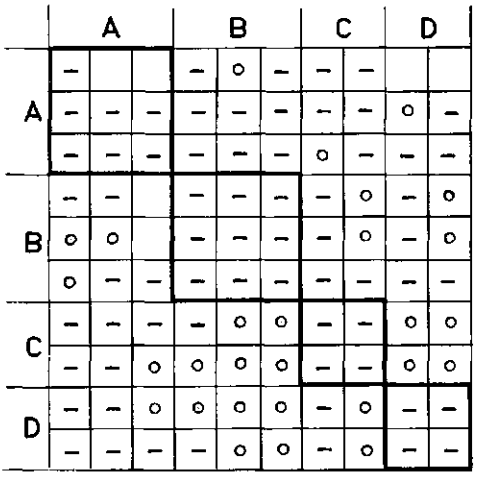


Fig. 40. Attempted pollinations in 10 F_1 plants from the hybrid *S. phureja* PI 225682–1 × PI 225702–2 (key as in Fig. 38).

7.1.4 S. phureja PI 225682–1 × PI 225702–2

The six flowers of *phu* PI 225682–1 pollinated by *phu* PI 225702–2 produced two berries with 27 and 101 seeds.

The results from a diallel using ten grafted F_1 plants showed a similar irregular pattern as that observed in the other hybrids (Fig. 40). Nevertheless the plants could be classified into four intra-incompatible groups, the inter-group matings giving erratic results.

	A	B						C		D		E	
A	–	–	–	–	–	–		–	–	–	–	–	–
B	–	–	–	–	–	–	–	–	–	–	–	–	o
	–	–	–	–	–	–	–	–	–	o	–	o	–
	–	–	–	–	–	–	–	o	o	–	o	–	o
	o	–	–	–	–	–	–	–	o	–	o	o	–
	–	–	–	–	–	–	–	–	o	o	o	–	o
		–	–	–	–	–	–	o		–	o	–	
C	o	–	–	–	–	o	–	–	–	o	o	o	o
	–	–	–	o	–	o	o	–	–	–	–	o	o
D	–	o	o	o	–	–	o	o	o	–	–	–	–
	o	–	–	–	o	–	–	o	o	–	–	–	–
E	o	–	–	o	–	o	–	o	o	o	–	–	–
	–	o	–	–	o	–	–	o	–	–	o	–	–

Fig. 41. Results of the attempted pollinations in 13 F_1 plants from the hybrid *S. phureja* PI 243461–1 × PI 225682–22 (key as in Fig. 38).

7.1.5 S. phureja PI 243461–1 × PI 225682–22

In the cross *phu* PI 243461–1 × PI 225682–22, the six flowers pollinated all showed berry set with an average of 119 seeds per berry.

Of the hybrid progeny 13 grafted plants were used for the diallel cross (Fig. 41). The irregular crossing behaviour was observed, but five incompatibility groups could be detected, one of which (with one plant) appeared to be incompatible when used as a female with all plants of the other groups.

7.1.6 S. phureja PI 243461–1 × PI 225702–2

The three attempted pollinations on *phu* PI 243461–1 using pollen of *phu* PI 225702–2 gave three berries with seed set varying from 370 to 710 per berry (average 570).

The six grafted F_1 plants studied in the diallel cross (Fig. 42) could be placed in

	A	B			C	
A	–	o	o	o	–	–
B	o	–	–	–	o	o
	o	–	–	–	o	o
C	o	o	o	o	–	–
	o	o	o	o	–	–

Fig. 42. Attempted pollinations in 6 F_1 plants from the hybrid *S. phureja* PI 243461–1 × PI 225702–2 (key as in Fig. 38).

three incompatibility groups. Each group was intra-incompatible and showed cross compatibility as male and female with the other two groups, except Group A which was female incompatible with Group C.

7.2 **Incompatibility behaviour in S. stenotomum WAC 425 × S. phureja PI 225682-22**

According to Dr. Hermsen's records, 43 crossings were attempted on *stn* WAC 425 using *phu* PI 225682–22 as pollinator. The resulting 3 berries contained 315 seeds.

Hybrid plants of this cross were studied in 1966, but their unexpected and irregular behaviour led to a second experiment, with other plants, in 1967. A similar behaviour was observed in the 1967 investigations.

	A		B			C				D					E							
A	–	–	o	–	–	–	–	–	–	–	–	–	–	–	–	o	–	–	–	–	o	–
	–	–	o	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
B	o	o	–	–	–	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	
	o		–			o		–							o	o						
	o	o	–	–	–	o	o	X	o	o	o	o	o	o	o	o	o	o	o	–	o	–
C	o	–	o	o	o	–	–	–	o	o	–	–	–	–	o	o	o	o	o	o	o	
	o	–	–		X	–	–	–	o	–	o	–			o	o	o	o				
	o	o	o	o	o	–	o	–	–	o	o	o	o	o	o	o	o	o	o	o	o	–
	–	o	o	o	o	o	–	–	–	o	o	–	o	o	–	o	o	o	–	o	o	o
D	o	–	o	o	–	–	–	o	o	–	–	–	–	–	o	o	o	o	o	–	o	o
	o	–	o	o	o	–	–	o	o	–	–	–	–	–	o	–	o	o	o	o	o	–
	o	–	o	o	o	o	–	o	o	–	–	–	–	–	o	o	o	o	–	o	–	o
	o	–	o	o	o	–	–	o	o	–	–	–	–	–	–	o	o	–	–	–	o	o
	o	–	o	o	–	–	–	o	o	–	–	–	–	–	–	–	o	–	–	o	–	–
E	o	o	o	o	o	o	o	o	o	o	o	o	o	o	–	–	–	–	–	–	–	–
	o	o	o	o	o	o	–	o	–	o	–	–	o	–	–	–	–	–	–	–	–	–
	o	o	o	o	o	o	o	o	o	o	o	o	o	o	–	–	–	–	–	–	–	–
	–	o	–	o	–	–	o	–	o	o	o	o	o	o	–	–	–	–	–	–	–	–
	o	o	o	o	o	o	o	–	o	o	o	o	o	o	–	–	–	–	–	–	–	–
	–	o	–	o	–	o	–	–	o	o	o	o	–	–	–	–	–	–	–	–	–	–
	o	o	o	o	–	o	o	–	o	o	o	o	–	–	–	–	–	–	–	–	–	–
	o	–	o	o	–		–	o		o	X	o	–	o	–	–	–	–	–	–	–	–

Fig. 43. Results of the attempted pollinations in 22 F_1 plants from the hybrid *S. stenotomum* WAC 425 × *S. phureja* PI 225682–22 (key as in Fig. 38).

The 1966 study included 22 grafted F_1 plants, which all showed good growth and flowering, with 43–98% stainable pollen (average 83%). The results of the diallel crossing could be arranged in five incompatibility groups (Fig. 43). Generally the groups were intra-incompatible inter-compatible with some exceptions showing sporadic incompatibility and few unexpected seed sets.

Group A (2 plants) showed female incompatibility with the other four groups, also with pollen grains from parent *phu*. Parent *phu* pollen was effective on almost all plants of Groups B, C, D, and E and *phu* gave berry set with pollen of nearly all plants of the five groups.

Seed set in these diallel crossings was higher than in the parental cross (average per berry about 300). There were no great differences in seed set per berry, either between the groups or within each group when used as male or female partner (except for Group A which showed female incompatibility). Large differences in seed set were observed in the pollinations with parent *phu* and its reciprocals. When *phu* was used as the female partner, seed set (89 per berry) was less than one third of that observed when *phu* was used as male partner (326 per berry).

In *S. phureja* PI 225682 female fertility was somewhat lower than in other phurejas and in its hybrid with *S. stenotomum*.

The 18 grafted F_1 plants of *stn* × *phu* raised in 1967 were used in a diallel crossing attempt. The results could be arranged in five incompatibility groups with frequency of 4:3:5:3:3. One group (4 plants) was incompatible as female with all plants of the other four groups, and some of its individuals showed male incompatibility with all plants of some of the other four groups. These four groups showed cross compatibility in both directions. Female incompatibility and some seed set were unexpectedly observed in some pollinations.

7.3 A hypothesis to explain the incompatibility behaviour

The incompatibility behaviour in *S. phureja* × *S. phureja* and *S. stenotomum* × *S. phureja* can be explained by assuming the presence of two loci. These loci are expected to be multiallelic and the incompatibility behaviour is believed to be gametophytically controlled.

The first locus (*S*) must be epistatic over the second (*R*), but no dominance relationship need exist between the *S* or *R* alleles. One of the *R* alleles, R_{fi}, when present in a homozygous condition, has the property of preventing fertilization by all pollen grain genotypes, including its own. This implies that $R_{fi}R_{fi}$ plants are female incompatible with all *S* and *R* genotypes.

The hypostasis of locus *R* may be one of the reasons why a homozygous genotype ($R_{fi}R_{fi}$) is required to produce female incompatibility. That hypostasis will cause the incompatibility behaviour to be controlled by the *S* locus, except in $R_{fi}R_{fi}$ females.

Five to seven *S* alleles and at least two *R* alleles have been discovered. More alleles may be present at the *R* locus, but their hypostasis prevents their detection.

The hypothesis leads to the following expectations.

(1) If the alleles at the R locus of two parents are such that $R_{fi}R_{fi}$ cannot occur in their F_1, the incompatibility behaviour is determined by the S locus only. In such a case two or four intra-incompatible inter-compatible groups are expected in F_1 when the parents are heterozygous and have respectively one or no S alleles in common.
(2) Two $R_{fi}R_{fi}$ individuals are reciprocally cross incompatible, irrespective of their S genotypes.
(3) If one parent is homozygous for the R_{fi} allele whereas the other is heterozygous or lacks this allele, the crossing with ♀ $R_{fi}R_{fi}$ will be incompatible. The success of the reciprocal cross depends entirely on S genotypes. The grouping in the eventually obtained progeny will depend on S alleles as well as on the absence or presence of R_{fi} in the other parent.
(4) When a cross is attempted between two parents, both heterozygous for S and R_{fi} allele and sharing no common S allele, the cross will be compatible in both directions. The twelve genotypes expected among F_1 plants will form five intra-incompatible inter-compatible groups in the frequency of 4:3:3:3:3 (see Fig. 44). The group with the frequency 4 will be $R_{fi}R_{fi}$ and therefore female incompatible with pollen from the other four groups. This female incompatible group will consist of 4 S allele genotypes, in contrast with each of the other 4 incompatibility groups, which comprise only one

		A				B			C			D			E		
$S_1S_3R_{fi}R_{fi}$ (1)	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
$S_1S_4R_{fi}R_{fi}$ (1)	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
$S_2S_3R_{fi}R_{fi}$ (1)	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
$S_2S_4R_{fi}R_{fi}$ (1)	A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
$S_1S_3R_aR_{fi}$ (2)	B	–	o	o	o	–	–	–	o	o	o	o	o	o	o	o	o
$S_1S_3R_aR_{fi}$ (2)	B	–	o	o	o	–	–	–	o	o	o	o	o	o	o	o	o
$S_1S_3R_aR_a$ (1)	B	–	o	o	o	–	–	–	o	o	o	o	o	o	o	o	o
$S_1S_4R_aR_{fi}$ (2)	C	o	–	o	o	o	o	o	–	–	–	o	o	o	o	o	o
$S_1S_4R_aR_{fi}$ (2)	C	o	–	o	o	o	o	o	–	–	–	o	o	o	o	o	o
$S_1S_4R_aR_a$ (1)	C	o	–	o	o	o	o	o	–	–	–	o	o	o	o	o	o
$S_2S_3R_aR_{fi}$ (2)	D	o	o	–	o	o	o	o	o	o	o	–	–	–	o	o	o
$S_2S_3R_aR_{fi}$ (2)	D	o	o	–	o	o	o	o	o	o	o	–	–	–	o	o	o
$S_2S_3R_aR_a$ (1)	D	o	o	–	o	o	o	o	o	o	o	–	–	–	o	o	o
$S_2S_4R_aR_{fi}$ (2)	E	o	o	o	–	o	o	o	o	o	o	o	o	o	–	–	–
$S_2S_4R_aR_{fi}$ (2)	E	o	o	o	–	o	o	o	o	o	o	o	o	o	–	–	–
$S_2S_4R_aR_a$ (1)	E	o	o	o	–	o	o	o	o	o	o	o	o	o	–	–	–

Fig. 44. Hypothetical diallel crossing in F_1 plants where the parental genotypes are S_1S_2–$R_aR_{fi} \times S_3S_4\ R_aR_{fi}$. Five incompatibility groups are expected to show the inter-group reaction as presented (key as in Fig. 38).

		A		B			C		
$S_1S_3R_{fi}R_{fi}(1)$	A	–	–	–	–	–	–	–	–
$S_2S_3R_{fi}R_{fi}(1)$		–	–	–	–	–	–	–	–
$S_1S_3R_aR_{fi}(2)$	B	–	o	–	–	–	o	o	o
$S_1S_3R_aR_a(1)$		–	o	–	–	–	o	o	o
$S_2S_3R_aR_{fi}(2)$	C	o	–	o	o	o	–	–	–
$S_2S_3R_aR_a(1)$		o	–	o	o	o	–	–	–

Fig. 45. Hypothetical diallel crossing in F_1 plants if parents are $S_1S_2R_aR_{fi} \times S_1S_3R_aR_{fi}$. The expected 3 incompatibility groups should react as observed (key as in Fig. 38).

S genotype. Each of the four *S* genotypes present in the $R_{fi}R_{fi}$ group (A) will be incompatible as male on the individuals from one of the other groups (B, C, D, E) with the identical *S* genotype, but male compatible on the other three groups.

(5) If parents are *S* allele heterozygous but have one *S* allele in common and both carry R_{fi} allele, the F_1 plants in the six expected genotypes will form three incompatibility groups in the frequency of 2:3:3 (Fig. 45). One of these groups (A = $R_{fi}R_{fi}$) will show female incompatibility to the other two groups (B and C), and one of the two *S* genotypes present in the $R_{fi}R_{fi}$ group will be male incompatible on Group B whereas the other *S* genotype will be male incompatible on Group C.

7.4 Observed results and hypothetical expectations

A comparison between the results of the diallel crossings in *phu* × *phu* and *stn* × *phu* with those based on the preceding hypothesis shows that the observed results could be explained within the frame of the hypothesis with or without slight modification.

In the F_1 of *phu* PI 225702–2 × PI 225682–22 (Fig. 38) five intra-incompatible inter-compatible groups A, B, C, D and E were found with the respective numbers of 3:3:2:5:3. Group A showed female incompatibility to all other groups, with three exceptions involving Group E (here either environment or minor genes or both were at work, as well as in the following crosses). The pollen grains of the plant indicated in the first column of Fig. 38 were ineffective on styles of Group E, while the plant indicated in the second column showed male incompatibility on Group C and the plant in the third column showed the same on Group B. This behaviour agrees with the hypothesis (see the theoretical scheme in Fig. 44). The parent phurejas PI 225702–2 and PI 225682–22 may be assumed to have the genotype $S_kS_dR_aR_{fi}$ and $S_aS_bR_aR_{fi}$, respectively. The discrepancy between the expected and the observed frequencies of the five groups is small and the observed numbers fit well.

The behaviour observed in the hybrid *phu* PI 243461–1 × PI 225682–22 (Fig. 41) can be explained in the same way as the previous cross (for the theoretical expectations

see Fig. 44). The five groups obtained in the frequency of 1:6:2:2:2 fit the expected five incompatibility groups. PI 243461–1 will be assumed to have the genotype $S_kS_eR_aR_{fi}$ (one S allele in common with PI 225702–2: see below).

In accordance with the assumed genotypes of the parents in the hybrid *phu* PI 225682–22 × PI 225702–2, the F_1 plants should show five incompatibility groups as did the reciprocal hybrid. But as shown in Fig. 39, only four intra-incompatible groups occur, one of which (A) is female incompatible with the other three. This means that one of the incompatibility groups is missing. In the parental cross, seed set was less than in the reciprocal (288 *v.* 600 seeds per berry). These results may tentatively be explained by assuming that the alleles S_a and S_k are linked with two complementary recessive lethals functioning in the plasmon of PI 225682. Accordingly the lethality will be expressed when the genotype S_aS_k is present in the plasmon of *phu* PI 225682. As a consequence both the complete S_aS_k group and the S_aS_k plants from Group A ($R_{fi}R_{fi}$) will be lacking. Therefore only four incompatibility groups are expected in the ratio of 3:3:3:3 and one of these groups (A) will be female incompatible with the other three groups. The observed frequency 3:4:5:2 fits the expectation.

The hybrid *phu* PI 225682–1 × PI 225702–2 (Fig. 40) also showed the presence of four incompatibility groups in the frequency of 3:3:2:2, one of which (A) is female incompatible with the other groups. As mentioned before, the S alleles of *phu* PI 225682–1 are identical with those in *phu* PI 225682–22, and as both clones belong to the same *phu* introduction they are expected to have the same plasmon. Therefore the results in this cross must be the same as those in the foregoing hybrid and there is a general agreement with expectation: only four incompatibility groups (instead of five) occur and the observed numbers fit the expected ratio.

In *phu* PI 243461–1 × PI 225702–2 three incompatibility groups were discovered in the progeny (Fig. 42). These parents have an S allele in common (S_k) and are heterozygous for the locus R. Thus six genotypes are expected in the progeny and these will function as three incompatibility groups in the ratio 2:3:3 (see theoretical scheme in Fig. 45). The observed groups show the frequency of 1:2:3 and fit the expected ratio. But according to the hypothetical expectation, one of the three groups should be female incompatible with the other two groups and should show different male incompatibilities on them. As apparent from Fig. 42, this expectation is partly fulfilled: Group A, which is assumed to be the female incompatible group showed male compatibility and was fertilized by Group B, but it behaved in the expected manner with Group C. This unexpected behaviour of Groups A and B may be due to some modifiers[19] affecting the plants with the genotype $R_{fi}R_{fi}$. Such plants were usually reduced in number compared with plants of the other groups.

The hybrid *S. stenotomum* × *S. phureja* checked in 1966 (and 1967) has shown that

19. The effect of such supposed modifiers has been observed in all the investigations. In fact, their presence, leading to seed set on the female incompatible plants ($R_{fi}R_{fi}$), proved that allele R_{fi} really controls female incompatibility and not female sterility.

the F_1 plants can be arranged in five intra-incompatible groups. One of the groups is female incompatible with the other groups. This confirms the expectation based on heterozygosity of both parents at the *R* locus and their having no common *S* alleles (see Figs. 43, 44). Including the lower number of plants in the female incompatible group, the observed numbers fitted the expected ratio.

As the phureja parent has been assumed to have the genotype $S_a S_b R_a R_{fl}$, the stenotomum parent will be assumed to be $S_g S_h R_a R_{fl}$. In this case *S. stenotomum* proves to have *S* alleles in the same allelic series as those present in *S. phureja*.

8 Late blight resistance in S. verrucosum and some other populations

The studies of late blight in *S. verrucosum* and some other populations were carried out in 1968 and 1969 at the Institute of Phytopathological Research at Wageningen.[20]

The physiologic races of *Phytophthora infestans* used were 4, 1.4, 1.2.3.4 and 1.3.4.7.8 and the two incompletely identified isolates of the fungus B 19 and 331.

B 19 was isolated from variety Multa in the autumn of 1966 in the province of North Brabant, the Netherlands. Multa was considered to be one of the best 'field resistant' varieties. B 19 is pathogenic on different potato varieties and selections considered to have good resistance, such as Arka and MPI 19268. It also attacks the differential hosts of Black with the genes R_1 and R_4.

Isolate 331 was found in 1968 on the potato clone H 65–221 from the C.I.V. Breeding Station at Ottersum, the Netherlands. The potato clone was known to be resistant till 1969, and the observed attack was not serious. Isolate 331 is known for its ability to attack differential hosts with the genes R_1, R_3, R_4, R_7 and R_8.

Almost all studies were made on seedlings of selfed plant populations. The comparisons and discussion are thus based on these assessments.

The terms 'virulence' and 'aggressiveness' will be used respectively to refer to races with a wider host range and to races that are more prolific.

8.1 Generalization and classification of seedling reaction

For artificial infection of seedlings three suspensions were used, with 3, 10 and 20 spores per mm^3 suspension.

Although the higher concentrations were more destructive, they gave the same effect as the lower concentration, but it occurred 2–3 days earlier (5 days *v.* 7 at 18 °C; 7 days *v.* 10 at 16 °C).

Independent of suspension concentration, in the susceptible verrucosum populations, all seedlings were susceptible and the fungus showed rich sporulation. Checked on the same day, the seedlings sprayed with concentrated suspensions generally showed complete death and were almost decayed while their stems were also affected, whereas seedlings sprayed with diluted suspension were still carrying some greenish, free-sporulating leaves and many of their stems seemed to be in good condition and a

20. The author is much indebted to the Director of this Institute and to the head of the Mycology Department and Dr. J. C. Mooi who kindly supplied all facilities needed to carry out the investigations. Dr. J. C. Mooi kindly put at the author's disposal all races, isolates and information about them.

few – less than 1 % – might escape infection.

The drawback of the higher concentrations is their destructive effect on the seedlings, especially at a continuously high humidity. In such cases, the leaves are too wet and show rotting, while the interference of other organisms is sometimes observed. Such complications impede screening. As a rule only the data of the diluted concentration will be presented here.

About 7 days after inoculation, seedlings were screened and were found to show a wide range of reactions, especially in those populations which segregated into resistant and susceptible seedlings. The reaction was arranged in 5 classes.

In Class 1, the seedlings showed no trace of sporulation nor any lesions.

In Class 2, seedling leaves had necrotic lesions of varying sizes (generally small), neighboured or surrounded by yellowish areas of differing sizes. Sometimes lesions and/or yellowish areas were hardly observable, but in many seedlings of *S. verrucosum* Haw 1532, dead areas of the leaves as well as yellowish surroundings could be clearly observed because of their large sizes. Seedlings of Class 2 were free of sporulation.

In Class 3 small lesions were present showing sparse sporulation, generally at the rims of some leaflets of each seedling.

Class 4 contained seedlings showing varying degrees of sporulation; large sporulating areas of different sizes on one or more leaflets, but with large parts of the leaflets and the stems being green and healthy.

In Class 5 rich sporulation covered nearly all leaves and stems, the latter broken in extreme cases. The plants looked soaked.

Class 5 and particularly Class 4 could have been split into more classes according to relative seedling reaction and the time needed to reach the sporulation stage. In Class 3 as well as with some seedlings in Class 4, the sporulation was sometimes limited to the lower leaves. Complications arose when some of these plants had lost their lower leaves and did not show symptoms on the higher ones. Such plants were traced and classified if possible. Otherwise they were cancelled and the assessment was made on another treated seedling tray.

Absence, presence, frequency, type and size of the necrotic lesions which had been observed in some seedlings, especially those under Class 2, are probably due to a complex of factors. Occurrence of these lesions seems to be affected by plant genotype, environmental conditions, the fungus race and the suspension concentration. Plant populations with *S. tuberosum* ancestry showed the lesions more frequently than pure *S. verrucosum*.

A seedling was considered to be resistant if it showed complete lack of sporulation (Classes 1 and 2). Those seedling populations in which Classes 4 and 5 predominate and in which Classes 1 and 2 do not occur were considered susceptible.

Although plants reacting somewhat like those of Class 3 were reported by Toxopeus (1958a) to possess genes for resistance, the present author hesitates to classify them as resistant, because some of such plants died after 1–3 weeks, and because Toxopeus was handling populations other than *S. verrucosum*. Hence, it is questionable whether the level of 'resistance' in seedlings belonging to Class 3 is sufficiently high for parents

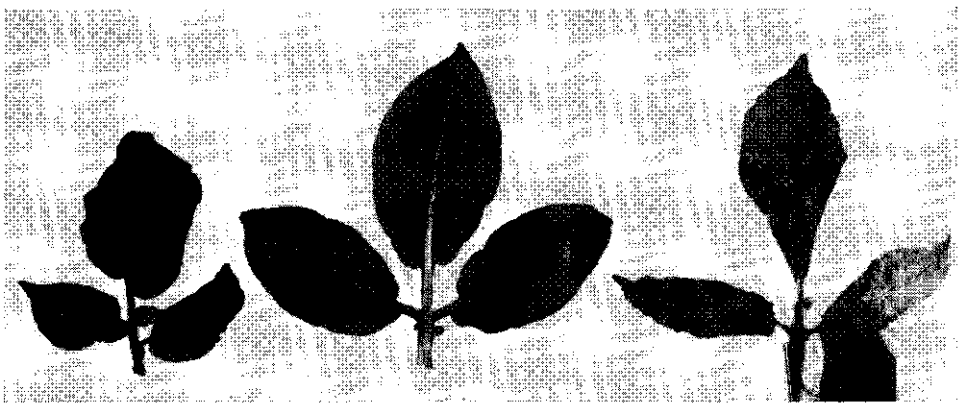


Plate 23. Reaction of detached leaves from full-grown plants of variety Libertas (left) and *S. verrucosum* PI 160228 parthenogenetic (middle) and CPC 2623a (right) to Race 1.3.4.7.8.

intended for use in crosses made to obtain starting material for a resistance breeding programme.

Several seedlings which showed resistance were transplanted to pots in the glasshouse. Except for less than 1 %, all grew normally and remained healthy. With the exception of one population, they were not rechecked for resistance in the adult stage. The exception was the parthenogenetic plants of verrucosum PI 160228 (see 6.2) which were resistant in the seedling stage to Race 1.2.3.4 and were checked at the flowering stage against Race 1.3.4.7.8 using detached leaves. The plants showed resistance whereas the controls from variety Libertas and *ver* CPC 2623a showed rich sporulation (Plate 23).

It may be assumed that the resistance in the seedling stage persists in advanced growth stages, as it is known that young potato plants are more susceptible to late blight than adult plants (Butler & Jones, 1949; Umaerus, 1963).

8.2 **The 1968 investigations**

In Table 38, data are presented on the reaction to four *Phytophthora* races by seven *ver* introductions, three F_3 populations of Hybrid 1 (*ver* PI 195172–237–6 × US–W 42; see 6.6) and two selfed progenies of the backcross of Hybrid 1 to the *ver* parent.

Some generalizations from the investigations made on *Phytophthora* can be reported here.

The *S. verrucosum* introductions varied from almost completely resistant to completely susceptible (Plate 24). *S. verrucosum* PI 275258, followed by PI 195172, showed the highest resistance to the four races, whereas CPC 2247 and EBS 2632 showed almost complete susceptibility to them.

S. verrucosum CPC 2514 and Haw 756 gave only a few segregants resistant to the four races. The progeny of CPC 2644, although expressing complete susceptibility to

Table 38. Reaction of seedlings one week after inoculation with *Phytophthora infestans* (1968).

	Race 4		Race 1.4		Race 1.2.3.4		Race 1.3.4.7.8	
	resistant[1]	susceptible	resistant[1]	susceptible	resistant[1]	susceptible	resistant[1]	susceptible
Solanum verrucosum PI 195172 I_1	12	52	24	40	18	46	12	52
PI 275258 I_1	19	45	40	24	53	11	43	21
Haw 756 I_1	6	58	6	58	5	59	5	58
CPC 2514 I_2	3	61	1	63	4	60	1	63
CPC 2644 I_2	0	63	27	37	29	35	22	42
CPC 2247 I_2	0	63	2	62	0	64	0	64
EBS 2632 I_1	2	62	1	63	0	64	0	64
F_3 populations of Hybrid 1[2]: M 18 pl. 6	9	55	8	56	10	54	8	56
pl. 11	27	37	19	45	37	27	14	50
pl. 16	0	64	0	64	0	64	0	64
Selfed backcross of Hybrid 1 to *S. verrucosum* parent								
pl. 23	0	64	0	64	2	62	0	64
pl. 93	28	36	8	56	23	41	37	27

1. No sporulation observed.
2. See 6.6.

Plate 24. Seedling populations of *S. verrucosum* CPC 1339 (above) and PI 275258 (below) inoculated with Race 1.2.3.4 and checked one week after inoculation.

Race 4, showed better results than the PI 195172 in its resistance reaction to the three Races 1.4, 1.2.3.4 and 1.3.4.7.8. The seedling population of the same origin as CPC 2644, raised from seeds received from Hawkes (Haw 1542), when sprayed with the four races showed the same reaction as CPC 2644 obtained from Marks, i.e. susceptibility to Race 4 and segregation into resistant and susceptible when tested against the other three races.

The F_3 and the selfed backcross populations differed in their reaction. Some were almost completely susceptible to all races (M 18 pl. 16, and BC pl. 23), whereas M 18 pl. 6 showed some resistant segregants with numbers comparable to all races. Populations M 18 pl. 11 and BC pl. 93 gave a relatively high proportion of seedlings resistant to the four races used.

Tests made on seedlings of the hybrids between *ver* and the following species, *phu* (PI 225682–22), *gig* (Haw 42), *ktz* (Haw 4003), *vrn* (PI 230468), *mga* (PI 210034) and *ifd* (PI 283077) not included in Table 38, all showed susceptibility to *Phytophthora*. Assuming the female parent *ver* was susceptible, some resistant F_1 individuals would have been saved if the males were resistant, unless the resistance is incompletely dominant (Dionne et al., 1960) or is recessive. Some resistant segregants were found in the F_1 population of *ver* PI 275258 × US–W 42.

8.2.1 Presence of polygenes

The observed consistency in the reaction to the four races leads to the speculation that resistance in CPC 2514, Haw 756 and M 18 pl. 6 is controlled by 'race non-specific' genes because no clear differential reaction to races occurs. Such behaviour is known as 'field resistance', 'horizontal resistance' or 'uniform resistance'[21] (van der Plank, 1968, 1969).

Such polygenes must have come from *S. verrucosum*, as the haploid US–W 42 proved to be susceptible to *Phytophthora* (from backcross of Hybrid 1, F_1 × US–W42, 107 seedlings tested against Races 4 and 1.2.3.4 proved to be completely susceptible).

The presence of such polygenes is supported by:

(1) the variability of the different seedlings in their reaction to the suspension of one race,

(2) the different degrees of susceptibility existing between the susceptible populations,

For instance in 1968, EBS 2632, CPC 2247 and CPC 1339 showed all their seedlings to be susceptible to Race 1.2.3.4, but quantitative differences between the three *ver* populations were observed with respect to the presence of non-sporulating host organs, the spread of the fungus and the degree of sporulation.

21. It has also been termed 'general resistance', 'polygenic resistance', 'minor gene resistance'... etc. (see van der Plank, 1963; Caldwell, 1968). It is known that such 'race non-specific' resistance is conditioned by polygenes; though oligogenic inheritance is not ruled out, it has never been reported in potato as far as the present author is aware. In this text when 'polygenic resistance', 'field resistance', 'horizontal resistance', 'uniform resistance' and 'race non-specific resistance' are used, all refer to the same type of resistance.

On the basis of these differences EBS 2632 was considered the least susceptible, followed by CPC 2247, whereas CPC 1339 showed the most severe symptoms. The 1969 investigations, in which the same Race 1.2.3.4 was used on different *ver* populations, gave results comparable to those in 1968. The susceptible populations could again be classified into susceptible (PI 255544), more susceptible (PI 195171 and CPC 1339) and most susceptible (CPC 2623a). The differences between CPC 1339 and CPC 2623a is in the number of days (1–2) after which a certain degree of infection is reached.

(3) the reaction of the *ver* plants grown in the field in 1968 compared with the reaction of different tuberosum varieties grown adjacently.

The *Phytophthora* attack was epidemic in 1968, even on varieties known to have good 'field resistance'. Most of these almost completely succumbed whereas the majority of the *ver* plants grew normally. The *ver* plants were given grades 9 and 10, whereas the most resistant varieties were given grade 5 (the higher the grade, the better the resistance). Those varieties classified as 5 were known to have grade 8 and 9 in normal seasons. This means that *ver* has not only a 'field resistance' but also a better resistance than that known in the cultivated varieties. It is also believed that the system conditioning this 'horizontal' resistance in *ver* is different from and more efficient than that present in the commercial varieties (see 8.3 and 9.6).

8.2.2 Presence of major genes

The test[22] applied to the data in Table 38 revealed significant differences in reaction to the four races, not only between seedling populations but also within seedling populations. Race 4 attacked more seedlings[23] and in addition in the populations showing resistant segregants, the class of infection was higher than with the other races.

The genetic differences within seedling populations determining their differential reaction to different fungus races suggested the presence of major genes[24] (see van der Plank, 1968). The differences in reaction to races, and the segregation into resistant and susceptible seedlings in *ver* populations pointed to the presence of segregating major genes which are probably of a higher order than those that could be attacked by Race 1.3.4.7.8 (see also 8.4, 5, 6).

8.3 **The 1969 investigations**

In 1969 the tests with *Phytophthora* were continued using Race 1.2.3.4, chosen because of its lower aggressiveness and its relatively high virulence.

22. Contingency tables were used to test the significance of the differences.
23. Note also the unexpected behaviour of CPC 2644, in which all the seedlings sprayed with Race 4 showed infection but spraying with the other three races resulted in segregation into resistant and susceptible seedlings.
24. Resistance based on genes with major effects has been given different names like: 'specific', 'vertical', 'hypersensitive', 'major-gene', 'racial', 'race-specific', 'differential'... etc. (see van der Plank, 1963, 1968, 1969; Caldwell, 1968). Any of these names will be used in this text. The genes controlling this type of resistance in *S. demissum* have been referred to as *R* genes. This type of resis tance is usually inherited monogenically or oligogenically.

Seeds obtained in 1968 by selfing some tetraploid varieties and selections were germinated. The seedlings were subjected to *Phytophthora* sprinkling to compare their reaction with that of *S. verrucosum*.

The tetraploid selections were Black 5030 (34), Black 3751 (5) and Black 6036e (13). Although their genotypes were unknown, these selections came from complex hybrids of *S. demissum-S. phureja* crossed with *S. tuberosum*. These selections probably possessed both *R* genes and polygenes.

The six varieties used were Humalda, Dr. MacIntosh, Oberarnbacher Frühe, Radosa, Libertas and Gineke with degrees of field resistance in leaves of 4, 5, 6, 7, 8 and 8 respectively (according to the Dutch official List of Varieties, the higher the number, the better the resistance). The Dutch List of Varieties reported Radosa to be R_3, the other five varieties being genetically *r*.

Two *ver* populations, PI 195172–237–9 (which had been selfed twice) and CPC 1339 were used as controls in the investigations of 1969. The experience gained from their reaction in 1968 pointed to the 'good resistance' in the first population and the 'high susceptibility' in CPC 1339.

In their reaction to infection the tetraploids showed a variability resembling that mentioned earlier for *ver*. The Black numbers like the resistant *ver* populations showed good resistance, with most of the seedlings grouped in Class 1. The tuberosum varieties showed a wider variation in their reaction; most of their seedlings belonged in Classes 3, 4 and 5.

The reaction of some of the tetraploids and *ver* controls, when sprinkled with Race 1.2.3.4, is given for one of the experiments in Table 39. The distinction between Classes 3 and 4 was not possible in seedlings of Libertas due to their variation in both classes.

The *ver* populations (both the resistant and the susceptible) gave clear class reaction, contrasting with the tetraploids. But it must be admitted that the segregating populations of *ver* also showed variation in their reaction to infection.

In comparing susceptible seedlings of *ver* CPC 1339 and CPC 2623a with Libertas

Table 39. Classification of some selfed seedling populations of *Solanum verrucosum* and some tetraploid *S. tuberosum* based on reaction pattern to *Phytophthora infestans* Race 1.2.3.4 (1969).

		Number of seedlings per class					Total
		1	2	3	4	5	
S. verrucosum	PI 195172–237–9	110	11	0	0	0	121
	CPC 1339	0	0	0	58	0	58
	CPC 2623a	0	0	0	0	63	63
S. tuberosum tetraploid	Black 5030(34)	65	10	20	1	0	96
	Libertas	12	9	59		14	94
	Gineke	0	9	14	53	8	84
	Radosa	1	1	8	75	11	96

For explanation of the five classes: see text.

and Dr. Mac Intosh, clear differences between *ver* and the varieties were observed in the frequency of sporangiophores and the presence of sporangia. The tuberosum varieties showed a higher frequency of sporangiophores, which also possessed more branches than those on *ver*. Furthermore these branches carried more conidia than those on *ver*, even when the leaflets had a similar number of hyphae. Such behaviour might point to differences in the factors conditioning the host-pathogen relationship in tuberosum varieties and verrucosum.

As appears from Table 39, a quantitative difference exists between the reaction of *ver* CPC 1339 and CPC 2623a, and a qualitative difference exists between these two and *ver* PI 195172–237–9.

The reaction to Race 1.2.3.4 in the seedling populations investigated in 1969 from *ver* and the tetraploids is presented in Table 40.

The reactions of the resistant and the susceptible controls agreed well with their behaviour in 1968. The susceptible control *ver* CPC 1339, of which all tested seedlings were susceptible in 1968, gave only one resistant seedling out of 363 plants checked in 1969. The resistant control *ver* PI 195172–237–9 gave 89% and 94% resistant seedlings

Table 40. Resistance and susceptibility in some selfed populations of *Solanum verrucosum* and some tetraploid varieties of *S. tuberosum* to *Phytophthora* Race 1.2.3.4 (1969).

		Seedling reaction		Resistant seedlings (%)
		resistant	susceptible	
S. verrucosum	PI 195172–237–9	284	18	94
	PI 161173	59	1	98
	PI 275255	47	5	90
	PI 275256	127	1	99
	PI 275259	56	4	93
	Haw 1532	100	19	84
	Haw 2246	52	52	50
	PI 275260	5	58	8
	PI 310966	9	34	21
	PI 195171	0	120	0
	PI 255544	0	64	0
	CPC 2623a	0	123	0
	CPC 1339	1	362	0
S. tuberosum	Black 5030(34)	75	21	78
	Black 3751(5)	82	13	86
	Black 6036e (13)	80	16	83
	Libertas	24	102	19
	Gineke	9	75	11
	Radosa	2	94	2
	Humalda	0	84	0
	Dr. Mac Intosh	3	125	2
	Oberarnbacher Frühe	7	142	5

Resistant = no sporulation.

in 1968 and 1969, respectively (statistically insignificant deviation).

It is clear from Table 40 that some *ver* introductions showed better resistance than others. For instance, *ver* PI 161173, PI 275255, PI 275256, PI 275259 and Haw 1532 segregated high numbers of resistant seedlings, as did PI 275258 (tested in 1968). Haw 2246 showed about the same behaviour as CPC 2644, about half of its segregants being resistant to Race 1.2.3.4. *S. verrucosum* PI 275260 and PI 310966 gave lower numbers of resistant seedlings while PI 195171, PI 255544 and CPC 2623a showed no resistant seedlings at all.

The comparison of the tetraploids revealed that the Black selections showed good resistance, comparable to that of *ver*, however, some verrucosums such as PI 161173 and PI 275256 expressed better reaction than the Black selections.

The tuberosum varieties proved to be inferior to the Black populations. They behaved like the susceptible *ver* introductions or the introductions that gave a low number of resistant segregants (PI 275260, PI 310966..), although the fungus on the varieties sporulated more profusely than on *ver*, as mentioned before.

The tuberosum varieties showed slight differences among themselves: Libertas and Gineke gave higher numbers of resistant seedlings than Radosa, Dr. Mac Intosh and Oberarnbacher Frühe, whereas Humalda showed no resistant seedling at all. It is interesting to note that these results correspond with the degree of 'field resistance' in the varieties. Radosa showed a low number of resistant seedlings, probably because of the presence of R_3 from *S. demissum* in this variety (seedlings possessing R_3 in their genotypes are expected to be susceptible to Race 1.2.3.4).

8.4 Support for the presence of major genes in S. verrucosum

In 8.2.1 some remarks were made on the presence of 'field resistance' (caused by polygenes) in *S. verrucosum* and the possibility of the presence of major genes was discussed under 8.2.2.

The data in Tables 38 and 40 show the presence of different degrees of resistance to different races in verrucosums and the segregation of several *ver* introductions into resistant and susceptible seedlings. This would indeed suggest the presence of different major genes in heterozygous condition segregating in inbred populations.

Black's selections, too, probably have *R* genes from *S. demissum* and they showed a behaviour comparable to that of some *ver* introductions. This points to the possible presence of major genes in *ver* of a type similar to those present in *S. demissum*.

Some experiments were carried out in 1969 to throw more light on the question of whether major genes are indeed present in *S. verrucosum*.

Two isolates of *Phytophthora*, B 19 and 331 ,were used on some seedling populations to test their resistance and to compare the reaction with that observed when Race 1.2.3.4 was used. The reaction of some *ver* and Black introductions to Isolate B 19 is presented in Table 41, and the data of the test against Isolate 331 are reported in Table 42.

Plants from some of the resistant seedlings from the 1968 investigations (Race

Table 41. Results of testing selfed seedling populations of some *Solanum verrucosum* and tetraploid *S. tuberosum* selections for resistance to Isolate B 19 of *Phytophthora* (1969).

		Seedling reaction		Resistant seedlings (%)
		resistant	susceptible	
S. verrucosum	PI 195172–237–9	46	23	67
	PI 195172–237–9 pl. 3[1]	50	14	78
	CPC 2644[1]	60	4	94
S. tuberosum	Black 5030 (34)	23	9	72
	Black 3751 (5)	28	1	97
	Black 6036e (13)	15	9	62

1. The parent was resistant to Race 1.2.3.4.

Table 42. Results of testing selfed seedling populations of two *Solanum verrucosum* plants and one F_4 from Hybrid 1 and two tetraploid selections of *S. tuberosum* for resistance to Isolate 331 of *Phytophthora* (1969).

		Seedling reaction		Resistant seedlings (%)
		resistant	susceptible	
S. verrucosum	PI 195172–237–9 pl. 3[1]	21	30	41
	PI 195172 I_1[1]	24	36	40
	F_4 M18 pl. 6[1]	4	60	6
S. tuberosum	Black 5030 (34)	19	13	59
	Black 3751 (5)	27	2	93

1. The parent was resistant to Race 1.2.3.4.

Table 43. Results of testing selfed seedling populations of *Solanum verrucosum* and related populations from Hybrid 1 for resistance to *Phytophthora* Race 1.2.3.4 (1969).

		Observed reaction		Resistant seedlings (%)	Expected ratios res: sus	P from χ^2-test
		resistant	susceptible			
S. verrucosum	PI 195172–237–9 pl. 14	62	0	100	1:0	
	CPC 2644	62	0	100	1:0	
	F_4 M18 pl. 6	122	1	99	1:0	
	Backcross pl. 93	24	0	100	1:0	
	CPC 2514	40	15	73	3:1	0.70–0.50
	F_4 M18 pl. 11	76	19	80	3:1	0.30–0.20
	Backcross pl. 23	46	10	82	3:1	0.30–0.20
	PI 195172 I_1	121	5	96	15:1	0.30–0.20
	PI 195172–237–9 pl. 3	93	6	94	15:1	0.95–0.90

In 1968 all parents had shown resistance to Race 1.2.3.4 in the seedling stage.

1.2.3.4) which had been maintained in the glasshouse, were selfed and their inbred progenies were tested in 1969 against the same race (Table 43) and some against the two isolates.

As apparent from Table 41, the seedling populations showed good resistance to Isolate B 19, which attacked variety Multa. Plants from verrucosum and Black selections were found to show resistance also to the other Isolate, 331.

A comparison between Tables 41 and 40 for the reaction of PI 195172–237–9, showed that this inbred was well resistant to Race 1.2.3.4 (94% resistant seedlings) but more susceptible seedlings appeared in the test with Isolate B 19 (67% resistant seedlings). The inbred progeny of the resistant plant 195172–237–9 pl. 3 showed 78% resistant seedlings to Isolate B 19 (Table 41), 41% to Isolate 331 (Table 42) and 94% to Race 1.2.3.4 (Table 43), whereas the resistant CPC 2644 gave no susceptible seedlings when tested by Race 1.2.3.4 and it segregated when checked for Isolate B 19. The comparison between the inbred progeny of the resistant plant of PI 195172–I_1 (Tables 42 and 43) showed the percentage of the resistant seedlings to be 96% when tested against Race 1.2.3.4 and 40% when tested by Isolate 331. The population of F_4M 18 pl. 6 behaved very distinctivly when sprayed by Race 1.2.3.4 (99% resistant) and by Isolate 331 (6%).

The Black selections also showed some differences in their reaction to Race 1.2.3.4 and to the Isolates B 19 and 331.

The data on the reactions of *ver* to Race 1.2.3.4 and the two Isolates B 19 and 331 reveal the presence of statistically significant differences in reaction upon inoculation with the three strains of the fungus. Such differences indicate the presence of major genes in *S. verrucosum*, as already concluded from the data in Table 38 (see 8.2.2).

Whether the major genes in *S. verrucosum* are identical with the *R* genes in *S. demissum* cannot be stated with certainty. The difficulty of differentiating between 'hypersensitive' reaction and one due to a high level of polygenes, and the inconsistency in the appearance of what may be described as a 'hypersensitive' reaction do not allow definite conclusions about the identity of the *S. verrucosum* major genes and the *R* genes from *S. demissum*. Nevertheless the data point to the presence of 'race-specific' resistance in *S. verrucosum*.

Until more definite conclusions can be drawn about the identity of *S. verrucosum* major genes with *R* genes from *S. demissum* (similarity is expected, see 8.5, 9.6), the former will be referred to as verrucosum major genes (*Vm*) (*Rv* may also be used).

8.5 Inheritance of resistance to Phytophthora in S. verrucosum

Because of the heterozygosity of *S. verrucosum* populations, the presence of both genes with major effects (*Vm*) as well as polygenes, the complications in separating the two types of resistance, and the fact that most of the data were collected from progenies of several selfed plants in each *ver* introduction, no clear idea about the inheritance of resistance to *Phytophthora* races could be obtained. For this reason and to check the efficiency of testing in the seedling stage, progenies obtained from selfing the plants raised from the resistant 1968 seedlings were tested in 1969 (Table 43).

These seedlings generally showed good resistance to Race 1.2.3.4 (the same race

which the parental seedlings had been tested against). Most of them belonged to Class 1 (no lesions), but even those classified as susceptible showed poor sporulation. Such seedlings when left for another week, showed the spread of the sporulation into larger areas (about 1/3 to 1/2 the surface of the leaflets). Also the fungus spread only over those leaflets which had shown the first infection, while all other plant parts remained free of it. Such behaviour was completely different from that observed in the normal susceptible seedlings of most *ver* populations particularly the susceptible ones, where the fungus usually covered the seedlings and death resulted within 2 to 3 days after the first check.

Obviously such seedlings had some resistance which reduced the degree of fungus spread in plant tissues. This may be one of the components of 'field resistance' (see van der Zaag, 1959).

As apparent from Table 43, some of the seedlings' progenies had resistant individuals only (four) whereas others showed different proportions of resistant and susceptible seedlings (five). From the 123 seedlings checked in F_4 M 18 pl. 6, the single susceptible seedling observed can be ignored as originating from the segregation of some modifiers. The observed segregation of the five populations mentioned in Table 43 may give some idea about the nature of inheritance of resistance to Race 1.2.3.4 in those populations.

We shall follow here the design adopted by Black et al. (1953) and tentatively assume that the *Vm* genes of *S. verrucosum* and the physiologic races interact in a manner similar to that known with the *R* genes of *S. demissum* (see 9.6). In that case the non-segregating four inbred populations of Table 43 (195172–237–9 pl. 14, CPC 2644, F_4 M 18 pl. 6 and BC pl. 93), must have originated from parents either homozygous for at least one *Vm* gene higher in order than Vm_1, Vm_2, Vm_3, Vm_4 (Vm_5 Vm_5, for instance) or heterozygous for several *Vm* genes (other than Vm_1 to Vm_4), so that the populations have been too small to catch the rare susceptible recessive segregants.

Inbred populations of CPC 2514, F_4 M 18 pl. 11 and BC pl. 23 segregating in a ratio 3 resistant: 1 susceptible, can be assumed to have parents with one dominant heterozygous resistance gene, Vm_x ($x > 4$).

Inbred progenies of PI 195172 I_1 and 195172–237–9 pl. 3, showing the ratio 15 resistant : 1 susceptible, are expected to have parents with a genotype Vm_xvm_x–Vm_yvm_y (both x and $y > 4$ and $x \neq y$). Only $vm_xvm_x\,vm_yvm_y$ (one out of sixteen) is susceptible to Race 1.2.3.4.

The segregations in Table 43 suggest that the resistance in *S. verrucosum* to Race 1.2.3.4 of *Phytophthora* is a dominant character inherited monogenically. However, the check of the parental populations of the progenies presented in Table 43 and the test of hybrids between *ver* and susceptible haploids demonstrate that such dominance may not be complete, at least not in all cases. Complete dominance of the resistance was observed in the hybrid *S. verrucosum* PI 160228 (parthenogenetic) × AH 66–94–28 when detached leaves were tested against Race 1.3.4.7.8. All leaves of the parent *S. verrucosum* and the ten F_1 plants checked proved to be resistant, whereas the leaves

of the male parent showed susceptibility. A possible effect of *S. verrucosum* plasmon on resistance can be considered, as an androgenetic plant from this hybrid has been found resistant.

Although the resistance to Race 1.2.3.4 in *S. verrucosum* has been assumed to be simply inherited and the relation between the *Vm* genes and races may be comparable to that between *R* genes of *S. demissum* and the fungus, many complications still remain to be elucidated, such as the unexpected behaviour of Race 4 on CPC 2644 (Table 38), and the segregation of F_3 M 18 pl. 6 and its F_4 progenies when tested against Race 1.2.3.4. The F_3 population showed few resistant seedlings and no differences in reaction to the four races used in 1968 (Table 38), but the F_4 showed complete resistance to Race 1.2.3.4 (Table 43) and almost complete susceptibility to Isolate 331 (Table 42). CPC 2514 also gave controversial results. The I_2 seedlings were nearly all susceptible to the four races used in 1968 (Table 38), but nevertheless, one of its resistant plants when selfed gave segregation (Table 43) pointing to the presence of a gene with major effect (see 9.6 for a possible explanation of such unexpected deviations).

The observation that selection in the seedling stage gives resistant inbred progeny points to the efficiency of selecting in young seedlings. Moreover, it is a simple procedure saving time, effort, space and equipment, and enables large numbers of populations to be handled.

8.6 The nature of Isolates B 19 and 331

Isolate 331 is the 'most pathogenic', Isolate B 19 is intermediate, whereas Race 1.2.3.4 is the 'least pathogenic' of the three strains of the fungus (see 8.4.). Some conclusions on the isolates can be drawn from the comparison between the segregation results in the inbred progeny of the plant PI 195172–237–9 pl. 3 when tested against Race 1.2.3.4 and the two isolates (Tables 41, 42, 43).

The inbred population segregated 15:1 (93:6) when tested with Race 1.2.3.4 but it showed segregation of 3:1 (50:14) when suspension of Isolate B 19 was used. This means that Isolate B 19 was able to attack genotypes with one higher *Vm* gene than Vm_1 to Vm_4 (*Vm* genes are assumed to be analogous in their function to *R* genes).

As Isolate B 19 can attack the differential host with the genes R_1, R_4[25] (thus also Race 1.2.3.4) it may be assumed that plant 195172–237–9–3 has the genotype Vm_1Vm_1-$Vm_4Vm_4Vm_xvm_xVm_yvm_y$. Such a plant will be resistant to Race 1.2.3.4 but its inbred progeny will segregate into a ratio of 15:1 as observed in Table 43.

The inbred progeny of the same plant when tested against Isolate B 19 (Table 41) segregated into a ratio of 3:1. Consequently Isolate B 19 can be assumed to be Race 1.4. x.

Inbred of the resistant CPC 2644 showed no segregation when tested with Race 1.2.3.4 and segregated into the ratio of 15:1 (60:4) when inoculated with Isolate B 19.

25. The ability of the Isolates B 19 and 331 to be pathogenic on certain differential hosts was known to the present author in 1970 from Dr. J. C. Mooi who believes Isolate B 19 to be Race 1.4.10.

So the genotype[26] of the parent of this population can be assumed to be Vm_1Vm_1-$Vm_4\,Vm_4\,Vm_x\,Vm_x\,Vm_y\,vm_y\,Vm_z\,v_zm$ (x, y and $x > 4$; $z \neq y \neq z$).

Isolate 331 is certainly a more virulent strain than Isolate B 19 and Race 1.2.3.4. This is certainly true when the results presented in Tables 41, 42 and 43 are compared and the ability of this isolate to attack the differential hosts with genes R_1,R_3,R_4,R_7, R_8 is considered. More investigations are needed to clarify the genotype of this isolate.

8.7 **Inbreeding and selection in S. verrucosum and their effect on resistance to Phytophthora**

As previously reported, it seems that major genes and polygenes are present in *S. verrucosum*, determining its resistance to *Phytophthora* races. Accordingly inbreeding in *ver* is expected to lead to reactions which may differ in the inbred progenies and the parental populations.

Several cases have already been mentioned in Tables 38 – 43, showing the reactions of the inbred *ver* populations to different races and isolates of *Phytophthora*. From these reactions it can be concluded that *S. verrucosum* is heterozygous for many genes controlling resistance and that inbreeding will lead to segregants showing different degrees of resistance.

The results of selfing the plants selected for resistance is shown in Table 43. Such resistant plants produced resistant inbred progenies, in which either only a few or no susceptible segregants at all were found.

Therefore it may be concluded that plants may either breed true or may segregate in accordance with their homozygosity or heterozygosity.

S. verrucosum CPC 1339 was found in the 1968 investigations to be susceptible to Race 1.2.3.4. One of the plants of this introduction was selfed and its inbred progeny was tested in 1969 against Race 1.2.3.4. All 164 inbred seedlings checked showed complete susceptibility, which means that inbreeding a completely susceptible plant leads to completely susceptible progeny.

Table 44 supplies data on the effect of inbreeding and selection in *S. verrucosum* on *Phytophthora* resistance; some data refer to different inbred generations. As shown in that Table , I_4 of PI 195172 showed good resistance to all four races compared with the original non-selected introduction (I_1). This means that selection has been effective.

Plant 9 of the selfed parent PI 195172–237–9 was selfed for two generations and a mixture of the selfed seeds (I_5) was germinated and tested against the four races for a comparison with I_1 and I_4. It was found that resistance had decreased by more selfing.

As reported in Table 44, the I_1 population of PI 195172 contained several susceptible seedlings whereas I_4 progeny from the selected resistant I_3 was only slightly

26. It must be kept in mind that in all these assumed genotypes the presence of dominant Vm_1, or Vm_4 gene is not necessary and the same results could have been obtained if these genes had been present in recessive condition.

Table 44. Resistance test results of some *Solanum verrucosum* populations in the seedling stage to four races of *Phytophthora* (PI 160228 populations tested in 1969, the others in 1968).

	Race 4			Race 1.4			Race 1.2.3.4			Race 1.3.4.7.8		
	res	sus	res (%)	res	sus	res (%)	res	sus	res (%)	res	sus	res (%
PI 195172 I_1	12	52	18.8	24	40	37.5	18	46	28.1	12	52	18.8
PI 195172–237–9 I_4	49	15	76.6	61	3	95.3	57	7	89.1	33	31	51.6
PI 195172–237–9–9 I_5	27	37	42.2	15	49	23.4	25	39	39.1	20	44	31.3
CPC 2514 I_2	3	61	4.7	1	63	1.6	4	60	6.1	1	63	1.6
CPC 2514 I_3	2	62	3.1	0	64	0	3	61	4.7	1	63	1.6
PI 160228 I_1 selfed							65	169	27.8			
PI 160228 I_1 parthenogenetic							110	0	100.0			

res: resistant; sus: susceptible.

Note: K. M. Graham selected plant PI 195172–237 as resistant to Race 1.2.3.4. Plant 9 was chosen from an inbred population sent to the late H. J. Toxopeus. Seed from the original PI number was sent to the present author from Sturgeon Bay, Wisconsin.

Plate 25. Parthenogenetic (left) and selfed (right) I_1 seedlings from *S. verrucosum* PI 160228 inoculated with Race 1.2.3.4 and checked one week after inoculation.

affected by the fungus. The non-selected selfed progeny of I_4 (= I_5) showed a marked decrease of resistance. Hence it is probable that the parental plants were not homozygous for the genes controlling resistance. It was also observed that susceptible seedlings from I_5 and I_1 had larger sporulating areas than the susceptible I_4 seedlings.

A comparison of I_2 and I_3 populations of *S. verrucosum* CPC 2514 revealed hardly any difference in the frequency of the resistant seedlings but the susceptible seedlings in I_3 were more heavily attacked and had larger sporulating areas than the susceptible I_2 individuals.

The comparison between the selfed I_1 and the parthenogenetic (2n = 24 chromosomes) I_1 populations of *S. verrucosum* PI 160228 revealed some quite interesting facts. Where the selfed population showed about 28% of its progeny to be resistant to Race 1.2.3.4, all parthenogenetic seedlings were resistant (Table 44 and Plate 25). Only three of the parthenogenetic seedlings showed a few brown flecks on one of their leaflets; the rest of the seedlings, 107, remained completely free of symptoms. Detached leaves from full-grown parthenogenetic plants tested against Race 1.3.4.7.8 also showed complete resistance but brownish lesions scattered on the leaves were observed (Plate 23).

The absence of segregation in the parthenogenetic I_1 population proved its complete homozygosity which was also manifested in its morphological uniformity. Such complete homozygosity is considered to be the highest expected degree of inbreeding.

This means that inbreeding in resistant populations accompanied by selection may lead to an invariably resistant inbred progeny.

9 Discussion

9.1 Variability, inbreeding and heterosis in S. verrucosum

9.1.1 Inbreeding in S. verrucosum introductions

According to Graham (1963), the self-compatible *S. verrucosum* is resistant to inbreeding. The results from the present investigations point to the opposite and demonstrate that many characters of *ver* can be affected by inbreeding.

It is apparent, however, that inbreeding effects are less harmful in *ver* than in commercial potato varieties, particularly on fertility (see Salaman, 1910; Salaman & Lesley, 1922; Krantz, 1924, 1946; Krantz & Hutchins, 1929; Guern, 1940 and Deshmukh & Verma, 1960). Although selfed populations of *ver* showed a wide range of variability for almost all characters studied, significant differences between successive inbred generations (I_1, I_2 etc.) have been demonstrated in several cases (Chapter 4).

Selfing of *ver* introductions may lead to different sub-populations. This offers possibilities for selection. An extreme example is the population obtained from one parthenogenetic seed (6.2).

Lamm (1945) found ten selfed progenies of his *ver* no. 4119 to vary in pollen stainability from 20–90%, although 90% of the parent pollen grains were well stained. Similar observations are reported in this paper. In addition, some new characters became manifested in the inbred populations: blunt spine appearance of pollen walls (5.1.2), bubble sterile pollen grains (5.1.2) and the undivided microsporocyte[27] type of male sterility[28] (5.1.3).

Because of their breeding system, the self-fertilizers might be expected to lack genetic variability especially within families. However, variability in self-fertilizers has been reported by several authors: Johannsen's classic investigations using the Princess variety of garden bean, (see Johannsen, 1926); the variability in populations

27. The undivided microsporocyte sterility differs from a similar phenomenon reported by Grun & Aubertin (1966b) in *Solanum* and by Gottschalk & Jahn (1964) in pea. These authors reported the presence of several micro nuclei in the PMC's. Such behaviour was exceptional ($< 5\%$ of the PMC's studied). In this material 4 nuclei were generally found in each PMC.

28. Some structures resembling embryos and embryo-sacs have been observed in the anthers with the undivided microsporocyte type of male sterility. This 'change in sex' has been reported by several authors (Koopmans, 1951 etc. in *Solanum*; Ghatnekar, 1965 in corn; Sand, 1968 in tobacco; see also Jain, 1969). It may be possible that such 'embryos' can be used in 'embryoid' production in a way similar to that reported by Nitsch & Nitsch (1969) and Sunderland & Wicks (1969) in *Nicotiana*.

of wheat, barley and oats (Roberts, 1929; Harlan, 1957); Knowles' (1943) studies on soft chess; the investigations of Allard & Golden (1954) on the red kidney bean and lima bean; Abdalla's (1964) studies on the self-fertilized field bean grown in Egypt and that of Imam & Allard (1965) on wild oats. The present and the above mentioned investigations proved that the self-fertilizers, irrespective of their breeding system, still maintain genetic variation. Allard et al. (1968) discussed this point and reported the following on inbreeding populations: 'individuals within a population are often heterozygous for many genes governing quantitative characters. The variability that is commonly observed within individual families is therefore not exclusively environmental or developmental but much of it can be ascribed to segregation'. The present investigations showed the self-compatible *S. verrucosum* to be heterozygous not only for genes controlling quantitative characters but also for genes controlling qualitative characters (blunt spine appearance of pollen wall and undivided microsporocyte type of male sterility).

9.1.2 Intraspecific hybrids in S. verrucosum

9.1.2.1 Heterosis in F_1

Hybrids between different verrucosum introductions show a better performance than their parents (see 4.1.2.3, 4.1.3, 4.2.2, 5.5, 5.8) and the wider the parents differ in a certain character, the greater is the heterosis for this character in the hybrids. Furthermore heterosis is more pronounced in the seedling stage and decreases with increasing age (see 5.5.1); this phenomenon has been reported by investigators on hybrid maize (Hayes et al., 1955).

Differences in combining ability have been observed, as some of the hybrids sharing the same male parent show varying performances.

The hybrid vigour observed in crosses between *S. verrucosum* introductions is less pronounced than that discovered upon crossing inbred lines of maize, but it is parallel to that observed in crossing self-fertilized crops as reported in pea (Keeble & Pellew, 1910; Gottschalk, 1970); in barley (Immer, 1941); in rice (Ramiah & Ramasamy, 1941; Jennings, 1966); in gram, sesame and chilli (Pal, 1945); in tomato (Larson & Currence, 1944); in soybean (Weiss et al., 1947); in flax (Carnahan, 1947); in egg plant (Pal & Singh, 1946; Odland & Noll, 1948); in *Nicotiana* (Smith, 1952); in wheat (Briggle, 1963); in bitter gourd (Pal & Singh, 1946); in pigeon pea (Solomon et al., 1957) and in mungbean (Bhatnagar & Singh, 1964).

Such investigations have proved that hybrid vigour can also be expected in self-fertilized populations, and that in the near future, hybrid varieties may have the same popularity as hybrid maize, assuming that the problems limiting their commercial application will be overcome.

9.1.2.2 Inbreeding effects in F_2

A comparison between F_1 and F_2 generations has revealed a clear inbreeding depression accompanying selfing F_1 (4.2.4, 5.5.1, 5.8). This depression varies for plant age

and for the different characters. The seedling stem height measurements one and two months after sowing reveal more depression than adult plant stem height, which may be related to the strong performance of the hybrid plants in the early stages of growth. The characters showing the higher percentages of heterosis in F_1 are in general those expressing the greater inbreeding depression in F_2. For instance, the hybrid at the left side of Table 13 showed heterosis percentages of 61.7%, 87.9%, and 42.4% in number of seeds per berry, number of tubers per plant and weight of tubers respectively in the studies of 1966. These three characters as is also presented in Table 13, showed the largest inbreeding depression in F_2. This behaviour also points to a possible relation between heterosis in F_1 and inbreeding depression in F_2.

9.1.3 Fertility and self-compatibility of S. verrucosum

9.1.3.1 Male and female fertility

Generally the verrucosum introductions show over 70% stainable pollen grains (Chapter 4, 5.1.1). Salaman (1910) has reported *ver* to have abundant pollen, with 40% of which is rounded when wet (adding water was used to check pollen quality). Swaminathan & Hougas (1954) mention a stainability of about 90%. According to Magoon et al. (1958a) it varies from 71.2 to 89.0% with an average of 77.4%, whereas Matsubayashi (1961) even records 98.3%. This all means that *S. verrucosum* is highly male fertile.

The only exception is the clone CPC 1349, which has been reported by Hawkes (1956a) to be male sterile. This clone is no longer available and the cause of its male sterility is unknown (Hawkes, 1968, pers. comm.).

Although all *ver* introductions studied show good female fertility, some set three or four times as many seeds per berry as others (Chapter 4,5.1.1).

No observable association has been found between male and female fertility in pure *ver*.

9.1.3.2 Self-compatibility

Contradictory reports have been published about the self-compatibility of *S. verrucosum*. Propach (1940), Lamm (1945), Hawkes (1956a, b, 1958b, 1963), Marks (1958, 1965a), Malheiros-Gardé (1959a), Buck (1960), Grun & Radlow (1961), Pandey (1962c), Graham (1963) and Dionne & Graham (1963) reported self-compatibility of *ver*. Swaminathan & Hougas (1954) have pointed to 'self-fertility' of many clones of this species.

Magoon et al. (1958a, b), Pushkarnath (1961) and Cipar et al. (1964b) reported self-incompatibility of *ver*. Magoon et al. and Cipar et al. found no differences in seed set after selfing *ver* and other species, e.g. *S. pinnatisectum*. Colchicine-doubled *ver* has been reported by Magoon et al. (1958b) to be largely self-compatible.

Swaminathan & Hougas have investigated *ver* PI 195172 and CPC 1339; Magoon et al. PI 195171 and PI 160228; Cipar et al. PI 160228, while Pushkarnath did not mention specific introduction numbers. All these introductions, together with some

others (see Chapter 4, 5.1.1, 5.2) have been investigated by the present author and all have indeed proved to be self-compatible, good seed set being obtained after artificial selfing without any difficulty.

If Magoon et al. (1958a, b), Pushkarnath (1961) and Cipar et al. (1964b) have used spontaneous berry set as a criterion for self-compatibility then the reason for their mentioning self-incompatibility of *ver* is obvious. It is the inability of certain *ver* introductions to set spontaneous berries in the absence of a pollinating agent (see 5.3).

9.1.3.3 The origin of self-compatibility in S. verrucosum

The inbreeding depression in *ver* and the heterosis observed after crossing within this species allow the speculation that *ver* introductions do not basically differ from self-fertilizers, at least in the populations investigated for the effects of inbreeding and crossing.

All diploid *Solanum* species (except five) are self-incompatible (Hawkes, 1956b). Assuming that self-incompatible species are the old ancestors (see 9.4.7) one may consider the self-compatibility of *S. verrucosum* as being a character evolved from self-incompatibility.

S. verrucosum is a species which is sensitive to inbreeding. It carries several harmful genes, has a flower structure favouring cross-pollination, needs a pollinating agent in most of its introductions to ensure berry and seed set and it must have become self-compatible in recent times. This is in accordance with the 'two power competition' hypothesis to be explained in 9.4.8.

S. verrucosum, though collected in Mexico, is believed to be non-indigenous to that country (Hawkes, 1958b). The morphological similarities and the success of crossing between this species and South American species, its failure to cross with Mexican species, the good chromosome pairing in hybrids between *ver* and different South American species and haploids (see Chapter 6) as well as the genome differentiation between South American and Mexican species (Hermsen & Ramanna, 1969; Ramanna & Abdalla, 1970) indicate that it is of South American origin. It is thus not surprising that Correll (1952) has placed *S. verrucosum* under series *Tuberosa* instead of under series *Demissa*.

The appearance of self-compatibility in *S. verrucosum* is believed to result from its migration and colonization of a new habitat in Mexico[29]. A breakdown of self-incompatibility had a selective advantage because of its leading to a reproductive mechanism able to build up large adapted populations from one or few seeds or tubers. In the earlier period of migration particularly, *ver* must have been scarce. In addition a 'thinning effect' must have taken place due to the attack by *Phytophthora*. Under such circumstances selfing will be more advantageous than outcrossing.

A similar hypothesis has been adopted by Hawkes (1958b) to explain the self-compatibility of *S. verrucosum*.

29. It is the author's intention to discuss the possible role – if any – of fragments in self-compatibility of *S. verrucosum* in another publication.

9.1.3.4 Artificial versus spontaneous selfing

The flower structure of *S. verrucosum* favours cross pollination. The pistils rise above the stamen tips (supra-staminal pistils). Berry set largely depends on flower manipulation.

Spontaneous berry set in the plants raised in the glasshouses is associated with the relative length of pistils and stamens: the populations with a short distance between both are those producing spontaneous berries (see 5.3).

Seed set in spontaneous berries in an insect-free glasshouse amounts to about half that in berries obtained after artificial selfing on the same plants (see 5.3). It may be assumed that, owing to the 'mechanism' regulating spontaneous berry set, such berries are induced by a lower number of pollen grains. A similar observation on seed set has been reported by Marks (1965a; Table 2) for the *Solanum* species *S. demissum*, *S. stoloniferum* and *S. polytrichon*. When selfed, these species gave 122, 122, and 109 seeds per berry, whereas the corresponding numbers in spontaneous berries were 60, 72, and 13.

Marks found the spontaneous berry set in several species to occur at the end of the season except in *S. demissum* and *S. stoloniferum*.

In *S. verrucosum*, spontaneous berry set which is generally observed at the beginning and end of the flowering season[30], might be attributed to plant manipulation (fixing raffia, selfing, crossing, collecting flowers and berries etc.).

Self-setting at the end of the flowering season (some authors call it end of season fertility) often occurs, not only on some self-compatible plants but also on some self-incompatible ones (Marks, 1965a). It seems thus justified that at the end of flowering season, the plants are in and/or under 'conditions' which promote berry and seed set. Such 'conditions' may weaken the self-incompatibility mechanism in self-incompatible species, may promote the development of unfertilized ovules[31], etc.

9.1.4 Glasshouse versus field -grown plants of S. verrucosum

The difference between growing conditions in the glasshouse and in the field may affect plant characters (see 4.1.2, 4.1.3).

Density of plant growth as well as the light and temperature effect may cause longer stems in the glasshouse. The reduced stainability of pollen grains in field-grown plants may be ascribed to the less favourable environmental conditions. Pollen has been checked on dry days only as stainability and pollen shedding are much reduced after rainfall.

Spontaneous berry set in the field-grown plants has mostly been abundant in all *ver*

30. Although no critical data are available, it has been generally observed that flowers produced at the end of the season are small in size. Most of the small-sized flowers have been found to possess shorter pistils favouring spontaneous berry set.

31. For deep insight into the phenomenon of end of season berry set, the study is required of plants at the end of season, the effect of end season environment on seed set, the nature of such seeds, etc.

plants even in populations showing poor or no berry set in the glasshouse. As insects are expected to play the major role in achieving berry set in the field, many *ver* plants grown in the insect-free glasshouses have not shown any berry set. As hardly any differences in flowering have been noticed between the field-grown and glasshouse plants, this factor cannot be the cause of difference in berry set.

The higher berry set on the hybrid plants in the field as compared with that on inbred populations is probably mainly due to their richer and longer flowering which will attract more insects.

The number of seeds per berry of the plants in the field is higher than that of the populations in the glasshouses. Several factors may have caused this. Most flowers in the field are expected to be repeatedly pollinated, whereas in the glasshouse each flower is pollinated only once. In addition, the operation of rigorous selection in the field may reduce the survival of weak and poorly seed setting berries. Cipar et al. (1964b) have found that after artificial selfing seed set is higher in plants grown in the field than in the glasshouse, average seed set per berry in *ver* PI 160228 is 84.1 in the field, 9.9 in the glasshouse. Such a difference is much larger than that found in the present studies where seed set in different populations in the glasshouse has been about 60% of that recorded for sister populations in the field (seeds per berry in *ver* PI 160228 in glasshouse has amounted to 118.5 which is much higher than that reported by Cipar et al.). Perhaps the cut-stem technique used by Cipar et al. has caused a reduction in seed set.

9.1.5 Inheritance of short stem in S. verrucosum CPC 1339

Data on inheritance of stem height in *ver* CPC 1339 are presented in 5.5. When the χ^2-test was applied only to the last measurement in hybrid CPC 1339 I_2–36 × PI 195172–237–9–13, seven out of ten F_2 populations fit the hypothesis, three populations (14, 31 and 35) did not. This proves that the way the data have been handled is more efficient than if any of the four measurements would have been used separately.

Tall stem has been found to be inherited through the action of two complementary dominant genes. Parent PI 195172–237–9–13 is assumed to possess two such genes whereas parent CPC 2247 has three any two of which will lead to tall stems. The absence of F_2 populations segregating 3:1 in the cross CPC 2247 × CPC 1339 is unexpected. The F_2 populations may have been a non-random sample of F_1 genotypes, although the probability of this being true is less than 5% (Mather, 1938).

Short-stem plants are known to occur in several crops. They have been referred to as dwarfs, semi-dwarfs, half-dwarfs etc., in pea (Keeble & Pellew, 1910), in barley (Miyazawa, 1921), in corn (van Overbeek, 1938), in rice (Jodon & Beachell, 1943; Nagao, 1951; Butany et al. 1959; Chang, 1967), in cotton (Balasubrahmanyan & Santhanam, 1950), in *Lolium perenne* (Jenkin, 1954; Cooper, 1958), in sorghum (Quinby & Karper, 1954), in castor beans (Zimmerman, 1957), in alfalfa (Busbice & Wilsie, 1966a, b), in wheat (Hermsen, 1967), and in tomato (MacArthur, 1931; Plummer & Tomes, 1958). In most of these crops, the 'dwarf' types are inherited

through one or two recessive genes, with a few exceptions (see Nagao, 1951; Miyazawa, 1921) where dwarfness has been reported to be dominant.

Dwarfness in sorghum, in rice and especially in wheat has been the subject of extensive studies. Unlike sorghum and rice, wheat dwarfness shows a more complicated pattern of inheritance. The recent studies of Hermsen (1967) assume the dwarfness in wheat to be controlled by the interaction of at least two dominant genes while three loci have been identified.

The crossings between dwarfs in rice (Jodon & Beachell, 1943; Nagao, 1951) and in pea (Keeble & Pellew, 1910) have given normally growing plants. This means that the complementation of dominant genes (two in each crop) are responsible for the appearance of normal height.

The results of the present studies on *S. verrucosum* agree with most of those mentioned on the inheritance of short stems in other plant species but it has been possible in rice and pea, to differentiate the dwarfing pattern[32] that accompanies the recessivity of any or both of the dominant genes.

9.2 Doubling the chromosome number of S. verrucosum

Doubling the chromosome number in *ver* has resulted in more vigorous plants with reduced fertility. The effects of doubling the number of chromosomes are in accordance with those found in *Solanum* (Johnstone, 1939; Livermore & Johnstone, 1940; Swaminathan, 1951) and as also reported in *ver* by Beamish & Shejkal (1955); Lebedeva (1959); Lebedeva & Lukovnikova (1960); Magoon et al. (1958b); and Matsubayashi (1961).

The only case in which chromosome doubling has improved seed set in *ver* is mentioned by Magoon et al. (1958b). This is not surprising as it agrees with the finding of the same authors that *ver* is 'self sterile'; consequently doubling the chromosome number of a 'self sterile' species may improve seed set after selfing.

9.3 Grafting S. verrucosum and some other populations onto tomato

Some 'gigantism' has been observed in grafted populations when compared with their non-grafted sisters (see 4.1.1 and Chapter 6).

Grafting is known to affect several plant characters[33]. Grafting effects on potato have been reported by Lamm (1945), Howard (1949, 1963), Lazareva (1950), Bains (1954), Bernadowski (1954), Ivančenko (1954), Dionne (1961a), Pushkarnath (1961), Marks (1965a) and Weinheimer & Woodbury (1966). Lebedeva (1950) found the selfed *S. schreiteri* and *S. punae* (= *S. acaule* ssp. *punae*) to yield a double amount of seed when grafted. The present studies have also shown a pronounced effect of grafting

32. Whereas this was not possible in the case of the segregating populations of hybrids involving *S. verrucosum* CPC 1339, it was possible to differentiate the growth types of recessive plants in selfed *S. phureja* (see 6.4.2.2).

33. See Swingle (1927), Zeevaart (1958), Sulbha & Swaminathan (1959), Hartmann & Kester (1968).

on seed set in *ver* CPC 2247: on the average it has increased it by about 54%.

The effect of grafting on potato may be explained by the change in competition for food between above and below ground plant organs: all food which would have been used for the production of tubers will become available for the above ground organs. But it is also possible that some substances are transmitted from scion to rootstock or reverse, which affect the expression of the characters in the scion.

However, grafting in some cases may be a selection for compatible combinations. In such cases, a comparison between grafted and non-grafted populations will not be a fair one because the incompatible graftings are ignored and the successful grafts may not represent a random sample of the genotypes present in the non-grafted pool. But this is certainly not the case in the present studies because of the high success of grafting.

9.4 **Barriers and interspecific crosses**

Dobzhansky (1947) and Stebbins (1950, 1958) have classified the isolating mechanisms forming barriers between species. In the present study at least two major types of isolating mechanisms have been discovered.

The first is a barrier to species crossing (classified under external barriers, according to Stebbins, 1950) manifest in reducing pollen germination and in arresting the penetration of pollen tubes in styles. It has been discovered in attempts to intercross the self-compatible species *S. verrucosum*, *S. polyadenium* and *S. etuberosum*, as well as in most self-incompatible × self-compatible pollinations.

The second major type of isolating mechanism is the hybrid sterility observed in hybrids of ♀ *S. verrucosum* with various species and haploids.

9.4.1 Self-compatible species and parthenogenesis in S. verrucosum

The three self-compatible species *ver*, *pld* and *etb* have failed to cross with each other. This is in accordance with their taxonomic classification, as species of series *Demissa* (*ver*), series *Polyadenia* (*pld*) and series *Etuberosa* (*etb*) are difficult to cross (Hawkes, 1958b). Sometimes seedless berries have been obtained and once (*ver* pollinated by *etb*) a parthenogenetic seed has been found in a small berry (see 6.2).

This parthenogenetic seed is expected to originate from a post-meiotic duplication of the chromosome set in a reduced cell in the ovule, probably the egg cell. Its selfed progeny has not only shown morphological uniformity but has also appeared completely resistant to Race 1.2.3.4 of *Phytophthora*, contrary to the I_1 of the mother *ver* plant which has segregated for resistance (see 8.7).

The depression observed for different characters in the parthenogenetic population as compared with the selfed one is most probably the result of complete homozygosity of the parthenotes (inbreeding depression), although an additional effect of the inherent genotype of the doubled gamete which has developed into a plant, is not excluded. The increase of seed set per berry in the parthenogenetic population is most

probably determined by the genotype and is not related to the reduced seed size, because such a relation has never been found in *ver* populations differing in number of seeds per berry.

The higher tuber yield of the parthenogenetic plants may be a result of their reduced vigour. In some plant populations, whether pure *ver* or hybrids between *ver* and tuberosum haploids, the higher tuber yield has been found to be accompanied by reduced plant vigour, poor flowering or non-flowering and the absence of seed set[34] (see 6.7,6.13.1).

A satisfactory explanation for the better tuber yield of the non-flowering and non-seed setting plants is based on the assumed competition between the above and below ground plant organs for assimilates. It is improbable however, that this explanation also holds good for the better tuber yield of the parthenogenetic population because it has shown normal flowering and better seed set than the selfed plants. The better tuber yield of the parthenogenetic population may be a result of its reduced vigour. Ivins & Bremner (1965) have reported 'in the potato plant there is competition for assimilates between foliage and tubers, and the balance can swing in one direction or the other depending on the treatment received'. It may thus be assumed that there is an optimum above ground vigour for producing the best tuber yield. A positive or negative deviation from that optimum may affect tuber production. Consequently *ver* PI 160228 has a surplus of growth in the above ground organs reducing its tuber yield, whereas the reduced vigour of the parthenogenetic population is nearer to the optimum above ground growth resulting in higher tuber production.

The increase in tuber yield reported by Bodlaender & Algra (1966) accompanying the use of the growth retardant B 995 points to the presence of an excess of above ground growth in their material.

If the hypothesis of the competition between above and below ground plant organs is correct, potato breeders can direct their efforts towards breeding and selection for an optimum above ground growth necessary for the production of high tuber yield.

9.4.2 Self-incompatible species and inheritance of incompatibility

Incompatibility studies in the two self-incompatible cultivated species *S. phureja* and *S. stenotomum* (Chapter 7) have shown irregularities in *phu* × *phu* hybrid populations and in *stn* WAC 425 × *phu* PI 225682–22. They have also revealed the occurrence of a group of plants female incompatible with the other groups. A two loci and gametophytic control of incompatibility offer a reasonable explanation.

These two loci are the already known multi-allelic *S* locus and a second one, *R*, which is believed to be multi-allelic too. The *S* locus is epistatic over *R*, and therefore the only 'expressed' locus, except in an $R_{fi}R_{fi}$ style. Such a style rejects all pollen,

34. An inverse association of flowering and fruiting with tuber yield has been reported by Krantz (1946) in commercial potato varieties.

irrespective of their *S* and *R* alleles[35], and therefore causes female incompatibility[36].

Pollen rejecting genes, like the R_{fl} allele which causes unilateral crossability have been reported in earlier studies on *Solanum*[37] by Pushkarnath (1953a), Malheiros-Gardé (1959a), and Pandey (1962a). The rejecting gene assumed by Pushkarnath (1953a) and Malheiros-Gardé (1959a) functions both in homozygous and in heterozygous condition, but the results of Pandey (1962a) and those presented here have demonstrated that homozygosity of the pollen rejecting allele is required for its functioning.

The irregularity frequently observed in the diallel crossing within the studied hybrid populations may be partly attributed to environmental conditions, but evidence is accumulating, also from other investigations, that different genetic backgrounds are involved in causing such irregular behaviour. Unexpected deviations from the simple one locus gametophytic system have been reported by Carson & Howard (1942) in the hybrid *S. rybinii* with *S. boyacense* (both are *S. phureja*), by Pal & Pushkarnath (1944) in *S. rybinii*, by Bains (1954) in *S. rybinii*, by Pushkarnath (1953b) in *S. rybinii* × *S. subtilius*[38], by Dodds (1956) in selfed *S. phureja* CPC 979 and by Pandey (1962b) in *S. simplicifolium* and *S. phureja* hybrids and reciprocals, in hybrids of *S. infundibuliforme* and in *S. phureja* and *S. goniocalyx*[39].

Cipar and his associates at Wisconsin have reported the presence of the one locus system in cultivated diploids and haploids of *S. andigena* and *S. tuberosum*. But their material has also shown some irregularities, such as the presence of self-compatible plants among hybrids between self-incompatible parents[40] (see Smiley, 1963; Cipar, 1964 and Hollenback, 1966) and the unexpected irregularity observed in the studies of Cipar (1964) and Cipar et al.[41] (1964a, 1967).

The use of tester sibs on hybrid plants to detect the incompatibility groups, as employed by Cipar et al. (1964a, 1967) does not allow the discovery of the complete behaviour pattern of all plants in the reciprocal matings. The irregularities present in the studies of Cipar et al. are unexpected for a one locus system. It is also possible that the rejecting alleles cannot be clearly detected in their material.

35. The alleles of locus *R* seem to have no great selective advantage because in heterozygous condition they are hypostatic to *S* alleles and in homozygous condition they will be eliminated through the female incompatibility of the $R_{fi}R_{fi}$ group.

36. The rejecting female incompatible allele R_{fi}, has a similar function as the R_{IC} allele reported by Pandey (1962a).

37. Such rejecting behaviour was reported also by Bukasov (1933), Emme (1936), Kovalenko & Sidorov (1933) and by Choudhuri (1944).

38. Most of the hybrids have shown female incompatibility to their four tester sibs and all show female incompatibility to both parents.

39. Some of the incompatibility groups behave as though female incompatible with others.

40. The presence of self-compatible plants among hybrids between self-incompatible parents may be explained as a result of the breakdown of self-incompatibility due to recombination of differing polygenic backgrounds brought together in hybrid plants, as suggested by Mather (1943). Another possible explanation will be presented in 9.4.8.

41. In Cipar et al. (1967), plants belonging to Group a (Fig. 2) have shown female incompatibility with plants of Group b.

The conclusion of Cipar et al. (1964a, 1967) that alleles of the same series are present in differing cultivated diploids, is supported by our finding that *S* alleles in *stn* belong to the same allelic series as those present in *phu*.

The only report on the presence of a one locus gametophytic system in the cultivated diploids without reference to irregularity is the general statement of Dodds & Paxman (1962), but they have not presented any data.

Until now two types of gametophytic incompatibility in diploid *Solanum* have been reported in literature: the one locus gametophytic system and the two loci gametophytic system. Pandey has demonstrated the presence of a two loci system in the Mexican species *S. pinnatisectum*, *S. ehrenbergii* and *S. bulbocastanum*. *S. pinnatisectum* in particular has been studied extensively (Pandey, 1962a). The dominance relationships of the *S* alleles and the interaction between the two loci made the system in *S. pinnatisectum* more complicated than that discovered in the present investigations.

Pandey (1960a) has suggested a geographical separation of the two loci system (Mexican species) and the one locus system (South American species). The present studies, however, indicate the presence of two active incompatibility loci in South American species. In addition Pandey (1962b) has found, that the Mexican species *S. michoacanum* has a one locus system. Therefore the geographical separation appears to be not as strict as supposed initially by Pandey.

In agreement with Pandey (1962a), the two loci system in the diploid species of *Solanum* is believed to have resulted from doubling the chromosome number of initial self-incompatible parents with 12 chromosomes[42]. The chromosome doubling will lead to self-compatibility or it will not (Lewis, 1943). If not, the survival of the induced tetraploids may be small. Taking into account that chromosome doubling may promote crossability, which in turn will improve fertility, selection for bigger tubers in the induced polyploids, and their vegetative propagation,[43] can compensate impeded fertility. Under such circumstances, the induced tetraploids will have a good chance to survive, irrespective of their self-incompatibility.

Selection against competitive interaction of the self-incompatibility alleles may also have taken place; consequently self-incompatibility will be maintained in the induced tetraploids. Also the possible loss of function and the suppression of the second locus (*R*) will lead to the wide spread of self-incompatibility (or re-evolution in populations which became self-compatible).

9.4.3 Self-compatible and self-incompatible species

Generally self-compatible species fail to fertilize self-incompatible ones, because pollen tube growth is arrested in the stigmatic region or in the style. The reciprocal pollinations have been successful only with *S. verrucosum* as the female partner.

42. See 9.5 for discussion about the basic chromosome number in *Solanum*.

43. Because of the vegetative propagation, modifier complexes affecting incompatibility will be maintained in the cultivated populations. These will lead to irregularity of the incompatibility behaviour in hybrids between the cultivated species as reported in these studies as well in others.

Pollen grains did not germinate in *S. polyadenium* × *S. phureja* whereas in *S. etuberosum* × *S. phureja* and *S. verrucosum* × *S. bulbocastanum*, pollen tubes grow normally, but male gametes either have not fertilized the eggs or the zygotes have not been able to develop (probably both cases occur).

Sometimes parthenocarpic berries[44] are formed and in one case (*etb* × *phu*), parthenogenetic seeds have developed. The set of parthenogenetic seeds in *etb* × *phu* and in *ver* × *etb* points to the possibility of using crossing attempts between such distantly related species to obtain homozygous diploid lines.

In some cases (*ver* on haploids and *ver* and *etb* on *phu*) self set seeds have been obtained on self-incompatible non-emasculated females instead of the expected hybrids. Here the self-incompatibility barrier is overcome by pollinating with pollen from self-compatible species. With similar methods, self set seeds may be obtained on self-incompatible plants.

9.4.3.1 Inbreeding in the self-incompatible S. phureja

The studies on selfed *phu* progeny have revealed the great heterozygosity of this species. The inbreeding effects have been found to be influenced by the two *Ng* loci and by minor genes (see 6.4.2.9). They show that differing genes may have unequal potentialities in contributing to the characters they condition, even when they have complementary action. This observation as well as the effect of minor genes (see 6.4.2.9) may be of importance in contributing to the understanding of heterosis and inbreeding depression.

The irregularity of chromosome pairing accompanying inbreeding in *phu* indicates that chromosome pairing in this species may be genetically controlled. This agrees with the assumption of Lamm (1945) that genotypical differences in chiasma frequency are present in inbred *phu*.

The improved stainability in the I_2 of *phu* as compared with its I_1 parents (6.4.2.8) suggests that some of the factors decreasing pollen stainability in the parents have been eliminated from the progeny by gametic selection on the male side.

The inbreeding depression observed in *S. phureja* is similar to the effects of inbreeding in *S. chacoense* reported by Pushkarnath (1943) and by Hermsen (1969), but more pronounced than that reported by Paxman (1958, 1960) in selfed *phu*, by Dodds & Paxman (1962) in inbreds of *gon* and by de Jong & Rowe (1969) in *S. phureja* – *S. tuberosum* hybrids.

Clear differences exist between the effect of inbreeding in the self-compatible *S. verrucosum* and the self-incompatible *S. phureja* (see Tables 9, 10, 11, 12 and 25). The inbreeding depression in *ver* CPC 2514 (which appears most sensitive to inbreeding) is still considerably smaller than that observed in selfed *phu*. Compared to inbreeding depression in *phu*, inbreeding depression in *ver* amounts to about 44% in

44. According to Rudorf (1966), the set of parthenocarpic fruits is favoured by polyploidy, and Bukasov (1933) expects that, if parthenocarpic berries are found, the cross will be successful, if sufficiently large numbers of pollinations are carried out. Choudhuri (1944) considers parthenocarpy a physiologic barrier between phylogenetically closely related species.

final stem height, 55% in compound L value, 33% in T value, 8% in pollen stainability, 94% in number of tubers per plant and 82% in tuber weight per plant. The self-incompatible species appears to be clearly less resistant to inbreeding than the self-compatible one, even though cross pollination is favoured in the latter.

9.4.3.2 Crossability of S. verrucosum with other species and haploids

The non-germinability of the seeds obtained with *ver* as male partner on H 141, TH 63–71–38 and TH 66–1–25, or as female partner with *phu* PI 225698 (see 6.5) is hard to explain. It may be that some kind of lethality suppressing seed germination is at work here. Cases of such non-germinability of seeds supposed to be of hybrid origin have been reported by Malheiros-Gardé (1959a), Marks (1965a) and Irikura (1968).

S. verrucosum as female partner is successful with several species and haploids. The failure of *blb*, *jam*, *cph* and *trf* to fertilize *ver* is expected in view of their taxonomic differences: species of series *Bulbocastana* and *Pinnatisecta* are known to be non-crossable with species of series *Demissa* (2.2).

In attempts to cross both colchicine induced tetraploid *blb* and *ver* the reaction has been the same as that at the 24-chromosome level: the growth of pollen tubes of *ver* is arrested on the stigma or in the upper part of styles, and those of *blb* penetrate the whole style. The mechanism functioning in the latter case seems to be the prevention of fertilization or failure of development of the fertilized ovules or both. Such a reaction has also been found by Malheiros-Gardé (1959b) in attempts to cross *ver* and other diploid Mexican species, contrary to Pandey's observation (1962c) that the failure of *ver* × *blb* is due to only few pollen tubes penetrating the style.

In the successful crosses with *ver*, barriers seem to reduce berry set, seed set and seed germination (see 6.5.3). Similar barriers were reported by Grun (1961).

9.4.3.3 Hybrid sterility and plasmon-sensitive genes, and plasmon differentiation of S. verrucosum

The successful interspecific hybrids between female *S. verrucosum* and various haploids and diploid species has shown one common characteristic: all hybrid plants are male sterile due to gene-cytoplasm interaction (see 6.6.3.1).

Male sterility is known to occur in a great number of plants (see Edwardson, 1956 and Jain, 1959). Hybrid sterility due to plasmon-genic interaction has been frequently mentioned[45]. It has been reported in *Solanum* by Lamm (1941, 1945, 1953), Ivanov

45. The following cases may be cited in different plants: *Streptocarpus* (Oehlkers, 1964), *Epilobium* (Michaelis, 1954), flax (Bateson & Gairdner, 1921; Chittenden, 1927; Chittenden & Pellew, 1927; Gairdner, 1929), sugar beets (Owen, 1942, 1945; Bolz, 1968), onion (Jones & Clarke, 1943), *Nicotiana* (East, 1932; Clayton, 1950), sorghum (Stephens & Holland, 1954; Maunder & Pickett, 1959), pearl millet (Burton 1958), *Saccharum* (Kandasami, 1961), wheat (Kihara, 1951; Fukasawa, 1953; Wilson & Ross, 1962; Hori & Tsunewaki, 1967), corn (Duvick, 1965), castor beans (Parkey, 1957), *Geranium* (Sansome, 1936), orchard grass (Myers, 1946), pepper (Peterson 1958), tomato (Andersen, 1963, 1964), carrots (Banga et al., 1964), *Petunia* (Edwardson & Warmke, 1967), cotton (Meyer, 1965; Meyer & Meyer 1965; Meyer, 1969); its presence in rice is questioned (Jennings, 1966; Shinjyo, 1969). In some of these cases, male sterility has been utilized for the production of hybrid seed.

(1939), Schnell (1948), Dionne (1961b), Pushkarnath & Kishore (1963), Koopmans (1951, 1952, 1954, 1955, 1959), Grun & Radlow (1960), Grun et al. (1962), Peloquin & Hougas (1961), Hougas & Peloquin (1962), Ross (1966), Howard (1968), and especially in the presence of *ver* plasmon by Buck (1960) and Grun et al. (1962).

Hybrid sterility is known as an isolating barrier to gene exchange between species (see Dobzhansky, 1947; Stebbins, 1950, 1958). The male sterility of the hybrids between female *S. verrucosum* and various diploid species and haploids can also result in isolating barriers to gene exchange (see also 9.4.11).

All 21 *S. verrucosum* introductions studied have sterilizing plasmon sensitive to dominant genes. Three types of such plasmons are present, each leading to a different expression of male sterility (Plate 13) in the presence of the dominant plasmon-sensitive genes. The *ver* introductions studied have been grouped according to the three sensitive plasmons [Tr^s], [Ps^s] and [Sv^s] (see 6.14).

The tetrad male sterility (*Tr*) has been reported before by Buck (1960) in crosses using *ver* PI 195171 and PI 195172 and by Grun et al. (1962) for PI 160228. Figure 9 on page 294 of Peloquin & Hougas' study (1960) shows a similar type of male sterility occurring among the pollen of the haploid US–W 20. The present author has found the expression of this type of sterility in the pollen grains of haploid TH 66–36–2. Toxopeus (1964) and Ramanna & Abdalla (1970) mention a similar type of sterility in hybrids with *S. polytrichon* as the female parent. It seems advisable to avoid the term 'lobed sterility' used by Grun et al. (1962), and to use the indication 'tetrad sterility' because it is descriptive (see 6.6) and to distinguish this type of sterility from the character 'lobedness of the pollen grains', which is controlled by different genes (see 6.6.3.4). The *Ps* and *Sv* types of male sterility have not been reported before.

Several authors have reported the successful use of *ver* as a female parent in crosses with different species (Propach, 1940; Magoon et al. 1958a, b; Malheiros-Gardé, 1959a; Buck, 1960; Matsubayashi, 1961; Pandey, 1962c; Toxopeus, 1964). Hybrids between *ver* and species with a higher ploidy level have been recorded by Swaminathan & Hougas (1954), Hawkes (1956a), Kawakami & Matsubayashi (1957), Marks (1958, 1965a). High percentages of pollen stainability in the hybrids with female *ver* have been reported by Magoon et al. (1958a) and Matsubayashi (1961). Buck (1960) has referred to *S. verrucosum* PI 161173 as having normal cytoplasm, whereas Pandey (1962c) has claimed the hybrid *S. verrucosum* × *S. simplicifolium* to be self-incompatible and he mentions two intra-incompatible inter-compatible groups upon sib-pollinations of the hybrids.

The present author has checked the *S. verrucosum* introductions used by Buck and Matsubayashi and most probably also those used by Magoon et al. (1958a), though they are not explicitly mentioned by them.[46] The introductions used by Buck and Matsubayashi (PI 161173 and PI 161128) have [Ps^s] plasmon. As mentioned before, the pollen grains of hybrids with the sterilizing [Ps^s] plasmon show 'almost normal' –

46. This is highly probable because I have tested the *ver* introductions used by Magoon et al. (1958b) and all other introductions of this species available at Wisconsin in that period.

but not real – staining, compared with those of the other two types of male sterility (see 6.6,6.13.2,6.14). Most probably the 'almost normal' staining of the pollen grains is the reason why this type of male sterility has escaped detection by the above-mentioned authors. The low berry set (7.4 to 28.6%) and seed set (2.5 to 11.0 per berry) on sib-mating the hybrids mentioned by Matsubayashi support our finding, that they are highly male sterile. The occurrence of any berry and seed set is due either to pseudofertility (modifiers or environment affecting the sterility) or to fertilization by the scarce good pollen grains that happen to occur among the sterile ones (see 6.6.1,6.13.2).

The female *S. verrucosum* parent (CPC 1349) used by Pandey (1962c) is reported by Hawkes (1956a) to be male sterile and is no longer available. It may be questioned whether that clone has non-sterilizing plasmon. Unfortunately neither the results from diallel crossing, nor berry and seed set are recorded in Pandey's publication. Modifiers leading to pseudofertility as suggested in Matsubayashi's investigations, have most probably also been operating in Pandey's material. They will not have greatly affected his results but will only have caused some irregular behaviour and a reduction in berry and seed set.

From the literature on potato, cases of hybrids showing 'normal' but non-functional pollen are known. They have been reported by Becker (1939) in *S. demissum* × *S. tuberosum*, by Hawkes (1956a) in hybrids within species of the series *Demissa*, and by Dionne (1961b) in hybrids of female *S. demissum* with *S. phureja*, *S. chacoense* and *S. tuberosum*. Two other cases are reported by Magoon et al. (1958c, d) with reduced pollen stainability (but still sometimes reaching 60%). In all these cases the female parent has been a species of series *Demissa*. As *S. verrucosum* is considered to be an ancestral species of this series, it is logical to suppose that here some type of male sterility similar to the *Ps* type is present. Accordingly, the ancestral *ver* of series *Demissa* may be assumed to be related to *ver* populations with [Ps^s] plasmon.

The colouring observed in many pollen grains in *Ps* sterility as compared with *Tr* and *Sv* types is believed to be due to the later blockage of microspore development in the former type of male sterility. No improvement in pollen grain germination has been observed in the *Ps* over *Tr* and *Sv* types. The stainability of pollen observed in *Ps* sterility does not mean absence of association between pollen stainability and pollen vitality. The comparison between the stainability in *Ps* sterile hybrids and pure *ver* (see Plate 13) or the hybrids with *ver* as the male partner, clearly reveals the difference between the stainability of *Ps* sterile pollen and good fertile pollen. Therefore the association between pollen stainability and its fertility (Poole, 1932) also holds good for *Ps* sterility. A good assessment on pollen stainability cannot be made unless a clear picture is available of the reaction of the known fertile pollen of related populations to the same stain.

The dominant plasmon-sensitive genes interacting with *ver* plasmons, leading to male sterility of F_1 hybrids are wide-spread (see 6.5.4,6.12). Various species and haploids belonging to the series *Commersoniana*, *Piurana*, *Cuneoalata*, *Megistacroloba* and *Tuberosa* possess such genes. It is most remarkable, that the plasmon-sensitive

genes *Tr*, *Ps*, *Ld*, *Sv* and *ns* are all present in homozygous condition, contrary to the genes controlling other characters which are always present in heterozygous condition (see e.g. segregation of characters in selfed *phu*, 6.4.2). The wide-spread and complete homozygosity of the plasmon-sensitive genes point to their importance in evolution (see 9.4.8).

The *Tr*, *Ps*, *Sv* and *ns* plasmon-sensitive genes are believed to control vital functions in their own plasmons, e.g. pollen production. *S. verrucosum* plasmons inhibit the normal action of such genes (see also Grun & Aubertin, 1965; Stebbins, 1958).

Whether *Tr*, *Ps* and *Sv* are distinct genes or belong to the same allelic series, or are even different symbols for one allele cannot be ascertained from the available data. They have been given different symbols to differentiate the distinct plasmons with which they interact. In the studies of Grun & Aubertin (1965), the *In* genes (of which there are at least 5) leading to indehiscence in [In^s] plasmon are reported to be independent.

Only *Tr* sterility has been investigated here in detail. US–W 42 and *S. goniocalyx* each have one *Tr* gene, whereas *S. stenotomum* WAC 780 and AH 66–88–14 each appeared to carry two *Tr* genes, any of which lead to the tetrad sterility in [Tr^s] plasmon of *S. verrucosum* (see 6.6.3.3, 6.8.1, 6.9, 6.11).

Buck (1960) has found the male sterility of the hybrids between *ver* and *chc*, *phu*, and *stn* to be controlled by a dominant gene from the male parent in *ver* plasmon, whereas in the present studies two dominant genes have been found in *stn*. The small size of Buck's populations (10 plants in each backcross) probably has impeded the establishment of the number of genes involved.

The other two characters related to male sterility and discovered in the backcrosses and segregating generations, are 'undivided microsporocytes' and 'non-shedding' of pollen grains (see 6.6.5). The first has never been mentioned before in any crop as far as the present author is aware. The second has been called 'pollenless' by Grun et al. (1962). The term 'non-shedding' is preferred by the present author, as long as it is not certain whether pollen sacs are indeed pollenless. In the present investigations, 'non-shedding' has been found to be controlled by the complementary action of three recessive *ns* genes in *ver* plasmon, whereas that of Grun et al. (1962) show only one recessive gene interacting with *phu* and *stn* plasmons. The differences in the material used by Grun et al. and by the present author are most probably the cause of this discrepancy.

The lobed characteristic of the pollen grains in the interspecific hybrids is controlled by *Ld* genes in [Ld^s] plasmon of *ver*. In Hybrid 1 (see 6.6.3.5), lobedness of pollen grains is assumed to be controlled by the combined action of two genes $Ld_1Ld_2, Ld_1ld_3, Ld_2ld_3$ involving three loci. *S. verrucosum* PI 195172 has been assumed to have the idiotype [Ld^s] $ld_1ld_1Ld_2Ld_2Ld_3Ld_3$. As the haploid AH 66–88–14 has two *Ld* genes, any of which lead to lobedness of pollen grains in [Ld^s] plasmon of *ver* PI 160228 (see 6.11), it may be assumed that locus Ld_3 in *ver* PI 195172 is a dominant inhibitor (*II*) which suppresses the action of either Ld_1 or Ld_2. The idiotype of this PI 195172 is, according to this modified hypothesis, [Ld^s] $ld_1ld_1Ld_2Ld_2II$. Therefore it

may be assumed that this character is controlled by one dominant *Ld* gene in the [Ld^s] plasmon. *S. verrucosum* PI 195172 carries one of these genes, but its effect is suppressed by a dominant inhibitor. Grun & Aubertin (1965) found restorer genes in *S. chacoense* to be epistatic to indehiscence (*In*) genes. Without the presence of such restorers, the plants would have been male sterile. The restorers reported by Grun & Aubertin (1965) have a similar effect on *In* genes like the suppressing effect of the inhibitor *I* on *Ld* genes found in the present studies. The only difference is that the restorer genes were reported to be specific in their effects, whereas it is not sure whether the inhibitor *I* is specific to any of the two *Ld* genes.

The study of the hybrids between BC pollinators and the haploids AH 66–94–28 and TH 67–17–2 (see 6.16) has affirmed that the character 'lobed pollen grains' is controlled by gene-cytoplasm interaction (absence of any lobed pollen in the hybrids). The haploids AH 66–94–28 and TH 67–17–2 can be assumed to have [ld^r] plasmon. The presence of self-incompatible plants in those hybrids rules out the possibility suggested by Buck (1960), that self-incompatibility genes lead to male sterility in *ver* plasmon. It has been proved that the *S* alleles of US–W 42 are masked in the male fertile backcross plants.

The strong linkage between the *Tr* and *Ld* genes in AH 66–88–14 (see 6.11) demonstrates the presence of duplicate linkage groups:

(1) the group including Tr_e and Ld_e,

(2) the group including Tr_f and Ld_f.

The duplicate linkage groups most probably have originated from chromosome doubling (see 9.5).

9.4.4 The causes of male sterility of haploids extracted from tetraploids

The commercial varieties are heterozygous for several characters as has been proved from the studies of their selfed progenies (see Chapter 8; Krantz, 1924, 1946; Krantz & Hutchins, 1929; Guern, 1940; Deshmakh & Verma, 1960), from the variability observed among the extracted haploids (Peloquin & Hougas, 1960), and from the heterozygosity detected within the haploids (see 6.6).

The heterozygosity of the commercial varieties has probably been preserved because of their vegetative propagation. Under such circumstances it is not surprising that extracting haploids, as an efficient inbreeding method, will enable this variability to be explored.

Male sterility of haploids may have resulted from the recombination of recessive genes (which may also be plasmon-sensitive), affecting pollen fertility. The disturbance of chromosome sets and plasmons by haploid extraction may thus affect fertility.

Owing to the heterozygosity of the commercial varieties for recessive genes affecting pollen fertility, and to their expectedly 'mixed' plasmons, the extraction of haploids as a means of obtaining homozygosity of recessive genes, of halving the chromosome number, of having disturbed and 'segregating' plasmons, will show several of these haploids to be male sterile.

9.4.5 The uncovering of recessive characters in self-incompatible plants and its prevention

Recessive characteristics in haploids US–W 42 and AH 66–94–28 have been uncovered upon inbreeding, which becomes possible after the introduction of self-compatibility (see 6.6, 6.16.2). Inbreeding in *S. phureja* has shown harmful effects and has led to almost worthless progeny from the fertility point of view, especially on the male side (see 6.4.2).

The self-incompatible species have proved to carry several deleterious recessives, which are masked by their dominant alleles. The self-incompatibility genes guarantee cross-pollination and consequently maintenance of such recessives in heterozygous condition. Bringing such recessive genes into homozygous condition will badly affect the self-incompatible populations, may endanger their persistence and will lead to 'reproductive wastage'. Accordingly any mechanism operating against the introduction of self-compatibility alleles into self-incompatible populations will have a selective advantage. This mechanism does exist on the side of self-incompatible species as will be discussed under 9.4.7, 8.

9.4.6 Self-compatibility, self-incompatibility and unilateral incompatibility

A self-incompatible plant is a plant unable – under normal conditions – to produce seed upon selfing, irrespective of the functioning of its male and female gametes. Self-incompatibility has been proved by East & Mangelsdorf (1925) to result from the function of alleles at the *S* locus. The presence of an S_i allele (S_i = self-incompatibility allele at the *S locus*) in the style will prevent fertilization by a pollen grain carrying an identical S_i allele.

On the other hand, a self-compatible plant can produce normal seed set upon selfing due to the absence of functioning S_i alleles or due to the presence of 'self fertility' alleles. These 'self fertility' alleles are frequently symbolized by S_f. The present author prefers to use the term self-compatibility instead of self fertility and consequently will use the symbol S_c (S_c = self-compatibility allele) instead of S_f.

Unilateral incompatibility is a term applied to the one-way success of crossing between self-incompatible (SI) and self-compatible (SC) species; success is usually achieved when the SC species is the female partner (see 9.4.6.3 for details).

9.4.6.1 S_c and S_i alleles?

When AH 66–94–28 has been fertilized by pollen from backcross plants of Hybrid 1, three hybrid plants out of 15 set no berries after selfing and have proved to be self-incompatible (see 6.16.2). The numbers of 12 SC : 3 SI fit the ratio of 3:1 which is expected if S_c belongs to the same allelic series as S_i[47], because the pollen mixture of

47. Even when the plant which set one seedless berry is considered to be self-incompatible, the numbers of 11 SC: 4 SI still fit into the ratio of 3:1.

the backcross plants of Hybrid 1 is expected to contain (75%) S_c gametes and (25%) S_t gametes (see 9.4.6.2).

In all other crops studied till now, it has been reported that the S_c allele belongs to the same allelic series as the self-incompatibility alleles (see e.g. East, 1932; Townsend, 1965).

9.4.6.2 Absence of self-incompatible plants in segregants of hybrids between self-compatible and self-incompatible species

Neither the F_2 from the hybrid AH 66–94–28 × BC (see 6.16.2), nor the F_2 and F_3 of Hybrid 1 (see 6.6.3.1) have shown any definite self-incompatible plant. Whenever the segregants have shown good pollen, they have always set seed after selfing a reasonable number of flowers. This also applies to backcross plants of Hybrids 1, 3, 4 to *ver* parents and their inbred progenies. This means the absence of any self-incompatible plant in segregating generations of hybrids between self-compatible and self-incompatible species.

Suppose that *S. verrucosum* has the genotype S_cS_c (self-compatible) and US–W 42 is S_xS_y (self-incompatible). Then the hybrid plants between *ver* and US–W 42 (Hybrid 1) will be S_cS_x and S_cS_y. The self-compatibility of some of the hybrid plants must then be due to the ability of S_c pollen to penetrate S_cS_x and S_cS_y styles. The absence of SI plants in both F_2 and F_3 points either to the inability of S_x and S_y to penetrate S_cS_x and S_cS_y styles, respectively, or to the changed or weakened specificity of the self-incompatibility alleles by introduction into an SC background.[48] In this thesis, evidence is presented that the S specificity is neither lost nor changed: when the backcross plants of Hybrid 1 have been used as males on AH 66–94–28 and TH 67–17–2, self-incompatible hybrids have been found in the progenies (see 6.16.1, 2; 9.4.6.1). Therefore the second assumption is ruled out.

The hypothesis that absence of SI plants in the segregating populations of hybrids between SC and SI plants is due to the inability of S_t pollen to function normally in an S_cS_t style with an identical S_t allele will be adopted here; it agrees with the principal function of oppositional S alleles. Accordingly selfing S_cS_x plants will result in S_cS_c and S_cS_x progeny, because of fertilization by S_c pollen only. Both genotypes will be SC through their functional S_c pollen; S_x pollen will be inhibited in the S_cS_x style.

The important conclusion is that: in the gametophytic system of incompatibility, the self-compatibility and the self-incompatibility alleles have independent functions. Pollen carrying S_c allele is not inhibited in its pistil and fertilizes the egg cell bringing about self-compatibility, whereas pollen carrying S_t allele cannot effect fertilization when an identical S_t allele is present in the style. When brought together, S_c and S_t alleles act independently. There is no dominance relationship between them. Such independence of action runs parallel to the independent action of two self-incompatibility alleles when present or brought together in the same plant.

48. Martin (1968) has reported the loss of S specificity in the pollen when self-incompatibility is transferred into SC *Lycopersicon*.

In the literature (see Sirks, 1927; East, 1929, 1932), some authors who crossed SC and SI species, have reported the F_1 to be self-compatible owing to the dominance of self-compatibility over self-incompatibility; they have recorded SI segregants in F_2 and have considered these plants as segregants with the recessive self-incompatibility alleles. The presence of such 'self-incompatible' plants may be explained in several ways. Genetic backgrounds affecting the action of S alleles could account for the occurrence of self-incompatibles (see East, 1932). Self-incompatible F_2 segregants are also expected when F_1 plants are sib-mated ($S_cS_x \times S_cS_y$). It is also possible that some sterile F_2 segregants may have been misclassified as self-incompatible. Segregation for recessive genes (not S alleles) which prevent fertilization by S_c pollen may also be involved (for details see 9.4.8).

Support for the idea that SI segregants result from causes other than the fertilization of S_cS_x plants through S_x pollen is derived from the following.

(1) The findings of East (1932) that all F_2 plants from the cross between SC *Nicotiana langsdorffii* and SI *N. sanderae* are self-compatible, unless other modifiers are functioning.

(2) The studies of Williams & Silow (1933) on *Trifolium pratense* which have shown that selfing an SC plant (S_1S_f) produced only SC progeny (S_1S_f, S_fS_f).

(3) The investigations of Rinke & Johnson (1941) on *Trifolium pratense* where the F_1 hybrids between SC and SI plants as well as 180 out of 182 F_2 plants have been reported self-compatible.

(4) The heterozygous SC (S_fS_x) plants from *Trifolium hybridum* yield only SC plants on selfing (Townsend, 1965).

(5) The present results where all F_2 and F_3 segregants of the hybrids between *ver* and haploids are found to be self-compatible.

(6) In the earlier studies on crossing SC and SI species there has always been a shortage of SI segregants in the segregating populations (3:1 expected). For instance, East (1919) reported the segregation of 144 SC: 37 SI in selfed F_1 of the hybrid SC *N. langsdorffii* and SI *N. forgetiana* whereas 38 SI plants from about 200 F_2 have been recorded in the cross between *N. langsdorffii* and SI *N. alata*. Also Baur (1919) has reported 22 self-incompatible plants among 212 F_2 plants from the hybrid between SC *Antirrhinum majus* and SI *A. molle*.

Sirks (1927) in discussing the problems of incompatibility has rejected the 'real dominance' but has assumed the presence of SI segregants as due to the ability of S_1 pollen to grow in S_cS_1 style (S_1 in S_0S_1 style according to Sirks), and he states: 'the ratios found in the F_2 generation are then the results of the proportions in the rapidity of growth between S_0 and S_1 pollen in the quantity of pollen which has been used for the pollination'.

The gametophytic control of specificity is basic in the oppositional hypothesis. The S_c allele belongs to the same locus as the self-incompatibility alleles. Why then should we assume the dominance of S_c allele, though all others act independently? The early investigators have probably been very much influenced by the rediscovery of Mendel's Laws (dominance according to F_1 phenotype, segregation in F_2 populations). In

addition, the oppositional hypothesis had not yet been discovered at that time. These factors may have affected the explanation of the data in early investigations. Actually East (1932) has rejected the earlier explanations for the presence of SI plants in F_2 progeny from crosses between SC and SI species.

9.4.6.3 Current hypotheses on unilateral incompatibility

In several plants where the cross self-compatible × self-incompatible generally succeeds, the reciprocal is usually not successful. This phenomenon has been called unilateral incompatibility, unilateral hybridization, unilateral inhibition, SI × SC inhibition.

Unilateral incompatibility occurs between species of the same genus, of different genera and even of different families (Lewis & Crowe, 1958). It has been reported for *Nicotiana* (Anderson & de Winton, 1931; Pandey 1964, 1968, 1969c), *Petunia* (Mather, 1943; Bateman, 1943), *Lycopersicon* (Mc Guire & Rick, 1954; Martin, 1961b, 1964, 1967), *Antirrhinum* (Harrison & Darby, 1955), *Solanum* (Malheiros-Gardé, 1959a; Buck, 1960; Matsubayashi, 1961; Grun & Radlow, 1961; Grun & Aubertin, 1966a; Pandey, 1962c; Marks, 1965a; also these studies, see 6.4, 6.5.1).

Self-compatible species not following the unilateral incompatibility rule have been referred to as Sc-genotypes and were considered of recent origin (Lewis & Crowe, 1958). They present the hypothesis that self-compatible (SC) species have evolved from self-incompatible (SI) ones by mutations in the sequence:

$$SI \longrightarrow Sc \longrightarrow Sc' \longrightarrow SC$$

The authors assumed a dual function of the *S* alleles: inhibition of their own pollen and of pollen of SC plants.

Grun & Radlow (1961), who have used *S. verrucosum* with success as male partner on *S. chacoense*, *S. subtilius* and *S. soukupii*, have reported the presence of two forms of SI species: one accepting pollen of self-compatible species, the other rejecting them. Grun & Aubertin (1966a) mention the unilateral incompatibility to be controlled by independent dominant genes, 'non-acceptors'.

The studies of Martin (1961a, b, 1963, 1964, 1967, 1968) on *Lycopersicon* have shown inhibition in SI × SI, SI × SC and SC × SC combinations. Self-incompatibility and unilateral incompatibility have in most cases been found to be associated, with dominant major genes. Unilateral incompatibility between SC species is assumed by him to be a remnant of their previous self-incompatibility. Martin (1963) has presented a hypothesis of balanced polygenic control of substances affecting pollen tube growth and stylar inhibition to account for unilateral relations (see also Martin, 1964, 1967, 1968).

In 9.4.10, the present author will discuss these current hypotheses in comparison with his own hypothesis presented in 9.4.8.

9.4.7 Evolutionary aspects of self-compatibility and unilateral incompatibility

Cross-fertilization offers better possibilities for maintaining and increasing variability than does self-fertilization. Therefore it is generally accepted that self-fertilizing plants have been derived from cross-fertilizing ones[49] (Stebbins, 1950, 1957; Mather 1955; Lewis & Crowe, 1958; Crowe 1964).

Consequently, it may be speculated that SC species have evolved from SI ancestors, at least in the families where self-incompatibility predominates. This is in agreement with Stebbins (1957), Lewis & Crowe (1958) and Martin (1968) but contradictory to the reports of East (1929) and Mather (1943). Supporting the idea of SC developing from SI ancestors is that: all detectable mutations of S alleles are inactivators and loss mutations (see Lewis, 1951, 1954, 1961; Lewis & Crowe, 1954; Pandey, 1956, 1959, 1967, 1969a, 1970; Lundqvist, 1963; de Nettancourt, 1969), self-compatibility can be brought about by inbreeding SI populations (Lundqvist, 1960, 1968), and the S specificity in pollen has been lost when transferred into SC species (Martin, 1968). The conclusion that SC species may have been developed from SI ancestors sounds logical as the chance of an S_c allele surviving and increasing in frequency in an SI population is better than the chance of an S_i allele building up a self-incompatibility system in an SC population. As far as the present author is aware no case of mutation from S_c to S_i has ever been reported.

Whether the ancestor species is self-compatible or self-incompatible, both SC and SI plants are believed to have been present together in the original population as 'Two Powers'. Fertilization by pollen carried by pollinating agents will lead to gene exchange between SC and SI plants. The transfer of genes from SI plants to SC ones will enrich the evolutionary potentialities of the latter, but the effect on self-compatibility will be minimum, as the S_c pollen will still grow in the S_cS_i style, whereas the S_i pollen will be inhibited in the hybrid style with an identical S_i allele (see 9.4.6.2). On the other hand the transfer of pollen from SC to SI plants will introduce self-compatibility into the latter. This will not only lead to uncovering deleterious recessives masked in the heterozygous SI population (see 6.4.2, 9.4.5), but will also affect spread and occurrence of SI plants by increasing the frequency of S_c alleles. Natural selection will operate against the expected 'reproductive wastage' (Stebbins, 1958) and 'poorly adapted genotypes' (Dobzhansky, 1947); by favouring cross-pollination, it will encourage the development of barriers against the introduction of S_c-carrying pollen into SI populations (see also Grun & Radlow 1961). Accordingly, and favoured by natural selection, a barrier will develop in SI plants leading to rejection of pollen from SC plants (see 9.4.8).

The competition between SC and SI species and the harmful effects of introducing self-compatibility into SI species are not contradictory to the evolution of SC plants from SI ones. The change from self-incompatibility need not be a one step change;

49. On the other hand, very old self-fertilizing crops do exist (Stebbins, 1957), and it is possible that some self-fertilizers are the older ancestors.

it can occur gradually through accumulation of polygenes weakening the self-incompatibility mechanism. Even if the change to self-compatibility is sudden, this does not imply that the newly evolving SC plants will be strict self-fertilizers because not all SC plants are strict self-fertilizers. The harmful effects of a sudden change to self-compatibility may be partly tolerated by vegetative propagation of the newly developed SC plants, or they may be endured if other mechanisms favouring cross-pollination are functioning on the side of the newly evolving SC plants, such as the suprastaminal pistils of *S. verrucosum* (for mechanisms promoting cross-fertilization, see Elliott, 1958). Several SC plant species are known which still have efficient mechanisms promoting cross-fertilization.

9.4.8 A 'two power competition' hypothesis to account for unilateral incompatibility

In 9.4.7 it has been assumed that both self-compatible (SC) and self-incompatible (SI) species have been present within the same area as 'two competing powers'. Because of the adverse effects of introducing self-compatibility into self-incompatible species, a strong barrier favoured by natural selection has developed in SI plants against fertilization by pollen from SC ones. This barrier will be assumed to consist of specific genes, *UI*, for unilateral incompatibility (see 9.4.6.3).

These *UI* genes inhibit fertilization by pollen grains carrying S_c (self-compatibility) alleles. They are probably associated with genes controlling the most distinctive mechanism of SI species, i.e. the self-incompatibility, in order to warrant a strong barrier. This is why the SI $\times$ SC inhibition is a general phenomenon, whereas the reciprocal usually succeeds. The present studies as well as those of several other authors investigating different plant species (see 9.4.6.3) point to the general occurrence of this SI $\times$ SC inhibition.

Once the *UI* genes occur, not only can SC species no longer fertilize SI species, but in addition the fertilization of SC species by pollen from SI species renders the hybrids self-incompatible and prevents them from accepting pollen from SC parent, owing to the function of *UI* genes introduced to hybrids from the SI parent. This function of *UI* genes corners the SC populations and endangers their persistence by continuous crossing and backcrossing by SI populations and hybrids.

The SC power in its competition with the SI power counteracts the function of *UI* genes by one or more of the following reactions. By the development of mechanisms that allow its being kept apart by its self-fertilization. By developing barriers against gene exchange with SI and probably other SC species.

Thus some of the species become almost dead ends of the evolutionary pathway depending only on mutations and accidental hybridization for enriching their potentialities.

The 'old' SC species will not accept S_t-carrying pollen, nor will the SI species be fertilized by their S_c-carrying pollen.

Other SC species, such as newly developed ones depending on SI species as a pollen source, or SC species which need a pollinating agent to be fertilized (e.g. *S. verruco-*

sum), and especially perennial SC species and those which propagate by tubers, will develop plasmons sensitive to specific genes from the SI populations. (Such genes will be of prime importance for SI species, see 9.4.3.3).

The plasmon-sensitivity will lead to sterile[50] hybrids and the hybrid sterility will thus act as a barrier to gene exchange (see 9.4.11). These sterile hybrids, if perennials or vegetatively propagated like *Solanum* will form a 'dead barrier' in the meeting zone of the two species.

Backcrossing the male sterile hybrids with SI species will not be of prime importance as the hybrids will continue to be sterile, but backcrossing with SC parents (provided that the SI parents are heterozygous for *UI* genes) will result in self-compatible male fertile segregants, enriching the potentialities of the SC population.

The presence of plasmon-sensitive genes in the male sterile hybrids may also affect the multiplication rate of different plasmon particles in favour of those leading to male fertility. Male fertile self-compatible plants (or branches) may originate in this way. These can practise self-fertilization and contribute to the pool of self-compatible population. In any case, the SC population will take advantage of the presence of such sterile hybrids, so that sensitive plasmons will be favoured by natural selection promoting speciation, even though they lead to reproductive wastage.

Where insects act as pollinating agent (as in *S. verrucosum*), the species needs to develop a sterilizing plasmon able to produce sterile hybrids with 'normal looking' flowers and 'normal' pollen production (in quantity and appearance) to attract the insects. This may be accomplished by the [Ps^s] plasmon (see 6.13.2 and 9.4.3.3): sterilizing the hybrids, keeping them attractive to insects and acting as a sieve for genes from SI plants. Through the sieving function, pollen from SI plants will get lost during insect visits to *Ps* sterile hybrids, thus increasing the chance that pure *S. verrucosum* plants with [Ps^s], [Tr^s] and [Sv^s] plasmons will be fertilized by fertile pollen collected from pure *S. verrucosum*.

If this view is correct, the presence of chronological differences accompanying the development of [Tr^s], [Ps^s] and [Sv^s] plasmons may be considered. Such chronological differences may be affected by the dependence of the species on pollinating insects. *Ps* sterile plants, though perhaps attracting insects, lead to wastage of sterile gametes, and though in *Tr* and *Sv* sterile plants this wastage will be smaller, their flowers look unhealthy. Consequently a change from [Ps^s] into [Tr^s] or [Sv^s] plasmons needs to be preceded or accompanied by the (nearly) independence of the plants on spontaneous mechanisms ensuring self-set seeds. This behaviour is generally observed in populations with [Tr^s] plasmon, but not in that with [Sv^s] (see 5.3, 6.14). On the other hand it may be possible that the earlier change from normal plasmon (resistant) into a sterilizing one has been similar to [Tr^s] and [Sv^s] plasmons. The change from any of these to [Ps^s] plasmon may have happened when insect attraction and sieving function have become necessary. This sequence is also expected, as some introductions with

50. The discussion will continue assuming F_1 to be male sterile but female fertile; however, this does not rule out the possibility of female organs being affected too.

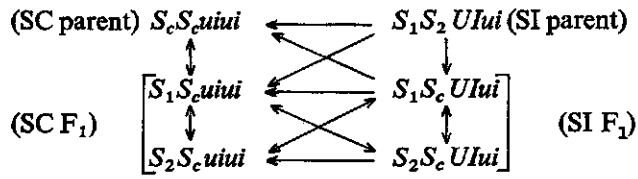
[Ps^s] plasmon can give seed set without manipulation (see 5.3, 6.14).

Within the frame of the two power competition hypothesis one may anticipate that:
(1) The *UI* genes have developed through the challenge of hybridization between SI and SC populations. Active *UI* genes may be absent if the ancient SI ancestors never met SC relatives; the unilateral incompatibility mechanism may also have been lost later through mutations or selection. Both absence and loss of activity may especially be expected, when SC relatives do not exist or are isolated from the SI populations.

The SI × SC inhibition will be clear in the wild populations where both SC and SI relatives occur within the same areas.
(2) The sensitive plasmons of SC species have developed in the counteracting competition subsequent to crossing of SC and SI populations, particularly after the development of *UI* genes.
(3) There are various SC as well as SI species. Crossing between isolated 'old' SC species and SI ones will fail in both directions.
(4) When no other barriers are present, crosses between SC and SI species, in the absence of active *UI* genes, will be reciprocally compatible. The hybrid plants will be self-compatible due to fertilization by S_c-carrying pollen only. Backcrossing will be possible in all directions if the SI species is *S* allele heterozygous. Selfing F_1 will lead to SC segregants only, because of the independent action of S_c and S_t alleles. Theoretically SI F_2 segregants may be obtained only upon sib-mating F_1 unless modifiers affecting S_t alleles are present.
(5) In the absence of other barriers and presence of active *UI* genes, the cross between SC and SI plants will be successful in one direction only: when SC is the female partner.

If the UI genes are present as homozygous dominants in the SI parent, the hybrid plants will be self-incompatible and will reject pollen from the SC parent because of the presence of *UI* genes. The hybrids can fertilize the SC parent. Sib-mating, and backcrossing to the SI parent will be controlled by the self-incompatibility alleles.

If the SI parent is heterozygous for *UI* genes, some of the F_1 plants will be self-compatible due to the absence of the active rejecting *UI* allele(s). For instance, if the unilateral incompatibility is controlled by the dominant allele *UI* which rejects S_c-carrying pollen and the SI parent is heterozygous at the *UI* locus, the following diagram represents the expected crossability of parents and F_1 (success in the direction of arrows only):



(6) Crosses between self-incompatible plants can lead to self-compatible progeny if S_c allele is present in one of the parents.

For instance, crossing an SI plant (S_1S_2uiui) as a female with another SI plant (S_3S_cUIui) will give rise to 25% SC F_1 plants (2 genotypes out of 8). If both parents had had an S_i allele in common, half of the F_1 plants would have been SC. A cross between SI ♀ (S_1S_cUIui) with SI ♂ (S_1S_2uiui) will lead to one fourth SC F_1 (= S_2S_cuiui) and some of the SI F_1's (= S_1S_2UIui, S_2S_cUIui) will be crossable only in one direction. Similar behaviour may explain the one-way success of crossing between SI species, and the occurrence of SC plants in hybrids between SI plants (see Smiley, 1963; Cipar, 1964; Hollenback, 1966 and 9.4.10).

(7) Different S_c alleles may occur and consequently different matching[51] *UI* genes are expected to exist. The corresponding anticipations will be the occurrence of one way success in crossing between self-compatible species as well as the presence of SI plants among hybrids between SC ones (see 9.4.10).

The unilateral incompatibility mechanism – like any other character – may be controlled by dominant genes or recessive ones or by both. However, for the sake of simplicity, here unilateral incompatibility is assumed to be controlled by dominant genes, *UI*. This may sound, however, rather arbitrary, but it has to be kept in mind that unilateral incompatibility alleles (rejecting fertilization by S_c-carrying pollen) are expected to function as dominants to the non-rejecting alleles, otherwise they will not form a strong barrier between SC and SI populations.

The unilateral incompatibility manifests itself by arresting S_c-carrying pollen tube growth in styles of SI species (see Plate 13–7). Also, the mechanism of self-incompatibility functions through the control of pollen tube growth. Unilateral incompatibility, however, has proved to be a stronger mechanism than self-incompatibility controlled by *S* alleles. This is clear from several studies: Bateman (1943), McGuire & Rick (1954), Lewis & Crowe (1958), Malheiros-Gardé (1959a, Fig. 2), Pandey (1962c, 1964), Martin (1963). It is also supported by the results in 6.4.1 and 6.5.1.

The presence of specific *UI* genes in SI species controlling the unilateral incompatibility is apparent from the ability of the same *S. verrucosum* to pollinate certain SI plants, but not others. In the present studies, AH 66–94–28 accepts *S. verrucosum* pollen but other SI plants reject S_c-carrying pollen (see also Grun & Radlow, 1961; Grun & Aubertin, 1966a; Buck, 1960; Matsubayashi, 1961; Pandey, 1962c). The presence of such genes has also been reported by Grun & Radlow (1961), Grun & Aubertin (1966a) and Martin (1964, 1967).

9.4.9 Support for the two power competition hypothesis from Solanum and other genera

9.4.9.1 From Solanum

In the literature on *Solanum*, several cases on crossing between SC and SI species have been reported. All can be explained within the frame-work of the two power

51. In this discussion, the matching UI_1 gene is the one able to prevent fertilization by pollen from SC plants carrying the S_{c1} whereas UI_a will prevent fertilization by S_{ca} etc.

competition hypothesis.

Malheiros-Gardé (1959a) and Pandey (1962c) mention the occurrence of unilateral incompatibility in crosses between SC *S. verrucosum* and SI species from different series. The F_1 hybrids obtained using *ver* as the female parent have been reported to be self-incompatible. These results can be explained (see also 9.4.3.3) with the two power competition hypothesis as follows: the SI species carry rejecting *UI* genes in homozygous condition. These genes will cause the SI × SC inhibition of the parents and lead to self-incompatibility of the F_1 plants because of rejecting fertilization by S_c-carrying pollen.

An interesting finding related to the investigations of both Malheiros-Gardé and Pandey is the behaviour of *S. vernei* and its hybrids with *S. verrucosum*. In the investigations of Malheiros-Gardé, *S. vernei* has rejected *S. verrucosum* pollen whereas some F_1 plants from *S. verrucosum* × *S. vernei* have accepted them. Closely related plants from *S. vernei* used by Pandey have proved to be reciprocally crossable with *S. verrucosum* and both reciprocal F_1 hybrid populations have been reported self-compatible. These results can be explained by assuming that the original vernei stock used by both authors consists of heterozygous as well as homozygous (both dominant and recessive) plants for the *UI* genes. Malheiros-Gardé happened to use heterozygous plants of vernei, which will reject S_c pollen of *S. verrucosum*, but will give F_1 segregants (with *uiui* resulting from *S. verrucosum* × *S. vernei*) which will accept such pollen, whereas Pandey has accidentally used a homozygous *S. vernei* (*uiui*) not rejecting S_c pollen of verrucosum, in the parental crosses (leading to success of reciprocal crosses) and in the F_1 (leading to an SC F_1).

The studies of Buck (1960), in which SI species did not accept *S. verrucosum* pollen but the F_1 (*S. verrucosum* × SI species) did (possibly some F_1 plants, but this is not reported) suggest heterozygosity of the SI species for *UI* genes: only recessive *uiui* hybrids will accept verrucosum pollen. This is supported by the fact that only one clone of *S. phureja* and one clone of *S. chacoense* have accepted verrucosum pollen.

The work of Matsubayashi (1961) has shown that SI species generally reject verrucosum pollen as also did F_1 of *S. verrucosum* × SI species (Matsubayashi, 1969, pers. comm). The hybrid *S. phureja* × *S. verrucosum* obtained by Matsubayashi (1961) has been self-pollinated (pers. comm.). These results fit our hypothesis assuming SI species to carry *UI* genes rejecting S_c pollen whereas in case of their absence the reciprocal cross could also be made and F_1 will be SC.

On the basis of the two power competition hypothesis our own data can be explained as follows. *S. polyadenium* differs greatly from other species used in that it is hardly expected to cross with any of them. *S. etuberosum* pollen was inhibited in styles of SI species, whereas its style did not arrest pollen tubes from SI species. If it is accepted that penetration of pollen tubes deep into styles – without seed set – means non-failure of pollinations, it may be assumed that *S. etuberosum* can be included within species showing unilateral relations.

The self-compatibility of *S. verrucosum* is a newly acquired character. This species is the best of the three SC species on which to study the unilateral incompatibility

due to absence of any apparent barriers affecting its crossability with many SI species, except *UI* genes.

With the exception of AH 66–94–28, all the SI species and haploids used carry *UI* genes preventing fertilization by S_c pollen of *S. verrucosum*. The absence of active *UI* genes (probably the *uiui* genotype is present) in AH 66–94–28 has led to the success of *S. verrucosum* as a male parent and the self-compatibility of the hybrid plants.

Some F_1 plants of hybrids between *S. verrucosum* and males US–W 42, *S. stenotomum*, *S. goniocalyx* and AH 66–88–14 has accepted *S. verrucosum* pollen. This indicates *UI* heterozygosity of the SI male parents, leading to segregation in the F_1 into plants accepting S_c pollen (which will also be self-compatible) and others rejecting them. One of the F_1 plants in Hybrid 1 (see 6.6.1) has produced seedless berries on selfing, whereas the other 10 have shown seed set. It can be assumed that this plant having *UI* gene unlike the others (which are expected to be *uiui*) will not allow fertilization by S_c pollen. The different reactions of the F_1 hybrids of *S. verrucosum* PI 195172 and PI 160228 with US–W 42 to parent verrucosum pollen (see 6.6.2, 6.11) may be explained on two assumptions. First, it has been purely accidental that no recessive (*uiui*) plants of the hybrid with *S. verrucosum* PI 160228 have been used in the backcross to verrucosum, whereas *uiui* recessive plants have been taken from the hybrid with *S. verrucosum* PI 195172. Secondly – though this point needs further investigations before being accepted – differences may exist between SC genes and/or backgrounds of the two verrucosum introductions used.

The behaviour of hybrids with *S. verrucosum* PI 275258 (6.13.2) can be explained in a way similar to that presented for hybrids with *S. verrucosum* PI 160228.

Homozygosity for *UI* genes may be assumed in SI parents where their hybrids with *S. verrucosum* reject S_c pollen (see 6.11, 6.13.2).

9.4.9.2 From other genera

The two power competition hypothesis has also the support of investigations in other genera than *Solanum*.

In *Trifolium*, all cases of crossing between SC plants possessing the S_c (S_f) allele, and SI plants agree with the hypothesis. This refers, among others to the studies of Williams & Silow (1933) and Rinke & Johnson (1941) on *T. pratense*, of Atwood (1942) on *T. repens* and of Townsend (1965) on *T. hybridum*. 'Self-fertility' has been reported to be dominant by Rinke & Johnson (1941) and by Atwood (1942). All investigations on clovers point to the absence of active *UI* genes and no SI $\times$ SC inhibition has been reported. If this proves to be a general rule, it indicates that either the SI Trifoliums have never come across SC relatives or, if this has happened they have lost the rejecting mechanism.

In *Antirrhinum*, Sherman (1939) mentions hybrids between SC *A. majus* and SI *A. latifolium* to be SC. The SI parent has been fertilized by F_1 pollen producing only SC plants. This means that *A. latifolium* lacks *UI* genes, which causes its hybrids with *A. majus* to be SC, and leads to non-rejection of the S_c pollen from F_1.

In *Papaver*, Fabergé (1944) reports the success of the reciprocal crosses between SC *P. alpinum* and SI *P. nudicaule*. This points to the absence of active *UI* genes in the SI species. As expected, the F_1 has shown to be self-compatible (Fabergé believes in dominance of 'self-fertility').

In *Oenothera*, Crowe (1955) has reported the success of the reciprocal crosses between SC *O. trichocalyx* and SI *O. pallida, O. latifolia, O. deltoides* and *O. runcinata*. As expected in view of our hypothesis, Crowe has found the hybrids to be SC.

In *Lycopersicon*, Mc Guire & Rick (1954) have reported the hybrids between SC *L. esculentum* and SI *L. peruvianum* to be SI. Martin (1961a) has found a similar behaviour when crossing *L. esculentum* with SI *L. chilense*, and in 1964 when crossing SC and SI forms of *L. hirsutum* and also in 1967 after crossing *L. esculentum* with SI *L. hirsutum*. In all these cases, the SI × SC inhibition has been reported and dominance of self-incompatibility alleles has been considered the cause of self-incompatibility in the hybrids. The results of Mc Guire & Rick as well as those of Martin can be explained on the basis of the present hypothesis assuming the presence in SI species of active *UI* genes.

In *Nicotiana*, the earliest finding on unilateral incompatibility has been that of Anderson & de Winton (1931). They have reported the hybrids between the SC *N. langsdorffii* and SI *N. alata* and *N. sanderae* to be SC. One SI plant of *N. alata* (No. 15–2) has proved to be crossable with the SC species only as a male, half of its hybrid progeny being SC and the other half SI. The latter accepted *N. alata* pollen but rejected those of *N. langsdorffii*. To explain this behaviour, the authors assume Plant No. 15–2 to be heterozygous for a self-sterility allelomorph (S_F) with dual function, expressed in inhibiting its identical allelomorph and preventing fertilization by pollen from SC species.

On the basis of our hypothesis, the data of Anderson and de Winton can be explained as follows: the self-compatibility of the hybrids between *N. langsdorffii* and *N. alata* and *N. sanderae* is due to the absence of active *UI* genes in the SI species (crossing with SC species have been successful in both directions) and the ability of S_c-carrying pollen to fertilize the hybrids without active *UI* genes. *N. alata* No. 15–2 is *UI* heterozygous (*UIui*) and accordingly rejects S_c pollen. The reciprocal cross will succeed and half the hybrids will be SC (*uiui*), the other half SI (*UIui*). There is no need to assume a dual function of *S* alleles.

The study of East (1932) shows the SI *Nicotiana sanderae* to be reciprocally crossable with the SC *N. langsdorffii* and the hybrid plants are reported self-compatible. The success of the reciprocal cross implies the absence of *UI* rejecting genes in the SI species, and thus the self-compatibility of the hybrid plants.

Another case of crosses between SC and SI species of *Nicotiana* has been reported by Pandey (1964).[52] He distinguishes two kinds (M and N) of self-incompatible *N. alata*. The M plants reject pollen of the SC *N. langsdorffii*, but their pollen are not rejected

52. Other studies by Pandey have also contributed to the current discussions on unilateral incompatibility (Pandey, 1962c, 1964, 1968, 1969b,c, 1970) (see Abdalla, in preparation).

in its styles, whereas the N plants are reciprocally crossable with the SC species. In hybrids with the SC species as female, the M plants show two classes with about equal frequencies: the first contains self- and intra-compatible hybrids, the second self- and intra-incompatible hybrids. The SC hybrids accept pollen from M and N plants of *N. alata* and are crossable in both directions with their SC parent, whereas the SI hybrids accept *N. alata* pollen, but are crossable only as males on the SC parent. Crosses between M plants segregate into plants which either accept S_c-carrying pollen or reject them (ratios about 1:3 and 1:1). Crosses between M and N plants segregate for acceptance *v.* non-acceptance of S_c-carrying pollen into 1:1. For an explanation, Pandey assumes the presence of *S* alleles (S_{FI}) at the *S* locus with a dual function, rejecting both their own and S_c pollen.

On the basis of our hypothesis, Pandey's data can be interpreted assuming *N. alata* to be heterozygous for *UI* genes. As this species is maintained by sib-mating, it is expected that some of the plants will be homozygous recessive, *uiui*. Such recessive genotypes (N plants) will accept S_c-carrying pollen, whereas plants carrying dominant *UI* genes (M plants) will reject S_c pollen. Crossing between SC female and M plants will show SI and SC segregants in F_1; SC are those with the genotype *uiui* and as expected, will accept pollen from SC parent. The SI F_1 hybrids (*UIui*), carrying *UI*, will reject their own S_c pollen and pollen of the SC parent, but will accept *N. alata* pollen due to its heterozygosity for *S* alleles. Crosses between M, and between M and N plants are expected to yield segregants accepting S_c pollen, because these are crosses between *UI* heterozygous plants or between heterozygous and recessive ones respectively. To explain differences in pollen tube growth it is not necessary to assume special S_{FI} alleles, because such differences are known to occur between 'normal' *S* alleles (see East, 1932; Mather, 1943; Bateman, 1943).

The discrepancies found in the ratios of self-compatible non-rejecting plants to the self-incompatible rejecting ones are simply explained by the assumption of two complementary dominant genes conditioning the unilateral incompatibility. The segregation 28 SC : 8 SI F_1 plants is clearly a ratio of 3:1 and not 1:1, as Pandey has assumed. Such a ratio is expected when a double heterozygous plant is crossed to the recessive SC *N. langsdorffii*. It may also be assumed to contain more than one ratio if the data are not pooled. Ratios equal or near to those obtained in the crosses among M plants and in crosses between M and N plants are expected on the basis of two complementary dominant genes controlling unilateral incompatibility. The linkage between the *S* alleles and *UI* genes is very probable in Pandey's investigations, as the unilateral incompatibility has been reported to follow the incompatibility groups.

In *Petunia*, Mather (1943; see also Bateman, 1943) mentions that the crossing between the SC *P. axillaris* (S_aS_a in his symbol for the self-compatibility alleles) and the SI *P. violacea* (S_1S_2) is successful only when the SC species is the female partner. The F_1 is reported to consist of SC (S_2S_a) and SI (S_1S_a) plants in equal numbers. Both groups are crossable in both directions and set seed as females with *P. violacea*, but the reciprocals are unsuccessful. Both groups are successful as males on *P. axillaris*, but pollination by the latter gives no seed set on the SI group, contrary to SC plants.

One S_2S_2 plant of *P. violacea* has given only SC F_1 when crossed to *P. axillaris*. This homozygous plant accepts pollen from both hybrid groups; its pollen has fertilized SI F_1 hybrids, but on SC hybrids they have been either unsuccessful (50%) or have given a partial set. Four flowers of this plant, pollinated by *P. axillaris* have shown no success.

Mather explains his results assuming 'S_a' gives no efficient pollen growth in styles of SI plants. *P. axillaris* has genes non-allelic with *S*, weakening the incompatibility of the *S* alleles, especially of S_2 and this leads to the self-compatibility of the hybrids. The function of S_2 has been reported to be strengthened when S_1 is present in the same style; that is why S_1S_2 is self-incompatible.

Mather's data can be explained according to our hypothesis as follows:
The S_1S_2 *P. violacea* is heterozygous for rejecting *UI* genes (*UIui*). Accordingly S_c-carrying pollen of *P. axillaris* is not able to fertilize *P. violacea*. The F_1 plants (female *P. axillaris*) will be either SC or SI because of segregation at the *UI* locus. The SC hybrids can be self-pollinated because of the function of S_c (S_a) pollen, and not as Mather has assumed because of fertilization by weakened S_2 allele. Both SC and SI hybrids are cross-compatible because all gametes of SI plants (S_1, S_2, S_c) can grow in the style of SC hybrids (in the absence of the identical *S* allele), and alleles of SC plants except the S_c allele can fertilize the SI sibs lacking identical *S* alleles. Both groups can be fertilized by the S_1S_2 parent, but the reciprocals fail because of the inability of S_1 and S_2 pollen to penetrate the S_1S_2 style and the rejection of the S_c allele by the *UI* gene. Both groups of hybrids will fertilize the SC parent, as S_c, S_1 and S_2 gametes function, but pollen of this parent will be rejected by the SI group due to the functioning of the *UI* gene.

As *P. violacea* is *UI* heterozygous it may be surmised that the homozygous S_2S_2 is also *uiui*; consequently it will produce only SC hybrids with *P. axillaris*, and it will accept pollen from SC hybrids. Such pollen is believed to be S_c, but not S_2 as suggested by Mather.

The only four pollinations attempted using S_2S_2 as a female to *P. axillaris* are too small in number to consider S_2S_2 as rejecting pollen from the SC species, because Mather himself has reported the difficulty of crossing the two species because of 'isolating genes'; on p. 218, he mentions that in 1939, 101 attempts to pollinate *P. axillaris* by *P. violacea* have failed.

As far as could be traced, all crosses involving SC species and their SI relatives with the gametophytic system of incompatibility fit the two power competition hypothesis. Whether it also applies to hybrids between SC and SI species with the sporophytic system of incompatibility in the latter cannot be decided until more information is available on the crossability between such species. Actually more complications are expected in case of SI species having sporophytic system of incompatibility, because of the 'dominance' relationships and the interaction of alleles in this system.

9.4.10 Comparisons between the two power competition hypothesis and other hypotheses[53]

Our hypothesis agrees with that of Lewis & Crowe (1958) on the general occurrence of SI × SC inhibition and the possibility that SC species may have evolved from SI ancestors. But it has to be kept in mind that wide crosses (between species of different genera and families, sometimes even between related species) will not only be conditioned by the action of *S* or *UI* loci. Many other barriers are expected to operate in such cases, so that the rule of SI × SC inhibition cannot be expected to function in all crossings. We have to restrict such inhibition to related populations. It has been assumed that the SI × SC inhibition is due to the functioning of specific genes, developed through natural selection preventing hybridization between SI and SC populations. Such a hybridization is possible only among related populations.

Our hypothesis does not agree with the assumption of Lewis & Crowe (and with that of Anderson & de Winton, 1931; Mather, 1943; Bateman, 1943; and Pandey 1964, 1968, 1969b, c) on the dual function of the *S* alleles. Such dual function may rule out the possible evolving of SC species from SI ones which is a basic point in Lewis & Crowe's hypothesis.

Our hypothesis does not agree with the idea that only recent self-compatible (Sc) species will not be prevented to fertilize SI species, whereas old self-compatible (SC) species will be prevented. In our hypothesis any S_c-carrying pollen will be inhibited in SI styles if these styles possess the matching rejecting *UI* genes.

The dominance relationships of S_c and S_i alleles assumed by Lewis & Crowe have been rejected and it is made clear that the self-compatibility of the hybrids between SC and SI species is conditioned by the ability of S_c-carrying pollen to fertilize the hybrids, and this makes the absence of matching *UI* genes a necessity.

On the other hand the presence of specific genes in SI species preventing fertilization by S_c-carrying pollen as reported by Grun & Radlow (1961) and Grun & Aubertin (1966a) seems quite plausible. Our hypothesis presented the role played by the assumed *UI* genes in the self-incompatibility of the hybrids between SC and SI plants and has also shown the necessity of barriers like the change of the plasmon in the competition between the SC and SI populations. But we do not share the opinion of Grun & Aubertin on the generalization of unilateral incompatibility covering all unilateral relations.

The hypothesis put forward by Martin (1963; see also Grun & Aubertin, 1966a) does not differentiate between the unilateral relations between plants, though the general rule is the SI × SC inhibition as found in his studies. Lumping all unilateral relations together is not recommended, because one should distinguish between a general behaviour and exceptions, between plants which reject only S_c-carrying pollen and those which reject any pollen. The exceptional one way success of crossing between SI plants can be explained within the framework of the already known

53. The other hypotheses on unilateral relations has been presented in 9.4.6.3.

incompatibility systems (see Chapter 7 and 9.4.2, 9.4.8).

The occurrence of one-way crossability between SC plants is probably a function of various interacting factors. Self-compatibility may be due to the presence of S_c alleles, to inbreeding effects, to the presence of genes suppressing the function of the *S* locus, to pseudocompatibility, to induced polyploidy (Lewis, 1943), or to the presence of centric fragments carrying an additional *S* allele (Brewbaker & Natarajan, 1960; see also Pandey, 1967). With all these possibilities and expected differences in the genetic backgrounds, it is probable that one-way success may occur between two SC plants.[54]

Our hypothesis does not exclude the occurrence of one-way crossability between SC species; on the contrary, it is the only hypothesis which can furnish a simple satisfactory explanation of this phenomenon. Such behaviour will imply the presence of different S_c alleles and consequently different matching *UI* genes in differing SC species.[55] Based on this assumption, one may anticipate the occurrence of SI plants within hybrids between SC ones. Martin (1964) has reported such occurrence in hybrids between the two SC lines, Baños and Surco, of *Lycopersicon hirsutum* (see also Martin, 1967).

To explain such 'unexpected' occurrence of SI plants within hybrid progeny of SC parents, Martin (1964) has assumed polygenic control, whereas Pandey (1970) surmises the 'production of some form of specificity'. But a polygenic control needs a continuous order of behaviour which is not present in Martin's material. The explanation by a 'generation' of secondary specificity-in such high frequency of F_1 plants, see Martin's 1964 data – as suggested by Pandey (1970) is not simpler than that based on *UI* genes.

On the bases of the two power competition hypothesis, Martin's results may be explained by the presence of *UI* genes in Surco matching the S_c alleles of Baños but not its own S_c. Thus both lines will be self-compatible because of the absence -in each- of *UI* genes matching its own S_c. The self-incompatibility of some F_1 plants may be attributed to the presence in such plants of interacting *UI* genes, brought from both parents, which will reject S_c pollen of F_1 plants leading to their self-incompatibility. Segregation for self-compatibility and unilateral incompatibility is expected in F_1 plants and has been reported by Martin (1964). Some unexpected deviations in Martin's study may be attributed to the function of some modifiers and/or recessive genes.

Though it may be understood from the studies of Martin (1964, 1967) that there are specific genes for unilateral incompatibility 'Although SI and UI were usually associated, sufficient cases of non-association were found to suggest some differences in genetic control' (Martin, 1964); in his publication of 1968, Martin assumes the *S* alleles to have two properties, 'the specific property of inhibition of pollen tube growth of

54. It is also possible that other factors, not related to *S* or *UI* genes function in the expression of one-way success between SC (or SI) species. Such factors may be morphological and/or physiological against the functioning of a 'foreign' pollen in a 'foreign' style.

55. But SC species with functional S_c alleles will not carry functional matching *UI* genes to its own pollen.

pollen containing an identical allele, and the general property of inhibition of pollen tube growth of pollen containing the self-fertility allele'. This means a return to the dual function of *S* alleles presented by Lewis & Crowe (1958) and others. The change in Martin's ideas is due to his observation that 'self-incompatibility in these studies was always associated with the *S* allele. In addition, unilateral incompatibility between hybrid and *L. esculentum* was only found when hybrids were self-incompatible', (Martin, 1968).

Martin's results are a logical sequence to our hypothesis, because only self-incompatible hybrids (between SC and SI species) will show unilateral incompatibility with the SC parent, because of the presence of active *UI* genes in these SI hybrid plants. These genes will prevent fertilization by S_c pollen of its own plants leading to self-incompatibility and by S_c pollen of the SC parent leading to unilateral incompatibility. Martin's opinion (1961a, 1964) that the self-incompatibility of the hybrids between SC and SI plants is due to the dominance of self-incompatibility alleles is not shared by the present author.

9.4.11 Does plasmon-genic sterility lead to species isolation?

Whereas Grun & Aubertin (1965) report that pollen sterility owing to gene-cytoplasm interaction will cause isolation of species, Caspari et al. (1966) do not entirely agree. Plasmon-genic sterility originating from SC × SI hybrids can be an isolating barrier between populations (see also Dobzhansky, 1947; Stebbins, 1958) with the following considerations:

(1) SI species will be protected by *UI* genes against the introduction of genes from SC species,

(2) SC species will be kept apart by their self-compatibility, their good seed set (even under adverse conditions) guaranteeing their expansion,

(3) plasmon types like [Ps^s] will act as a sieve (see 9.4.8),

(4) the sterile hybrid population will be a dead barrier and no gene flow can take place from it to either parent populations,

(5) plasmon-sensitive genes are believed to control vital mechanisms in the parent populations (see 9.4.3.3), consequently its elimination is hardly expected.

9.5 **Basic chromosome number in Solanum**

The basic chromosome number in *Solanum* has been assumed to be either six or twelve (see Koopmans, 1951; Prakken & Swaminathan, 1952; Swaminathan & Howard, 1953; Howard, 1960; Swaminathan & Magoon, 1961; Magoon et al., 1962). Evidence derived form the present study suggests that the basic chromosome number is less than 12, so that the 24-chromosome species of *Solanum* may be considered 'tetraploids'.

In addition to the arguments put forward by some of the above mentioned authors, the following evidence can be given here to sustain this view.

(1) The meiotic abnormalities in selfed *S. phureja* (see 6.4.2.7) suggest the possibility that there is more than one genome with a genetic control of chromosome pairing. The few irregularities in chromosome pairing in the hybrids of *S. verrucosum* with other species and haploids (see 6.6.4) suggest the possibility of slight structural differences between genomes of the species involved.

Introgression due to cross-fertilization and free exchange of genes is expected (Hawkes, 1962). It will, however, be difficult to distinguish the genomes except in isolated species. Extensive cytological and cytogenetical studies, and extraction of haploids and monoploids from species with 24 and 48 chromosomes are needed to throw some light on the possible presence of different chromosome sets.

(2) The duplicate linkage groups Tr_e–Ld_e and Tr_f–Ld_f in the hybrid *S. verrucosum* PI 160228 × AH 66–88–14 (see 6.11) is believed to originate from chromosome doubling. During the course of evolution, diploidization is expected and it reduces the number of genes controlling different characters. It is not surprising (with this assumed polyploidy) that more than one gene has been found to condition most of the characters studied and that some of them have been found to be complementary. It is possible, that those genes have been introduced from the separate chromosome sets of ancestral forms. Complementary factors may have evolved from duplicate genes in such diploidized polyploids.

(3) The two loci system of incompatibility discovered in the South American species *S. phureja* and *S. stenotomum* is believed to have evolved from doubling the chromosome number of diploid ancestors having 12 chromosomes (see 9.4.2).

(4) The parthenocarpic berries obtained upon pollinations within and between different species may suggest polyploidy of the diploid species, as parthenocarpy has been reported by Rudorf (1966) to be promoted by polyploidization.

Unreduced gametes may function in experimental hybridization (see Prakken & Swaminathan, 1952; Swaminathan & Howard, 1953; Magoon et al., 1962; Hermsen, 1969). Some cases have also occurred in the present studies: a triploid verrucosum plant in selfed *S. verrucosum* and tetraploids among verrucosum × phureja hybrids (see 5.4, 6.14). Obviously this may also take place in nature, and indeed diploids and triploids of the same species do occur in nature (Graham et al., 1959, 1). The function of unreduced parental gametes in nature is expected to lead to hybrids with higher ploidy levels. This, together with the spontaneous doubling of chromosome numbers in lines and hybrids, may have resulted in polyploidization in potato.

The absence in nature of 'diploid' ancestors (with 12 chromosomes) may be ascribed to their insufficient competitive ability as compared with the polyploids.

9.6 Late blight

The investigations on the reaction of *S. verrucosum* to *Phytophthora infestans* have proved that, within this species, susceptible, resistant and segregating introductions occur (see Chapter 8). This confirms the findings of Hekkel (1898), Stuart (1905),

Reddick (1940), Rudorf et al. (1950), Black & Gallegly (1957), Graham (1963) and others (see 2.4).

Hybrids of *ver* with other species have shown varying reactions as reported by Umaerus & Stålhammar (1969). Only when a parthenogenetic resistant *ver* parent is used, are all F_1 plants resistant.

The test of resistance to *Phytophthora* has shown that *ver* is present in nature in heterogeneous populations. It is advisable in this connection to screen the *ver* introductions for resistance to *Phytophthora* before including them in breeding programmes.

The 'lower' races of *Phytophthora* i.e. races with a narrow host range, especially Race 4, are more aggressive and in some verrucosum populations they attack more seedlings than the relatively virulent races. Such behaviour cannot be satisfactorily explained if the interaction between *ver* resistance genes and the fungus strains is similar to that between *R* genes of *S. demissum* and the races as reported by Black et al. (1953).

However, 'lower' races have been known to be more aggressive, whereas strains with a wider host range are reported to be less prolific (Black, 1952; see also Thurston & Eide, 1953; Gallegly, 1968; van der Plank, 1968, 1969). The aggressiveness of Race 4 has been reported by Mastenbroek (1966). The complications observed when using this race, and its presence on populations where it should be absent or rare according to the populations' genotypes, have been mentioned by Mastenbroek & de Bruyn (1955), Wilson & Gallegly (1955), Doling (1956), Pristou & Gallegly (1956), Howatt (1957), Gallegly & Eichenmuller (1959), Graham et al. (1959, 2),Toxopeus (1960), Mastenbroek (1966) and Mooi (1969, pers. comm.).

This 'general' unexpected behaviour of Race 4 may explain some of the deviations observed in our results (see 8.2, 8.7), but it hardly explains the reaction of CPC 2644. Such reaction may perhaps be explained by assuming that Race 4 carries undiscovered additional pathogenicity genes (not present in other races) and that the genotypes of CPC 2644 are such that all plants are attacked. However, it has to be kept in mind that deviation from expectation in reaction to Race 4 has been reported not only in populations of *ver* and other species possessing genes for resistance, but also in populations carrying *R* genes from demissum. Consequently, it is not incorrect to apply the analysis of inheritance of resistance in some *ver* populations (see 8.5) according to the scheme adopted by Black et al. (1953; see hypothesis in 9.7).

It has been suggested that resistance in *S. verrucosum* is conditioned by genes with major effects, referred to as 'verrucosum major genes', '*Vm* genes', and polygenes (see 8.2.1, 2, 8.4). Graham (1963) has reported the inheritance of resistance to Race 1.2.3.4 in *S. verrucosum*, to be polygenically controlled. He also reported 'there seems to be no clear distinction between a hypersensitive reaction to infection and one indicating a high degree of partial resistance'. Such difficulties have also been met with in our material.

Both major and minor genes occur in *S. demissum* (see Toxopeus, 1959a, b; Umaerus & Stålhammar, 1969). It is not surprising that they are also present in *S. verrucosum*, which is considered to be one of the ancestral species of series *Demissa*

which also includes *S. demissum*[56].

The presence of both major genes and polygenes in *S. verrucosum* is believed to be responsible for the complications observed in the populations showing resistant and susceptible segregants. Association between *Vm* genes and polygenes is expected and it is possible that the *Vm* genes are present in a polygenic background, which contributes considerably to resistance.

Some cases (see, e.g. the reaction of CPC 2514 and M18 pl. 6 in Tables 38 and 43) have been observed where *Vm* genes seem to be 'masked' by polygenes. Cases like this have been reported also in *ver* by Graham (1963). This author has found that inbreeding of partially resistant *ver* parents (score 3) may give rise to more resistant segregants (scores 1 and 2); score 1 is usually associated with hypersensitivity.

Ferris (1955) reports duplex R_1 plant to be resistant to the fungus which can attack simplex R_1 plant, but Toxopeus (1957) mentions a small effect of extra R_3 genes on resistance. The assumption that major genes are associated with polygenic backgrounds may explain why plants simplex for an *R* gene will be more susceptible than duplex plants. The so-called 'masking' effect found in Graham's studies (1963) as well as in our own experiments, may be explained by assuming the parent plants to be poor in polygenic background, though some possess genes with major effects. This polygenic background may have become richer in some plants after selfing and in these the function of major genes becomes obvious (see hypothesis in 9.7).

After selfing, *ver* populations have shown different reactions, depending on their genetic make up. By selfing, good resisters have been selected and the resistance is sometimes maintained at the same level as that in the mother plant. Selfing in the susceptible *ver* did not produce any improvement. This is in accordance with the results obtained by Graham (1963), who mentions that inbreeding of susceptible verrucosum results in wholly susceptible progeny with the same score (5) as their parents.

The results of inbreeding in resistant and susceptible populations of *ver* compared with inbreeding in cultivated varieties (see 8.3; also Stevenson et al., 1936; Bonde et al., 1940), as well as the comparison between the reaction of seedlings from *S. verrucosum* and the cultivated varieties, suggest that the systems controlling resistance in the two species are probably different. This leads to the assumption that the resistance in the commercial varieties (without genes from wild species) is controlled by a system different from that in the wild species (see also Toxopeus, 1959a; Umaerus, 1969).

Umaerus (1959, 1963) and van der Plank (1968) have suggested the resistance in such tuberosum varieties to be controlled by non-specialized genes (see also Toxopeus, 1959a). If this is true, resistant haploids, mutations and selfed progenies from susceptible varieties are expected to occur, and it is not surprising that resistant inbreds have been obtained from the susceptible variety Katahdin (Stevenson et al., 1936; Bonde et al., 1940).

56. Swaminathan & Hougas (1954), Hawkes (1956a,b 1958a, 1963, 1966), Marks (1955, 1959, 1960, 1965a), Kawakami & Matsubayashi (1957).

Selfing the commercial *S. tuberosum* varieties (see 8.3) has shown no, or only a few resistant seedlings.[57] A similar result has been mentioned by Toxopeus (1959a) and it is in harmony with the supposition that resistance in such varieties is controlled by non-specialized genes.

9.7 Breeding for resistance to Phytophthora and the position of S. verrucosum

S. verrucosum has proved to possess both major genes and polygenes. It is also expected that *Vm* genes of a higher order than those that can be attacked by the races used in these studies are present in this species. Some of the *ver* introductions have shown good resistance and almost all of their seedlings have been free from *Phytophthora* sporulation and several introductions have shown very resistant segregants.

The pathogen can be multiplied both asexually (Butler & Jones, 1949) and sexually (Gallegly & Galindo, 1957, 1958; Smott et al., 1957). Graham et al. (1960) have reported its ability to change its virulence by mutation and new races have appeared through passages on resistant plants. Similar findings have been reported by Reddick & Mills (1938), Rudorf et al. (1950), de Bruyn (1951), Ferris (1955); see also Gallegly (1968).

Variation in the pathogenicity of races is known (Yamamoto, 1965; Takase, 1968) and the factors affecting the pathogen are variable (Crosier, 1933). As *Phytophthora* is a multinuclear organism (Clinton, 1911; Marks, 1965b), different mutant nuclei may be present in the same hypha, and genotypic variation is expected (see Toxopeus, 1956; Denward, 1967). Recently, Leach & Rich (1969) have reported the occurrence of anastomosis, heterokaryosis and parasexuality leading to the development of new races.

Differences in the aggressiveness of races are known and the general opinion is that relatively virulent races are less prolific.

From this it would seem that the fungus is variable, but this is also true for the host. It has always been possible to find verrucosum seedlings resistant to any of the fungus strains used.

Breeding for resistance to *Phytophthora* has been practised within the *S. tuberosum* ssp. *tuberosum* since the 19th century (Sneep 1966; pers. comm.). The breeding using wild species, has not proceeded beyond incorporating one or a few *R* genes of demissum into some varieties. *S. tuberosum* is known to lack such *R* genes, but 'field' resistance has been found in it.

Gallegly (1968) has reported the following: 'The resistance of the multigenic-resistant potato varieties introduced prior to 1900 seemed to decline after a few years .... Later, as the *R* genes were discovered and introduced into commercial-type clones, new *R*-gene-specific pathogenic races of *P. infestans* appeared as fast as each new gene was identified'.

57. The crossing of such resistant seedlings may lead to the production of varieties possessing better resistance than the original ones. Heterosis is expected for other characters including tuber yield.

Generally, a discrimination between 'uniform' and 'differential' resistance is accepted. Some research workers point to the need of breeding for 'uniform' resistance, neglecting 'differential' resistance, or even considering *R* genes to be barriers to testing for 'uniform' resistance (Mooi, Hermsen, van Suchtelen; Hermsen, pers. comm.; Simmonds 1969), others try to combine both (Mastenbroek, Black; Hermsen, pers. comm.; Gallegly, 1968).

The breeding for resistance to *Phytophthora* may be deliberated along the following lines

(1) Use of uniform resistance. The main ideas behind the preference for uniform resistance are: plants do not react differentially to the fungus races and some kind of balance is achieved, minimizing the development of new races.

But it seems that uniform resistance does not last for ever. This has been reported by Toxopeus (1956) and Niederhauser (1962), as is also clear from the history of variety Multa which had been good 'field' resistant till recently when it was attacked by Isolate B 19. Gallegly (1968) believes that 'the change to increased aggressiveness seems to be a logical method by which *P. infestans* can overcome multigenic resistance'. However, we may expect the change of aggressiveness to be slow, though it is not certain whether this can also be expected with widespread varieties bred for uniform resistance.

(2) Use of differential resistance. Differential resistance is easier to incorporate. It delays the start of an epidemic and can contribute immunity to specific races. Its drawback is the expected rapid spread of specific races.

It is true that, as more investigations are carried out, more *R* genes will be discovered (see Black et al., 1953; Schick et al., 1958; Eide et al., 1959; Mc Kee 1962; Malcolmson & Black, 1966), and more races will be identified. But such new races are expected to be more virulent if the hosts carry several *R* genes. According to the information available to date, these races will be less aggressive.

The drawback of the use of resistance based on major genes can be minimized, by growing varieties without and with differing *R* genes in such places and rotations as to minimize the development of new races; by limiting the area under cultivation to one variety; by breeding late maturing varieties for resistance against races capable of attacking earlier ones (the latter can be left without incorporating any resistance into it). Another possible method may be the use of artificially blended varieties differing in their major gene constituent and genetic backgrounds.[58]

(3) Use of uniform resistance and the combination of many major genes in the same variety. Both types of resistance are being tested with the same fungus races. With the use of appropriate races, tests are expected to give results comparable to those obtained in tests in Mexico (see also Simmonds, 1969).

Both types of resistance have proved unable to last for ever and both can be present within the same species. The so-called uniform resistance can be 'broken' by

58. A good example has been provided by wheat breeders who succeeded against wheat stem rust, of which more than 250 physiologic races are known and more are continuously being added (see Borlaug, 1966).

not very complex races like Isolate B 19 (Race 1.4.x) which 'broke' the resistance of variety Multa. The presence of any major gene in the host leads to resistance to all races of the fungus lacking the overcoming pathogenicity. This resistance can be terminated if a race with the overcoming pathogenicity appears and becomes widespread.

From the above, it is clear that some connection may be assumed to relate both uniform and differential resistance. They need not be basically differentiated as suggested by van der Plank (1968). The present author will present the following hypothesis to combine both types of resistance.

The resistance has been divided into uniform and differential. The differential resistance is controlled by genes with major effects, whereas the uniform resistance is assumed to be controlled by two different systems in the different *Solanum* species. The first is the 'gene non-specialized' uniform resistance which is the resistance found in *S. tuberosum* cultivars and probably in some other species also. This resistance is assumed to be controlled through non-specialized genes (genes which condition other characters and do not directly contribute to the mechanism of resistance). The second system is based on specialized polygenes which confer 'gene specialized' uniform resistance. This second system is expected to be present in the species adapted to the native area of the parasite (for instance Mexican *Solanum* species which are in contact with *Phytophthora infestans*). One may assume that the specialized polygenes determine the 'primary general' resistance whereas the major genes control the direct work, switching towards specific races. The strength of the resistance will depend on the polygenic background which affects the behaviour of the major genes. It is also possible that the presence of different major genes may strengthen their allover function and most probably the presence of more major genes will be accompanied by richer polygenic background.

If this hypothesis is correct, it is expected that polygenes are the 'older' evolving genes, and that major genes have developed with the selection pressure imposed on host populations by the 'newly' evolving races of the parasite. When the parasite develops a new race able to attack the resistant host (host resistance occurs first), the latter will develop a major gene which is able to switch the resistance towards the new race.

Natural selection will favour plant genotypes with resistance to the parasite and the susceptible plants will gradually disappear. This will put the parasite at a disadvantage. Consequently natural selection will favour mutations in the parasite, which are able to overcome the resistance in the host. This will lead to a reproductive disadvantage in the host compared with that in the parasite. This implies that genes (and mutations) which confer resistance in the host will be favoured by natural selection and so on (see also Person, 1959).

On the basis of the above hypothesis, one may anticipate that related resistant host species may have similar major genes, but differ in their polygenic backgrounds on which the efficiency of such genes depends. If this proves to be correct, we expect that the related Mexican *Solanum* species will possess similar major genes.

On the basis of the preceding discussion and until more information on the fungus and the hosts are available, we prefer to consider the use of both types of resistance within the same variety as an efficient method of breeding for resistance. Both types are not basically differentiated according to the hypothesis presented above; they are expected to complement each other. As both major genes and polygenes proved to be present in the same wild species, which are still found in Mexico irrespective of the constant presence of the fungus, it is logical that incorporating both types of resistance within the same variety will contribute to good resistance.

Potato breeders need to pay some attention to tuber resistance. Unfortunately haulm resistance is not always associated with tuber resistance (Toxopeus, 1958b; Roer & Toxopeus, 1961; Lapwood & McKee, 1961). A significant contribution to breeding will be the incorporation of polygenic resistance into tubers (the haulm is assumed to be resistant).

There is no need to fear the *Phytophthora* fungus. It may be a plastic fungus but history has proved that the variability in plant species is even wider. There is still potato which is resistant in Mexico. Resistance can always be incorporated into varieties. The problems of the blight will always be present as long as there are potato and *Phytophthora.*

There is an urgent demand for the breeding of new varieties with higher yields of better quality and accompanied by specific important characters. The need for variety replacements, in search of better ones, will always be a challenge to old varieties, whatever resistance they possess.

Breeding for resistance to *Phytophthora* requires the use of every possible type of resistance from any species. Incorporating resistance from different wild and cultivated species is expected to produce the formulas needed for the commercial varieties. However, according to the hypothesis presented before, it is expected that related *Solanum* species may carry different polygenic backgrounds. With this in mind, it is possible that hybridization between such related species, in order to combine the resistance of both, may lead to adverse results. It is probable in some of these cases that resistance will not be achieved in the hybrid population, due to the recombination of different polygenic backgrounds.

S. verrucosum as a species having good resistance to *Phytophthora*, can benefit the breeding for resistance. One of its important characteristics is its crossability with many South American species and haploids from common potato. The use of other resistant species, especially diploid Mexican species, has been hampered by their very low crossability with *S. tuberosum*. *S. verrucosum* or its hybrids can be used in direct crossings with haploids, or, after being doubled or crossed and doubled, with the cultivated varieties. No disturbances owing to genome imbalance is expected as *ver* shares A_1 genome with tuberosum (Hawkes, 1958a). The difficulty of crossing *ver* with other species and the expected abnormalities of the hybrids (see also Section 9.8) are small when compared to the barriers to gene exchange between other Mexican species and *S. tuberosum.*

9.8 Potato breeding and the place of S. verrucosum

Potato breeding has been practised along different lines. It is probable that selection of clones has been used on potatoes throughout its cultivation in South America, and in its early cultivation in Europe. Breeding varieties from spontaneous seed set has been reported by Sneep (1966). Hybridization between different clones may also have been employed. Selfing commercial varieties to explore and use the variability has always been the task of breeders and continues up to the present time. Crossing inbreds has sometimes been used to regain the loss in tuber yields and it has given promising hybrids (Krantz & Hutchins, 1929).

Selfing commercial varieties has probably not contributed much to potato breeding, especially in breeding for resistance. As the common potato seems to have narrow genetic variation (Simmonds, 1964b), inbreeding is not expected to lead to much improvement.

After the drastic pandemic in potato caused by *Phytophthora* in the 1840's, breeding for resistance became especially urgent. The best germplasm of resistance against late blight has been discovered in wild Mexican species and these have been crossed with commercial varieties (see Rudorf et al., 1950). *S. demissum* and its hybrid derivatives with *S. tuberosum* have been used extensively for this purpose.

To overcome the difficulties of crossing wild species and tuberosum varieties, diploid species and their hybrids were either treated with colchicine to double their chromosome number, or crossed with the hexaploid *S. demissum* to obtain progenies with 48 chromosomes. Such 48-chromosome species and hybrids were used in crosses with the cultivated varieties (see Rudorf, 1950; Rudorf et al., 1950; Rudorf & Schaper, 1951; Beamish & Shejkal, 1955; Bukasov & Kameraz, 1959). In cases where the wild species were not directly crossable with cultivated ones, bridging species have been used to facilitate crossability (see Hermsen, 1966).

Another way around is now being explored: reducing the chromosome number in cultivated varieties to 24 by inducing unfertilized egg cells to autonomous development through pollinations with certain *S. phureja* clones (extraction of haploids). Great successes have been achieved by this method and the haploids with 24 chromosomes are now being used in crossing with other haploids and with wild and cultivated species (see Hougas & Peloquin, 1958a, b; 1960 a, b; 1962). Breeding at the diploid level, followed by a doubling of the chromosome number and further selection has been proposed by Chase (1963).

It may be expected that breeding at the so-called diploid level will be of great importance in the near future; the return to the tetraploid level may prove to be superfluous. The variability among haploids is promising and some haploid derivatives may outyield commercial varieties (Peloquin & Hougas, 1960; Hougas & Peloquin, 1965). Hybrids with haploids may have the capacity for high tuber production and have been reported by Hanneman & Peloquin (1969). Even if these 24-chromosome clones produce relatively small tubers, such tubers may be valuable for the canning industry and for other purposes.

Several factors favour breeding at the diploid level. The time needed for breeding is shortened, effort, space and money are saved and the breeder's task is simplified. The wide genetic variation from the diploid wild and cultivated species is more easily transferred, owing to the relative ease with which crosses can be made between haploids and the diploid species.

S. verrucosum as a self-compatible, male fertile species, comprising resistance to *Phytophthora* and may possess other good properties, can be used in breeding at the diploid level. As apparent from the results reported on Hybrid 1 (6.6.6.3), some backcross plants have outyielded the haploid parent in tuber production. It is also expected that selection in the backcross generations and the segregating populations will yield productive, *Phytophthora* resistant plants. The introduction of male fertility and self-compatibility will improve crossability which will lead to wider genetic potentialities. This will enhance the success of selection in the segregating populations. One of the most important characteristics of *S. verrucosum* as a *Phytophthora* resistant species is its good crossability with many other species.

The sterilizing plasmon of *S. verrucosum* and its self-compatibility may, to some investigators, appear to be limiting factors for the use of this species in breeding. This is not quite true. It seems that sterilizing plasmons occur not infrequently in *Solanum*. Hougas & Peloquin (1962) and Howard (1968) have found some haploid-species hybrids to be male sterile. This male sterility due to gene-cytoplasm interaction has also been reported by the use of tuberosum plasmon by Ivanov (1939) and by Lamm (1941, 1945, 1953). Apart from *S. verrucosum* and *S. tuberosum*, sterilizing plasmons seem to occur in *S. demissum* (Schnell, 1948; Dionne, 1961b; Pushkarnath & Kishore, 1963), in *S. stoloniferum* (Ross, 1966) and in some diploid species (Koopmans 1951–1959; Grun et al., 1962).

The sterility of hybrids with *S. verrucosum* as a female can be overcome by its use as the male partner. In these studies as well as in those of Propach (1940), Lamm (1945), Buck (1960), Hougas & Peloquin (1960b), Matsubayashi (1961), Grun & Radlow (1961), Pandey (1962c) and Marks (1965a), *S. verrucosum* has been used as male partner. In the absence of unilateral incompatibility genes, *UI* (see 9.4.8), there will be no problems. If *UI* genes are present, it may be possible to remove them by appropriate crosses with plants lacking them. Sib-mated populations from heterozygous self-incompatible species and haploids are expected to possess some plants lacking the rejecting *UI* genes. These plants could be fertilized by *S. verrucosum*. Therefore it is advisable that this species be used on several plants from each population to discover the recessive segregants for *UI* genes.

Even with *S. verrucosum* as the female partner, some F_2 populations have been obtained (Matsubayashi, 1961; Pandey, 1962c; these studies, 6.6.1).

Attention may be drawn to the following possibilities.

(1) Plants might be discovered – though rarely expected – lacking plasmon-sensitive genes. Crossing these with the desired plants, which will be used as male partners on *S. verrucosum*, can remove the effect of such genes. If, in addition, such plants are made recessive for the *UI* genes, the F_1 with female *S. verrucosum* will be self-comp-

atible and male fertile.
(2) It might be possible to obtain fertile plants, or plant branches, on sterile hybrids through cytoplasmic alteration, developing spontaneously or induced by special treatments as reported by Michaelis (1951, 1954; see also Caspari, 1948). Flowers on such plants and branches could be selfed and segregating progenies could be obtained.
(3) It might be possible that some cytoplasm would eventually be transferred to the sterile hybrids through pollen of male parents, resulting in partially or completely fertile backcrosses, which could be selfed or sib-mated.
(4) It might prove possible that non-sterilizing plasmon of *S. verrucosum* exists, or can be developed in the future from the sterilizing ones by special treatments.
(5) Male fertility restoring genes might be discovered in some plants. These could be used to change the sterile hybrids into fertile ones.
(6) Pollen of backcross plants from *S. verrucosum* × other species × *S. verrucosum* can be used to pollinate haploids and species (see 6.16.1, 2). Backcross plants can easily be obtained. Combined verrucosum characters (except its self-compatibility) and those from other species may thus be transmitted.

On the other hand it might be that some day, breeding in potato will be directed towards producing sterile plants to maximize tuber production. In this case the sterilizing plasmon of *S. verrucosum* will be profitable.

Summary

Solanum verrucosum (2n = 24) is a self-compatible, highly fertile species. This conclusion is drawn from the results of studies on 21 various introductions of this species. In spite of self-compatibility and good male and female fertility, the species generally needs a pollinating agent to ensure seed set owing to the supra-staminal pistil characteristic of its flowers.

S. verrucosum is heterozygous for genes controlling qualitative as well as quantitative characters. Differences between and variation within introductions of *S. verrucosum* occur. The species is not resistant to inbreeding. Pre-breeding and selection within the species is demonstrated to be effective and therefore is advisable for its efficient utilization in potato breeding.

Within *S. verrucosum*, F_1 hybrids show a better performance than inbreds. The segregating F_2 show inbreeding depression. Heterosis is more pronounced in early growth stages. In different characters, magnitude of heterosis in F_1 and degree of inbreeding depression in F_2 are associated.

In *S. verrucosum*, 'tall stem' is controlled by two complementary dominant genes, as is obvious in hybrids between the short-stemmed introduction CPC 1339 and the tall introductions CPC 2247 and PI 195172–237–9–13. A similar genetic basis determines early *v.* late flowering in CPC 1339 × PI 195172–237–9–13, late flowering of CPC 1339 being recessive. A significantly negative correlation has been found between flowering time and stem height.

The 'bubble sterility' (pollen showing breakdown of the cytoplasm, staining faintly and looking bubbled) occurring in *S. verrucosum* Haw 2246 is controlled by polygenes. The 'blunt spine walls' of pollen grains (pollen with many coarse blunts on their walls), discovered in the same introduction, behaves as a monogenic recessive character. Both female fertility (number of seeds per berry) and male fertility (percentage of stainable pollen) are found from the study of different populations to be under polygenic control.

Colchicine induced *S. verrucosum* plants with 48 chromosomes show a decrease in male and female fertility but an increase in vigour.

In *S. verrucosum*, grafting onto tomato improves seed set and results in richer flowering and in a more vigorous growth, also in some interspecific hybrids. Grafting has no significant effect on male fertility, either in *S. verrucosum*, or in the male sterile interspecific hybrids.

Upon inoculation with *Phytophthora infestans* Races 4, 1.4, 1.2.3.4, 1.3.4.7.8 and Isolates B 19 and 331, *S. verrucosum* reacts in different ways. Several introductions

have segregated for resistance, others have appeared to consist of either susceptible or almost resistant individuals only. Both major genes and polygenes are assumed to condition resistance. Selection appears effective.

Whether by direct approaches (the use of self-set seed) or indirect (the introduction of self-compatibility), haploids (parthenotes with 2n = 24 from *S. tuberosum*) and self-incompatible species, especially *S. phureja*, proved to be highly heterozygous and suffer greatly from inbreeding. Worthless plants, and segregation for several vegetative and generative characters occur in inbred populations from self-incompatible species. In some cases, the degree of inbreeding is associated with the recessivity of specific genes.

The self-compatible species *S. polyadenium* and *S. etuberosum* are neither crossable mutually, nor with any of the other species studied.

Though barriers reducing berry and seed set and hybrid seed germination are present, *S. verrucosum* is easily crossed with several other species and haploids, but usually only as a female partner: as a rule pollen from self-compatible species is inhibited in styles of self-incompatible ones (unilateral incompatibility).

Hybrids between female *S. verrucosum* and haploids and diploid species generally grow well. In two cases androgenetic plants (2n = 24) have been found among hybrid populations, and in one case a parthenogenetic seed (2n = 24) of *S. verrucosum* has occurred, giving rise to homozygous and homogeneous population. The parthenogenetic population shows reduced vigour but improved seed set and tuber yield and all its plants are resistant to *Phytophthora* Race 1.2.3.4.

All interspecific hybrids with different female *S. verrucosum* introductions are highly male sterile due to plasmon-genic interaction. The dominant plasmon-sensitive genes are wide spread in haploids and diploid species. In the 21 introductions of *S. verrucosum*, three plasmons, [Tr^s], [Ps^s] and [Sv^s], occur, each leading to diagnostic appearance of male sterility in the presence of the corresponding *Tr*, *Ps* and *Sv* dominant plasmon-sensitive genes. The male sterilities are 'tetrad sterility' (generally poor shedding and clumping of the faintly or non-stained sterile pollen grains in tetrads) in [Tr^s] plasmon, 'partly stained pollen sterility' (pollen production normal, but the sterile pollen does not show complete staining) in [Ps^s] plasmon, and 'striped vacuolar sterility' (very poor shedding of faintly stained sterile pollen, characterized by the presence of vacuoles and stripes) in [Sv^s] plasmon. All *S. verrucosum* introductions possess sterilizing (sensitive) plasmons, contrary to a report in literature.

In the backcrosses and segregating populations of the interspecific hybrids with *S. verrucosum* as a female, two additional types of male sterility have occurred: 'undivided microsporocytes' (failure of pollen mother cells to separate into 4 cells, irrespective of nucleus division; consequently microsporocytes are shed, each possessing 4 nuclei) and 'non-shedding' (pollen sacs hardly shedding any pollen). The former type (also discovered in pure *S. verrucosum*) is controlled by two complementary recessive genes, the latter by the presence of three complementary recessive *ns* genes in the [ns^s] plasmon of *S. verrucosum*.

The 'lobed appearance' of pollen grains (each grain having three lobes) is con-

ditioned by dominant *Ld* gene(s) in [Ld^s] plasmon of *S. verrucosum*. Generally plants having [Tr^s] plasmon also possess [Ld^s] plasmon. The *Ld* genes are wide spread, like the dominant *Tr* plasmon-sensitive genes which lead to male sterility. That is why tetrad sterility and lobed pollen are mostly associated in F_1 hybrids. Perhaps this is the reason why the lobed appearance of pollen has been reported by some authors as a type of male sterility. In our studies lobed pollen and male sterility have proved to be different characters. In one cross, duplicate linkage groups between *Tr* and *Ld* genes have been discovered with a crossing over value of 15%.

In all the above cases of plasmon-genic interaction, *S. verrucosum* possesses 'sensitive' plasmons, whereas other species and haploids are assumed to have 'resistant' plasmons.

Self- and cross-incompatibility behaviour in *S. phureja* and *S. stenotomum* × *S. phureja* are controlled by two loci, *S* and *R*. The incompatibility reaction is gametophytically controlled. The *S* locus is epistatic to *R*; the latter has an allele (R_{fi}) which, when present in homozygous condition, leads to female incompatibility. Irregularities observed in the studies of incompatibility may be due to recombining modifier complexes.

Some evidence is presented in the discussion suggesting that the basic chromosome number in *Solanum* may be less than 12.

The evolutionary aspects of self-compatibility and unilateral incompatibility is discussed. A two power competition hypothesis is presented to account for the relation, crossability and the expected behaviour of hybrids between self-compatible and self-incompatible species. Specific genes are assumed to be present in self-incompatible species, preventing fertilization by pollen carrying the self-compatibility alleles (S_c). The hypothesis has shown why such genes must have developed in the course of evolution, what the result of their functioning has been and how self-compatible species react toward their development.

Data from literature on the crosses between self-compatible and self-incompatible species (with the gametophytic system of incompatibility) from several genera of different families have been critically considered and are easily explained on the basis of this new hypothesis.

Breeding for resistance to *Phytophthora* has been discussed. A new hypothesis is presented to combine 'uniform' and 'differential' resistance. Both types of resistance are assumed to be controlled by specialized interacting genes for resistance. The polygenes are 'older' and control the 'primary general' resistance, the major genes govern the direct (switching) work towards specific races.

Breeding at the diploid level and the use of *S. verrucosum* and overcoming the barriers to its use in breeding are debated.

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* Original papers not seen by the author.