Partial resistance of tomatoes against Phytophthora infestans, the late blight fungus

L. J. Turkensteen

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Stellingen

1

Het onderscheid tussen fysiospecifieke resistentie (overgevoeligheidsresistentie) en andere typen van resistentie dient bij afwezigheid van kompatibele waard-pathogeenrelaties niet gemaakt te worden aan de hand van fenomenologische, maar op grond van biochemische criteria.

2

Als in aanmerking wordt genomen, dat de veiligheidstermijn voor de toepassing van het carbamaat 'Zineb' op andijvie 6 weken, op sla, spinazie en radijs 4 weken en op tomaten slechts 3 dagen is, dan dient de consumptie van grote hoeveelheden tomaten ontraden te worden.

Gids voor ziekten- en onkruidbestrijding in land- en tuinbouw, 1973: 174-175.

3

Het pathotype 2,4-p/1-t van *Phytophthora infestans*, dat in 1970 gevonden werd op tomaat in een proefveld met aardappels en tomaten, waarin de pathotypen 1,2,3-p, 1,3,4-p en 1,4,10-p waren geïntroduceerd, is ontstaan uit een heterokaryon van twee van de drie geïntroduceerde pathotypen.

Dit proefschrift.

4

De waargenomen variatie tussen monozoösporecultures gemaakt van een monozoöspore-uitgangsculture van *Phytophthora infestans* (Caten, 1969) behoeft niet uitsluitend op cytoplasmatische factoren te berusten, aangezien *P. infestans* een diploïd vegetatief mycelium bezit (Sansome & Brasier, 1973) en dientengevolge zoösporen niet vrij zijn van genetische variabiliteit.

C.E. Caten, 1969. Spontaneous variability of single isolates of *Phytophthora infestans*. Can. J. Bot., 48: 897-905.
E. Sansome & C.M. Brasier, 1973. Diploidy and chromosomal structural hybridity in *Phytophthora infestans*. Nature, Lond. 241: 344-345.

5

De veelgehoorde mening, dat het niveau van partiële resistentie in landrassen voldoende hoog zou zijn, is ongegrond, omdat dit niveau mede bepaald wordt door de selectiedruk uitgeoefend door het pathogeen.

6

De vrees, dat de oprichting van grote internationale landbouwkundige instituten, zoals CIMMYT, AVRDC en CIP, de beoefening van de plantenziektenkunde in de ontwikkelingslanden nadelig zal beïnvloeden (Buddenhagen, 1973), is ongegrond.

I.W. Buddenhagen, 1973. The international research institutes in relation to future development of tropical plant pathology. Second International Congress of Plant Pathology - Minneapolis, Minnesota - September 5-12, 1973. Abstract of papers: No 0805.

7

Ter vermijding van afwijkingen bij aardappel- en tomateplanten in kassen en fytotrons, zoals waargenomen tijdens perioden met windstil weer in de winterhalfjaren 1971/1972 en 1972/1973, dienen kweekruimten voorzien te worden van ethyleenfilters.

8

Adaptatie van *Phytophthora infestans* aan andere gewassen dan gekweekte aardappeld en tomaten is een mogelijkheid die niet uitgesloten kan worden.

9

In die gebieden van Europa waar 's zomers tomaten buiten worden geteeld, is de invoering van de jaarrondteelt van tomaten de voornaamste oorzaak van de toename van de frequentie en hevigheid waarmee de epidemieën van *Phytophthora infestans* bij tomaten optreden.

Dit proefschrift.

10

De conclusie van Gallegly (1964), dat de partiële resistentie van de tomatelijn W.Va 700 tegen 1-t-pathotypen van *Phytophthora infestans* polygeen bepaald is, is onjuist; de bevinding dat partiële resistentie kwantitatief vererft, mag niet zonder meer tot een dergelijke conclusie leiden.

Dit proefschrift.

11

Bij de introductie van nieuwe tomatecultivars die resistent zijn tegen *Cladosporium fulvum*, dient de teler rekening te houden met een toename van de kans op aantasting van deze cultivars door *Phytophthora infestans*.

12

In verband met de symmetrie in de terminologie van Robinson (1969) wordt voorgesteld de term pathotype te vervangen door hospideme.

R.A. Robinson, 1969. Disease resistance terminology. Rev. appl. Mycol., 48: 593-605.

13

De introductie van, en het werken met het paringstype A_2 van *Phytophthora infestans* in Europa is onverantwoordelijk.

E. Sansome & C.M. Brasier, 1973. Diploidy and chromosomal structural hybridity in *Phytophthora infestans*. Nature, Lond. 241: 344-345.

14

De recente introductie van het paringstype A_2 van *Phytophthora infestans* in Europa geeft aanleiding tot bezinning op de bestrijdingsmethoden tegen de "aardappelziekte" op aardappel en tomaat, dit in verband met de te verwachten veranderingen in de epidemiologie (overblijven van de schimmel in de grond) en de pathogeniteit (versnelde recombinatie van pathogeniteitsfactoren) van de schimmel.

Proefschrift van L.J. Turkensteen
Wageningen, 9 november 1973

Abstract

Turkensteen, L.J. (1973) Partial resistance of tomatoes against *Phytophthora infestans*, the late blight fungus. Doctoral thesis, Wageningen. ISBN 90 220 0498 8, (xv) + 88 p., 23 tbs, 15 figs, 103 refs, Eng. and Dutch summaries. Also: Agric. Res. Rep. (Versl. landbouwk. Onderz.) 810 and Meded. Inst. Phytopath. Res., Wageningen 633.

In the Netherlands, the source of inoculum of the late blight fungus on tomatoes is the late blight fungus on potato crops. In regions of Europe mentioned, where tomatoes are grown in the open, *P. infestans* on tomatoes is the main source of inoculum. Especially in Bulgaria and Hungary, the effect of year-round cropping of tomatoes on prevalence and severity of the disease is obvious.

In field and laboratory experiments, the pathogenicity to tomatoes of *P. infestans* originating from potatoes can be increased by serial passages through tomato foliage. In Europe, no other hosts than potatoes and tomatoes played a role in the tomato late blight epidemidogy. *Phacelia tanacetifolia* is added to the host list of the fungus. For the purpose of genetica classification, two laboratory methods, probit - log dosage analysis and components analysis, and one field method have been developed to assess the resistance of tomatoes against *P. infestans*.

In particular the components infection ratio, lesion extension and sporulation intensity were studied. In the field method, the 'apparent infection rate' r was used. For breeding purposes, a simplified field method was indicated. A comparison of the three methods was made.

The resistance of tomatoes increased considerably until at least 8 weeks after seeding. Partial resistance of W.Va 700 against the 1-t-pathotype of *P. infestans* is based on a single gene, here named Ph_2.

Voorwoord

Mijn oprechte dank gaat uit naar allen die op enigerlei wijze hebben meegewerkt bij het tot stand komen van dit proefschrift.

In het bijzonder wil ik melding maken van de vele waardevolle suggesties van mijn promotor, dr. J.C. Zadoks.

Het onderzoek is verricht aan het Instituut voor Plantenziektenkundig Onderzoek (IPO) te Wageningen. De gelegenheid die mij daar geboden werd in dit werk mijn eigen weg te gaan, is door mij zeer gewaardeerd. Het Laboratorium voor Fytopathologie van de Landbouwhogeschool stelde ruimte beschikbaar in de klimaatkamers. Beide instellingen wil ik gaarne mijn erkentelijkheid betuigen voor hun medewerking.

Van de medewerkers van het IPO wil ik in het bijzonder noemen dr. J.C. Mooi en de heer D. Looyen, die mij de aardappelziekte leerden kennen en met wie ik op plezierige wijze mocht samenwerken. De heer C.F. Scheffel ben ik zeer dankbaar voor het maken van de tekeningen en mevrouw O. Krechting-Janssen voor het typewerk.

Een reissubsidie van het Landbouwhogeschoolfonds maakte het me mogelijk om de problemen betreffende de aardappelziekte bij tomaat in enkele Europese landen van nabij te leren kennen, wat het onderzoek zeer ten goede is gekomen.

De heer W. Hoogkamer verleende mij zijn technische bijstand, die ik zeer op prijs heb gesteld.

Ten slotte bedank ik mijn vrouw en mijn schoonvader voor hun onbaatzuchtige steun en voor het in mij gestelde vertrouwen.

Curriculum vitae

De auteur werd op 4 september 1936 te Nijmegen geboren. Hij volgde een MULO-opleiding te Arnhem en doorliep de Landbouwwinterschool te Zutphen. Na zijn militaire dienstplicht, volgde hij de beide hoogste klassen van de Lorentz-HBS te Arnhem. Hij studeerde vanaf 1960 aan de Landbouwhogeschool te Wageningen en verkreeg in 1971 het diploma van landbouwkundig ingenieur. Vanaf mei 1970 werkte de auteur als promotie-assistent bij het Instituut voor Plantenziektenkundig Onderzoek te Wageningen ter voorbereiding van zijn proefschrift.

Samenvatting

Hoofdstuk 1 In de jaren 1965 tot en met 1968 kwam *P. infestans*, de verwekker van de aardappelziekte, in Nederland epidemisch voor op tomaten in koude en licht gestookte kassen. Vóór deze tijd was de ziekte in kastomaten betrekkelijk onbekend. Het onverwachte optreden van deze ziekte werd behalve door de voor aardappelziekte gunstige klimatologische omstandigheden in deze periode mede beïnvloed door de teelt van tomaten die resistent waren tegen *Cladosporium fulvum*. De resistentie tegen *C. fulvum* had tot gevolg dat chemische bestrijding van deze ziekte achterwege bleef, waardoor *P. infestans* de kans kreeg zich ongehinderd te ontwikkelen.

Omdat behandeling met fungiciden niet altijd afdoende is en residuen van fungicide-middelen het aanzien en de consumptie kwaliteit van tomaten nadelig kunnen beïnvloeden, is resistentie dringend gewenst. Er zijn weinig bronnen van resistentie bekend en het niveau van de gevonden partiële resistentie is ontoereikend om de gewassen zonder de toepassing van fungiciden tegen *P. infestans* afdoende te beschermen. Accumulatie van resistentie door middel van genetische technieken lijkt hier een oplossing, maar de veredeling met behulp van dit type van resistentie is moeilijk, omdat de genetische grondslag van partiële resistentie onvoldoende bekend is, en het effect van partiële resistentie in sterke mate bepaald wordt door milieu-invloeden.

Hoofdstuk 2 In die delen van Europa, waar de tomaat in de open lucht wordt geteeld, is de aardappelziekte de belangrijkste ziekte van de buiten-tomaten en de op drie na belangrijkste ziekte van de kas-tomaten. De algemene mening is, dat de ziekte in de laatste tien jaren veelvuldiger en met grotere hevigheid optreedt. De oorzaak wordt gezocht in de jaarrond-teelt in deze gebieden, die allengs in betekenis toenam. De invloed van de jaarrond-teelt op het optreden van aardappelziekte bij tomaat kan het best toegelicht worden aan het voorbeeld van Bulgarije en Hongarije, waar na de introductie op grote schaal van voorjaars- en najaarsteelt van tomaten in kassen omstreeks 1965 *P. infestans* een enorm probleem geworden is.

Hoofdstuk 3 Om de herkomst van de aardappelziekte op tomaat na te gaan, werd studie gemaakt van de waardplantenreeks van *P. infestans*. Een nieuwe waardplant, *Phacelia tanacetifolia*, kon aan de lijst worden toegevoegd. In Nederland bestaat alle reden om aan te nemen dat het inoculum van de aardappelziekte op tomaten afkomstig is van aardappel. Uit onderzoekingen met herhaalde passages over verouderd tomateblad en jonge zaailingen van tomaat is gebleken, dat de aanvankelijke geringe pathogeniteit voor tomaat van *P. infestans* isolaten afkomstig van aardappel aanzienlijk kon worden verhoogd. Overeenkomstige ervaringen werden opgedaan met oorspronkelijk voor tomaat niet-pathogene *P. infestans*-isolaten in een veldproef met tomaten.

Hoofdstuk 4 Het onderzoek richtte zich op partiële resistentie. De aanpak was tweeledig. Enerzijds werden laboratoriummethoden ontwikkeld voor het meten van partiële resistentie aan enkele, speciaal voor dit doel gekozen 'resistentiecomponenten', zoals 'infectie resistentie', 'uitbreidingsresistentie' en 'sporulatie resistentie'. Anderzijds werd getracht tot een methode van veldtoetsing te komen waarmee verschillen in partiële resistentie bepaald kunnen worden. Het meten van resistentie-componenten vereist de ontwikkeling van technieken voor nauwkeurige dosering van het inoculum. Om het effect van verschillen door milieuinvloeden zo veel mogelijk te beperken, werden de planten opgekweekt en getoetst in de klimaatkamers van het Laboratorium voor Fytopathologie van de Landbouwhogeschool.

Hoofdstuk 5 en 6 De meeste onderzoekingen werden verricht aan planten van de vatbare cv. Moneymaker en aan planten van de lijn W.Va 700, die een hoog niveau van partiële resistentie bezit en daarom waardevol wordt geacht voor de resistentieveredeling. De resistentie van beide tomatelijnen, gemeten aan het aantal geslaagde inoculaties, de uitbreiding van de lesies en de sporulatie intensiteit, neemt met de leeftijd van de planten toe tot ten minste 8 weken na het zaaien.

Hoofdstuk 7 De proefresultaten van 6 weken oude planten van de F_2: Moneymaker × W.Va 700 en de eerste terugkruising Moneymaker × (Moneymaker × W.Va 700) zijn niet en die van 8 weken oude planten zijn wel geschikt voor een genetische analyse van de resistentie van W.Va 700. Zowel de veldproeven als de laboratoriumproeven leidden tot de conclusie dat de resistentie van W.Va 700 tegen het 1-t-pathotype van *P. infestans* wordt bepaald door één enkel gen, dat volledige bescherming biedt tegen stengelaantasting en partiële bescherming

tegen bladaantasting. Dit gen wordt het Ph_2-gen genoemd.

Hoofdstuk 8 Gebleken is dat van de drie toegepaste methoden de probit - log dosis analyse de meest nauwkeurige, maar ook de meest bewerkelijke en duurste methode is. De andere laboratoriummethode, waarmee de groei van de lesies en de sporulatie-intensiteit werd bepaald, was aanmerkelijk minder bewerkelijk maar wel kostbaar. De opzet van de veldproeven, waarin na inoculatie van de besmette rijen iedere week de planten gedurende 40 uur werden nat gehouden door een automatische beregeningsinstallatie, bleek geschikt voor het bepalen van verschillen in partiële resistentie. De maat voor resistentie was de 'schijnbare infectiesnelheid' r, in logit eenheden per dag. Deze methode is aanmerkelijk goedkoper en eenvoudiger dan de beide laboratoriummethoden en is bruikbaar voor de praktische veredeling op resistentie tegen *P. infestans*. Daarbij moet dan wel rekening worden gehouden met het feit, dat bepaalde typen van resistentie, zoals de sporulatie beperkende resistentie van de cv. Atom, in dit soort proeven (continue monocyclische toetsingen) niet tot hun recht komen. Voor een praktische uitwerking van de resultaten, verkregen met de veldproeven, is een vereenvoudigde methode aanbevolen.

Contents

1 Introduction

In the years 1965 till 1968, *Phytophthora infestans* was epidemic on tomatoes in some glasshouse districts of the Netherlands (Anonymous, 1968) The disease caused serious losses in unheated or slightly heated glasshouses. Before 1965, late blight on tomatoes in glasshouses was hardly a problem in the Netherlands. Growers used cultivars susceptible to *Cladosporium fulvum*, and treated their crops with fungicides to control this disease. After the introduction of cultivars resistant to *C. fulvum*, growers ceased to use fungicides, thus giving *P. infestans* an opportunity to develop without hindrance (de Boer, 1966). In the years after 1968, late blight on tomatoes was found sporadically. In the Netherlands, no tomatoes are grown in the open, because of the late ripening of the crop and the high risk of late blight infection.

In countries like Belgium, France, Italy, Bulgaria and Hungary, late blight is considered to be the most important disease of outdoor tomatoes. In glasshouses and in plastic tunnels, *P. infestans* takes the third place among the diseases of tomatoes in the southern part of France, in Italy and in Bulgaria.

In heated glasshouses, late blight can be successfully prevented by means of climatic control. In unheated glasshouses and in the field, the disease can only be controlled by fungicides. This method of control is not always effective. Weather and soil conditions can be too bad to enter the crop, and there is a relatively long harvest period, during which the crop can not be treated in order to avoid undesirable residues. The susceptibility of modern cultivars is such that a delay in treatment is a great production risk; therefore resistance is desirable.

Unfortunately, resistance against *P. infestans* is rare in the genus *Lycopersicon* (Richards & Barratt, 1946). Attempts to utilize a gene for hypersensitivity have been made, but these have been unsuccessful as a compatible race of the fungus appeared already before the breeding program was completed (Conover & Walter, 1953). Other genes for hypersensitivity are not yet known. The experiences gained with this type of resistance against *P. infestans* in potatoes are unfavourable because of the frequent appearance of compatible

races (Schick, 1932; Niederhauser & Cobb, 1959; Malcolmson, 1969). Nowadays, the attention is directed towards partial resistance (see Chapter 4). The life-expectancy of this type of resistance is possibly longer than that of resistance based on hypersensitivity. Breeding for partial resistance is complicated as the genetic background of partial resistance is usually unknown. Many problems about this type of resistance have to be solved, and therefore more research is needed. The success of breeding for partial resistance against *P. infestans* depends partly on the stability of the pathogenicity of the fungus (see section 3.4).

The unexpected appearance of epidemics of *P. infestans* in the Dutch glasshouses gave rise to questions like: 'Which other hosts are important?', 'What is the relation of late blight on tomatoes to late blight on potatoes?' and 'How do tomato races of late blight overcome unfavourable periods?' These questions will be discussed in sections 2.3 and 3.2 and answered in section 3.5.

2 The disease

2.1 INTRODUCTION

The disease has been known to occur on tomatoes since 1847 (Payen, 1847), but very few data on crop losses in the 19th Century are available. At the end of the 19th Century, the growth of tomatoes on a larger scale became popular. Concurrently, the difficulties caused by late blight increased (Howitt, 1917; Reed, 1912; Wiltshire, 1915). The history of late blight in potatoes is well known and will not be discussed (Hänni, 1949; Leach & Rich, 1969; Umaerus, 1968; Upshall, 1969). At present, the disease is a permanent threat to outdoor tomatoes in Europe, and it is an important disease of glasshouse crops.

2.2 SYMPTOMATOLOGY

2.2.1 Symptoms on leaves

Symptoms caused by races, which are weakly pathogenic to tomatoes must be distinguished from symptoms due to races, which are strongly pathogenic to tomatoes (see section 3.4).

The weakly pathogenic races form slowly extending, brown to black coloured lesions, which become angular of shape, as the veins limit the growth of the pathogen. The rate of growth is up to 1 mm a day. The lesions, usually, show no sporulation, but sometimes a slight sporulation can be found on dead tissue. When conditions for late blight are favourable, the fungus may traverse the smaller veins, and the sporulation may be more intense. Normally, lesions cease to grow in an early stage; the majority of lesions does not exceed 2 to 3 mm in diameter. Senescent leaves, however, may be invaded completely, and these leaves can show an abundant sporulation.

The strongly pathogenic races form water-soaked lesions of about 4 mm in diameter, when kept under favourable conditions until two days after infection. Three and a half day after infection the lesions have a diameter of

10 to 15 mm, and sporulation begins in a small zone of apparently unaffected tissue, that surrounds the water-soaked tissue. The linear growth rate of a lesion is up to 5 mm a day. When conditions are less favourable, a typical pigmentation of the infected tissue appears, which results in a dendritical pattern spreading in all directions from the site of infection. Within a few days the centre of the lesion turns light grey to black.

2.2.2 *Symptoms on stems*

Races of *P. infestans*, that are weakly pathogenic to tomatoes cause small black spots of less than 1 mm diameter (rarely up to 4 mm ϕ). Races strongly pathogenic to tomatoes cause large lesions of a typical sepia colour. When the weather conditions are favourable, there may be an abundant sporulation. Often, concentric rings of more and less darkened tissue are present. After about a week, the colour of the lesions fades and becomes palish grey. During windy weather the stalk may break, as the affected tissue loses its elasticity.

2.2.3 *Symptoms on fruits*

Green fruits infected by *P. infestans* first show a reddish brown, featherlike pattern on the skin. Within a few days, the skin turns dark brown, and becomes lumpy, as the underlying tissue dries up. On large fruits, concentric rings of more and less darkly coloured tissue can be found. The rate of symptom development varies according to the pathogenicity of the races tested. Races strongly pathogenic to tomatoes invade the fruits systemically, while weakly pathogenic races usually cause local lesions. The infected tissue dries and becomes hard. Infected fruits can sporulate abundantly whenever the weather conditions are favourable, the fruits then looking white and woolly. Ripe fruits and colouring fruits are very resistant to *P. infestans*.

For differences between symptoms caused by *P. infestans* and *P. nicotianae* var. *nicotianae* (buck eye rot), see Weststeijn, 1973.

2.3 EPIDEMIOLOGY

2.3.1 Epidemiologic parameters

The mathematics of epidemics has been elaborated by van der Plank (1963). An epidemic can be characterized by the apparent infection rate, which is the regression coefficient of the fraction diseased foliage, expressed in logits, on time:

$$r = \frac{1}{t_2 - t_1} \, nl \, \frac{x_2(1 - x_1)}{x_1(1 - x_2)}$$

r = apparent infection rate
t = time
x_t = fraction of diseased foliage at time t
$nl = {}^{e}\log = \log_e$

The apparent infection rate r will be expressed in logit units per day; its dimension is t^{-1}

2.3.2 Rate of epidemics

The most surprising fact of late blight epidemiology is the rapidity, with which the epidemic can run its course. Within a fortnight after the observation of the first lesions the crop can be completely ruined. This is the consequence of the short latent period, the high growth rate of the fungus, the abundant sporulation, and the crowding of many susceptible plants together. Van der Plank (1963, p.184) found $r = 0.42$ for the susceptible potato cultivar Bintje under conditions favourable for late blight. In a field trial, the author found a value of $r = 0.43$ for the susceptible tomato cultivar Moneymaker. $r = 0.43$ implies, that within a fortnight the disease severity can go from 1 to 75 per cent.

2.3.3 Epidemics and chemical control

Chemical control reduces the disease incidence. The disease incidence never becomes zero, as the application of fungicides is never perfect. There always remains a source of inoculum somewhere in a crop. This is a permanent threat, which becomes manifest when conditions are favourable for *P. infestans*. The minimum interval between the last application of zineb or maneb and the

picking of fruits is three days in the Netherlands. The picking period is rather extended, as tomato fruits ripen in succession during a four-month period. This situation causes the grower many difficulties. Either he cannot spray, or he cannot harvest for some days; in both cases, crop losses can occur. In addition, problems of planning and labour arrangement arise.

2.3.4 Epidemics and the ways of tomato growing

In Bulgaria, Hungary, and Italy, growers noticed that in recent years the attacks of *P. infestans* have been more severe than before. In Bulgaria and Hungary, the year of change can be identified. Before 1965, late blight on tomatoes was known as a mild disease, appearing occasionally on tomatoes in autumn. In 1965, the disease caused serious losses, and from 1965 up to 1972 considerable losses occurred nearly every year. In Hungary, tomatoes in the open are damaged in late summer and autumn. In Bulgaria, late blight is also severe in glasshouses in May, June, September, and October.

Bulgaria In Bulgaria, tomatoes have been cultivated on large fields since the collectivization in the period from 1947 up to 1956. About 1965, tomato culture in glasshouses twice a year was initiated on a large scale in Bulgarian glasshouses. Before 1965, late blight attacks on potatoes and tomatoes occurred simultaneously. There was an attack of tomatoes only, if there was an epidemic of late blight on potatoes. After 1965, epidemics of late blight on tomatoes seem to be independent of epidemics on potatoes (Dr H. Helenkove, 1972, pers. commun.). The mutual dependence of *P. infestans* epidemics on tomatoes and potatoes is an epidemiological problem per se (see Section 2.3.5). Informants assume that the disease has become so serious because of the year-round cultivation of tomatoes, thus giving tomato races of *P. infestans* an opportunity to oversummer in the field crops, and to overwinter in the glasshouse crops.

Hungary In Hungary, the cultivation of tomatoes on large fields was introduced about 1965; the cultivation of tomatoes in glasshouses twice a year was begun in the same period. Before this time, tomatoes, potatoes, and other crops were grown in small fields. No reports of serious late blight attacks from the period up to 1965 are known (S. Hodosy, 1972, pers. commun.). In Hungary, where late blight is not important in glasshouses, colleagues suppose that the rise in prevalence and severity of the diseases is due to the

growing of tomatoes on large fields.

Italy In Italy, like in the other south-west European countries, a sudden rise in the severity of attack did not occur. The explanation is sought in a more gradual change of growing methods. Here too, tomato races of *P. infestans* may overwinter in glasshouse tomato crops. An exception has to be made for the southern part of Italy, where winters are so mild, that *P. infestans* can overwinter on plant remnants and seedlings. To explain the increased severity of late blight, Italian growers support the hypothesis that modern high yielding tomato cultivars are more susceptible than the old ones.

New cultivation methods In Bulgaria and Hungary, a sudden rise in prevalence and severity of late blight attacks on tomatoes coincided with the large scale introduction of new methods of tomato growing. Two epidemiologically important factors, cultivation on large fields and year-round cultivation, have been introduced.

In Bulgaria, tomatoes have been grown on large fields without serious damage by late blight. Therefore, the hypothesis that cultivation of tomatoes on large fields promotes epidemics is not sufficient to explain the increased attack in 1965 and following years. The effect of large fields on epidemics is twofold; the larger the field, the more likely the inoculum will come from within the field if it is present (Van der Plank, 1968, p. 132); the larger the fields, the greater the distance between the fields; and the greater the distances between fields, the smaller the chance, that one field infects the other (Van der Plank, 1960).

Another hypothesis is, that the year-round cultivation of tomatoes increases the disease incidence. If this hypothesis is true, there must be a specific reason why late blight is not serious in Hungarian glasshouses. The fact, that late blight in Hungarian glasshouses is of no importance, can be explained in two ways. First, Hungarian growers face attack by *Cladosporium fulvum* in their glasshouses, just as in the Netherlands. Consequently, they treat their crops with fungicides, therewith controlling *P. infestans* as well as *C. fulvum*. Second, the climate in Hungary is cooler than in Bulgaria. Therefore, the heating season for glasshouses in Hungary is longer than in Bulgaria. Heating reduces the humidity of the air, and this is known to be an excellent method of late blight control in heated glasshouses, at least in the Netherlands. (Anonymous, 1968a). The year-round cultivation of tomatoes seems to be the decisive feature in the development of serious late blight epidemics on

tomatoes, thus giving tomato races of late blight the opportunity to overwinter and to oversummer in tomato crops. The cultivation of tomatoes over large areas with a high frequency of tomato crops may be important too, because the opportunity for overwintering and oversummering of the pathogen is improved.

The relation between year-round cultivation and increased incidence of disease has been observed for other host-pathogen combinations (*Chrysanthemum morifolium* and *Ascochyta chrysanthemi*, Sauthof, 1963; *C. morifolium* and *Puccinia horiana*, Zadoks, 1967).

2.3.5 *Relations between epidemics on tomatoes and on potatoes*

In the Netherlands, no late blight attack of tomatoes have been reported when there was no epidemic of late blight on potatoes. Frequently, however, there are epidemics on potatoes without noticable attack of tomatoes. In 1968, when late blight was serious on potatoes and tomatoes, the disease hardly occurred on tomatoes grown in the Westland glasshouse district, where no potatoes are grown (Weststeijn, 1973). In the Netherlands, late blight epidemics of tomatoes evidently depend on late blight epidemics on potatoes.

In Bulgaria, if there was no serious attack on potatoes, there was no epidemic of late blight on tomatoes, before 1965. The situation was the same as in the Netherlands. After 1965, epidemics on tomatoes occurred independently of epidemics on potatoes (H. Helenkove, pers. commun.). It seems possible, that tomato crops in winter are more important as source of late blight inoculum for potatoes than potato tubers. The relation between epidemics on potatoes and tomatoes after 1965 seems to be the inverse of that before 1965.

In Israel, late blight is a serious problem on potatoes and tomatoes except in summertime. In the hot and rainless summer no potatoes are grown; in this period tomatoes are relatively free of infection (Palti & Netzer, 1963). Kedar et al. (1959) tested 25 isolates collected from potatoes on tomato differential hosts. Sixteen of these isolates were pathogenic to tomatoes, thus giving an impression of the importance of late blight on tomatoes as a source of late blight inoculum for potatoes. In the Netherlands, where there is no evidence that tomatoes are important as a late blight source for potatoes, isolates of late blight collected on potatoes never were markedly pathogenic to tomatoes. In Israel, late blight probably oversummers in the tomato crop.

In Italy, tomato races could be collected from potatoes (Ciccarone et al.

1961). Here, many regions have such mild winters that plant remnants and seedlings are not killed by frost, thus offering an opportunity to *P. infestans* to overwinter on tomatoes in the fields.

In the Mediterranean region of France, it is customary to pick the last green fruits in the fields at the end of the season, and to store them in glasshouses or plastic tunnels for ripening. Some of these fruits may be infected by *P. infestans*. When a new crop is grown in the same room, the young plants, which are very susceptible, may be infected by spores formed on diseased fruits. This mode of overwintering might be an explanation of the occasionally occurring severe attack of young plants in glasshouses or plastic tunnels in early spring.

In regard to the mutual dependence of late blight epidemics on tomatoes and late blight epidemics on potatoes, several types of interdependency can be differentiated. Where *P. infestans* overwinters in potato tubers mainly, late blight attacks on tomatoes depend on the development of late blight epidemics on potatoes. This type of one-directional relation is known to exist in the Netherlands (Weststeijn, 1973), and is supposed to have existed in Bulgaria before 1965. In regions, where tomato crops alternate with potato crops in an annual rhythm, the interrelation of the two late blight epidemics is expected to be two-directional. This type of interrelation may exist in Israel and in parts of Japan. In regions, where the climate allows the tomato host to persist continuously either as crops or as plant remnants, late blight epidemics of tomatoes are expected to be independent of epidemics on potatoes. In this case, late blight epidemics on potatoes might depend on late blight epidemics of tomatoes, again a one-directional relation. This type of interrelation is supposed to exist in some Mediterranean areas, and in Bulgaria after 1965.

2.4 ECONOMIC IMPORTANCE OF THE DISEASE

2.4.1 In the Netherlands

In the Netherlands, the disease is important mainly in unheated glasshouses. There is a gradual shift from unheated to heated glasshouses (Table 1). More and more, the cultivation of tomatoes in unheated glasshouses becomes economically marginal. Consequently an attack by late blight can have a decisive effect on the financial outcome of an individual grower. Interestingly,

Table 1. Area of tomatoes in heated and unheated glasshouses in the Netherlands in 1963 and 1972.

	1963	1972
Heated glasshouses	1430 ha	2200 ha
Unheated glasshouses	1400 ha	1000 ha

the disease is found with the occasional tomato grower who is either unexperienced, or speculating insofar as he treats his crops according to his expectations of market prices.

The economic importance of late blight depends, too, on the use of cultivars resistant to *Cladosporium fulvum*. At present, the genes for resistance to *C. fulvum* in the released cultivars have been made ineffective by the appearance of compatible races of this fungus (Koopmans & Strijbosch, 1969). Therefore, this disease has again to be controlled with fungicides. When these fungicides are also effective against late blight, the economic importance of *P. infestans* will be reduced to zero. In 1974, new tomato cultivars resistant to *C. fulvum* will be released again.

2.4.2 *In other European countries*

No data economic aspects of losses caused by the disease are available. During a trip through France, Italy, Bulgaria, and Hungary, informants stated that late blight was the most important disease of outdoor tomatoes, responsible for crop losses estimated at 5 to 30 per cent year, under conditions of regular treatment with fungicides. For glasshouse crops, the disease was said to take the third place in importance among diseases of tomatoes.

3 The pathogen

3.1 BIOLOGY

Phytophthora infestans is a heterothallic species (Smoot et al., 1958; Savage et al., 1968). In Mexico, both mating types A_1 and A_2 can be found in a one-to-one ratio (Gallegly & Galindo, 1958). In Europe, only the A_1-type is known; accordingly, sexual reproduction nor the resulting oospores have been found in Europe. The oospore is the only resting stage of *P. infestans*.

The asexual reproduction, however, is very important. *P. infestans* forms zoosporangia, which can be transported by air and water (rainsplash) (Uhlrich, 1957; Hirst, 1958; De Weille, 1964). The viability of these sporangia is of short duration. In dry weather, the zoosporangia loose their ability to germinate within a few hours (Crosier, 1934). The abundant production of zoosporangia under favourable conditions compensates for the poor ability to survive.

Zoosporangia of *P. infestans* can germinate in two ways. In the case of direct germination, the sporangium forms a hypha, and functions as a single infective particle. Direct germination is favoured by temperatures about 24°C, but it never exceeds 20% of the sporangia (Melhus, 1916; Crosier, 1934), In the case of indirect germination, the zoosporangia germinate by the formation of zoospores, of which up to 38 have been observed originating from a single zoosporangium (Laviola, 1968). The optimum temperature for indirect sporulation is about 12°C. The zoospores are propelled by two flagellae. The duration of the swarming stage depends on environmental factors among which temperature and availability of nutritional substances. At 21°C, most of the zoospores stop moving within 3 h (Crosier, 1934). At 3°C, the zoospores remain motile over 18 h (McKee, 1964). When the swarming stage ends, the flagellae disappear, and the formation of a germ-tube begins. Each zoospore functions as a single infective particle. The formation of germtubes by zoospores is not much affected by temperatures between 3 and 21°C (Crosier, 1934). At temperatures between 15 and 18°C, the first penetration of leaf tissue of a susceptible host is effective within 2 h after inoculation with

motile zoospores (Crosier, 1934; Pristou & Gallegly, 1954).

On good evidence, Mexico is considered as the centre of origin of *P. infestans*. First, it is the only region in the world, where both mating types of *P. infestans* are known to exist (Smoot et al., 1958), and where the sexual stage of *P. infestans* is supposed to be functional (Gallegly & Galindo, 1958). Second, there is much diversity in pathotypes of the fungus (Niederhauser & Mills, 1953), and great diversity in resistance in the *Solanum* species indigenous in this area. This diversity on both sides can only be explained by the prolonged presence of both partners in this region (Reddick & Crosier, 1933; Mills & Niederhauser, 1953).

3.2 HOST RANGE

3.2.1 Introduction

The host range of *P. infestans* includes a number of species belonging to the Solanaceae and closely related genera, and a few species belonging to rather distantly related genera. These species are food crops, ornamentals, and wild plants. It is important to know, whether some of these species apart from *Solanum tuberosum* and *Lycopersicon esculentum* can function as a source of late blight inoculum. Enumerations of late blight host species have been made by several authors (Table 2).

In some species the foliage was not susceptible, but the fungus was sporulating well on infected tissues of petals, fruits, tubers, or roots. Successful inoculations have been made on petals of *Brassica oleracea* L., *Phaseolus multiflorus* Lam. (Müller, 1950), *Nerium oleander* L., and *Vinca rosea* L. (Sztejnberg & Wahl, 1966), on fruits of *P. multiflorus* Lam., *Solanum capsicastricum* Link. (Müller, 1950), and *Phaseolus vulgaris* L. (Sztejnberg & Wahl, 1966), and on tuber tissues of *Beta vulgaris* L., and bulb scales of *Allium cepa* L. (Müller, 1950).

A few species showed more or less clear lesions after infection, but without sporulation. These species are: *Dahlia variabilis* Hort., *Anemona japonica* Hort., *Lactuca sativa* L., *Phaseolus multiflorus* Lam., *Brassica oleracea* (Müller, 1950), *Lyceum europeum* L., *Nerium oleander* L., and *Vinca rosea* (Sztejnberg & Wahl, 1966).

Table 2. Host range of *Phytophthora infestans*

Host	Author
Solanum L.	
S. antipoviczii Buk.	Reddick, 1930
S. aviculare Forst	Driver, 1957
S. boreale (A. Gray) Bitt.	Niederhauser & Mills, 1953
S. cardiophyllum Lindl.	Niederhauser & Mills, 1953
S. capsicastricum Link ex Schauer	Müller, K.O., 1950
S. crispum Ruiz et Pav.	Moore, 1943
S. demissum Lindl.	Niederhauser & Mills, 1953
S. dulcamare L.	De Bary, 1876; Hirst & Stedman, 1960
S. incanum L.	Nattrass & Ryan, 1951
S. indicum L.	Nattrass & Ryan, 1951
S. iopetalum (Bitt.) Hawkes	Niederhauser & Mills, 1953
S. jamesii Torr.	Niederhauser & Mills, 1953
S. lacineatum Ait.	Driver, 1957
S. melongena L.	Haskel, 1921
S. nigrum L.	Hirst & Stedman, 1960
S. panduraeforme Drége	Nattrass & Ryan, 1951
S. pinnatisectum Dun.	Niederhauser & Mills, 1953
S. pyracanthum Jacq.	Sztejnberg & Wahl, 1966
S. rostratum Dun.	Kotila, 1949
S. simile F. Muell.	Driver, 1957
S. sambucinum Rydb.	Niederhauser & Mills, 1953
S. stoloniferum Schlecht et Behe	Niederhauser & Mills, 1953
S. tuberosum L.	Montagne, 1845
S. villosum (L.) Lam.	Sztejnberg & Wahl, 1966
S. xanthacarpum Schrad. & Wend.	Russel, 1969
Lycopersicon Mill.	
L. chilense Dun.	Richards & Barratt, 1946
L. esculentum Mill.	Payen, 1847
L. hirsutum Humb.	Richards & Barratt, 1946
L. peruvianum (L.) Mill.	Richards & Barratt, 1946
L. pimpinellifolium (Jusl.) Mill.	Richards & Barratt, 1946
Solanaceous species not belonging to the genera *Solanum* and *Lycopersicon*	
Anthocercis viscosa R.Br.	Berkeley, 1846 (cfr De Bary, 1876)
Datura metel L.	Sztejnberg & Wahl, 1966
Datura stramonium L.	Sztejnberg & Wahl, 1966
Hyoscyamus aureus L.	Sztejnberg & Wahl, 1966
Hyocyamus niger L.	Reddick, 1928
Lycium halimifolium Mill.	Reddick, 1928
Lycium turcomannicum Turcz.	Reddick, 1928
Mandragora officinarum L.	Sztejnberg & Wahl, 1966
Mirabilis jalapa L.	Romero & Fourton, 1963
Nicotiana glauca Grah.	Sztejnberg & Wahl, 1966
Petunia hybrida Hort.	Hirst & Moore, 1957; Sztejnberg & Wahl, 1966
Physalis alkengi L.	Reddick, 1928
Physalis angulata L.	Peterson, 1947
Salpiglossis sp.	Peterson, 1947
Schizanthus grahami N.H.	Reddick, 1928; De Bary, 1876
Schizanthus pinnatus R. & P.	Peterson, 1947
Withiana somnifera L.	Sztejnberg & Wahl, 1966

3.2.2 *The tomato*

The genus *Lycopersicon* Mill. is closely related to the genus *Solanum* L. (Rick, 1960). The centre of origin of the genus *Lycopersicon* is sought in the coastal region of South America between the Andes mountains and the Pacific Ocean, from the equator to about 30^0 S.L., and in the Galapagos Islands (Luckwill, 1943; Rick, 1960). A second centre is supposed to be in Mexico, in the Veracruz Pueblo area in particular (Jenkins, 1947). The two *Lycopersicon* species in this centre, which is probably a secondary centre, are *L. esculentum* Mill. (cultivated tomatoes) and *L. pimpinellifolium* (Jusl.) Mill. (currant tomatoes). The latter is sometimes considered to be subspecies of *L. esculentum*. All species of *Lycopersicon* are rather susceptible to *P. infestans* (Richards & Barratt, 1946; Alexander & Hoover, 1955).

In the South American centre of origin of tomatoes, late blight was not known before 1947 (Cox & Large, 1960), and there has been no selection pressure for resistance to *P. infestans* until that time. The first encounter between the genus *Lycopersicon* and *P. infestans* must have taken place in Mexico, where the tomato was imported in pre-Columbian times (Jenkins, 1947), Mexico supposedly being a centre of origin of *P. infestans* (see Section 3.1). In evolutionary perspective, the period of confrontation is short. This shortness may at least partially explain the rarity of resistance to *P. infestans* in the genus *Lycopersicon*. The lines of *L. pimpinellifolium* with late blight resistance often seem to be of Mexican origin (Gallegly, 1952).

3.2.3 *The potato*

The potato is the most common host of *P. infestans* in Europe. There is much literature available on this subject, and it will not be discussed here Some aspects of the relations between the potato and *P. infestans*, relevant to the subject of late blight on tomatoes, are mentioned in 2.3.4, 2.3.5, 3.4.2, and 3.4.3.

3.2.4 *Solanum dulcamara L.*

In 1971, 60 *S. dulcamara* plants were grown from cuttings taken from plants at 5 different locations in the neighbourhood of Wageningen. They were planted in a 25 m^2 plot situated in an experimental field used for the testing of potatoes for resistance to *P. infestans*. The potato plots were inocu-

lated with the pathotypes 1,2,3-p, 1,3,4-p, and 1,4,10-p (see Section 3.4.1). Though the weather conditions were unfavourable for late blight development, the foliage of susceptible cultivars like 'Bintje' and 'Eersteling' were destroyed completely by late blight within a month after inoculation. Only a few lesions were found on plants of *S. dulcamara*. The infected leaves dropped within a few days after the first observation of the lesions.

In 1972, 6 *S. dulcamara* plants were grown in an experimental field used to test tomatoes and potatoes for resistance to *P. infestans*. The development of late blight was favoured by sprinkler irrigation (see Section 5.3.3). For inoculation, the pathotypes 1,2,3-p, 1,4,10-p, and 2,4-p/1-t were used. The conditions for late blight development were quite favourable, and a large amount of inoculum was available during the epidemics on potatoes and tomatoes, but only a few lesions were found on the leaves of *S. dulcamara*. The leaves, on which lesions were found, dropped within a few days after the first observation of the lesions. No sporulation was observed on the lesions.

Apparently, the *S. dulcamara* plants tested were very resistant to the pathotypes used (see also Section 3.4.8).

3.2.5 *Solanaceous hosts, excluding potatoes and tomatoes, with potential survival value to the fungus in crop-free periods*

Some perennial solanaceous plants provide *P. infestans* with an opportunity to survive periods without potatoes or tomatoes in the field. During crop-free periods, *P. infestans* has been found on *S. aviculare* Forst. and *S. lacineatum* Ait (Driver, 1957). In Kenya, the gap between two successive crops is a short one, as two crops per year are grown. In New Zealand (Auckland), the situation is somewhat more complicated, as no crop-free period is present. Both countries are characterized by a climate without frost.

In Israel, wild Solanaceae and cultivated plants of this family, except potatoes and tomatoes, do not support the propagation and dissemination of the pathogen either in winter or in summer (Sztejnberg & Wahl, 1966). Peterson (1947) concluded, that there is no evidence of overwintering of *P. infestans* on perennial Solanaceae in Long Island, U.S.A. According to Cox & Large (1960), there is no evidence for the overwintering of *P. infestans* in any solanaceous plant in parts of the United States marked by cold winters. Accordingly, it is unlikely that *P. infestans* overwinters in wild solanaceous plants in those parts of Europe, where frequent and/or prolonged periods with temperatures below 0^0C occur.

3.2.6 *Phacelia tanacetifolia, a new host species of P. infestans*

In 1972, *Phacelia tanacetifolia* Benth (fam. Hydrophyllaceae) was grown for apicultural purposes on a strip of land adjacent to an experimental plot, where potato and tomato cultivars were tested for late blight resistance. Overhead irrigation by sprinkling was applied to stimulate the development of *P. infestans*. When the weather conditions were favourable for late blight development, the late blight fungus was repeatedly found to sporulate on leaves and inflorescences of *P. tanacetifolia*. Late blight infection was marked by necrotic reactions, and the infected leaves turned darkly red. The late blight isolates obtained from the infected tissues were only slightly pathogenic to leaves of *P. tanacetifolia* taken from field grown plants, and subsequently tested in the laboratory by inoculating and incubating the leaves as described in 4.4. Though the leaves were covered with small necrotic spots (up to 2 mm), no sporulation was observed. The disease was never serious in the field, and, when the plants grew older, late blight was only sporadically found.

3.2.7 *Development of P. infestans on Datura stramonium, Mirabilis jalapa, and Schizanthus pinnatus*

Introduction In 1971, an experiment was designed to investigate the possibility of stimulating the development of late blight epidemics by overhead irrigation. There were three fields with different sprinkling regimes, and on each field there were plots with tomatoes, potatoes, and the ornamentals *Datura stramonium* L. (fam. Solanaceae), *Mirabilis jalapa* L. (fam. Nyctaginaceae), and *Schizanthus pinnatus* R. & P. (fam. Scrophulariaceae). The reaction of these ornamentals to late blight under the regime with the most frequent sprinkling is discussed below.

Materials and methods The field plot with ornamentals contained two rows of respectively 8 and 7 *Datura* plants, one row of 8 *Mirabilis* plants and three rows of *Schizanthus* plants (20 plants per m). The space between the rows was 50 cm, and the row length was 6 m. Sprinkling took place at night between 19 h 00 and 21 h 00 with one sprinkling period of 150 s per 15 min, thus giving a 2 mm precipitation per night. The potatoes had been inoculated on 71.06.16 with a 1,2,3-p-pathotype, and the first sporophores on the inoculated potatoes were observed on 71.06.23. The day of inoculation will be indi-

cated as the zero day of this experiment. The ornamentals were inspected daily for late blight symptoms. Lesions suspected to be caused by *P. infestans* were collected for reisolation of the fungus on potato tuber slices (see Section 3.3.2).

Mirabilis jalapa Many brown coloured spots with diameters up to 2 cm have been found on the leaves of the Mirabilis plants, but attempts to reisolate *P. infestans* from these spots were unsuccessful.

Datura stramonium On *Datura*, the lesions caused by *P. infestans* were few but large. The first two lesions were observed on day 34, and day 50, respectively. When the weather conditions were favourable for late blight development, the lesions expanded quickly, and abundant sporulation was visible on apparently healthy tissue surrounding the lesions. *P. infestans* was frequently isolated from infected tissue. When the weather was too dry for the development of late blight, the infected tissue dried, became brittle, and desintegrated. Ultimately, only an angular hole was left, and no fungus could be isolated from the surrounding tissue. The disease severity never exceeded 5%.

Schizanthus pinnatus On day 26, the first lesions due to late blight were found on *Schizanthus*. On day 56, 95% of the foliage was destroyed by *P. infestans* (Fig. 1). A heavy infection of the stalks caused lodging of the plants. Sporulation was abundant on leaves and stalks, almost without preceeding discoloration of the host tissues. The apparent infection rate was 0.23. Although the primary inoculum must have come from the late blight developing on the potato plots, the major part of the inoculum seems to have originated from late blight developing within the crop, as evidenced by the presence of abundant sporulation on *Schizanthus*.

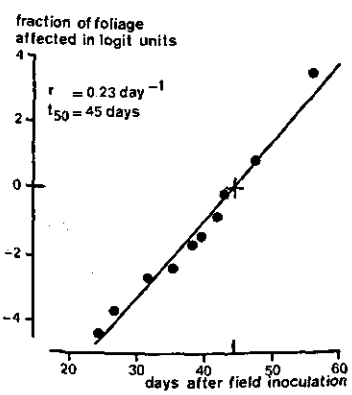

Fig. 1. *Schizanthus pinnatus*, Wageningen. Progress of a *Phytophthora infestans* epidemic in a field experiment with artificial inoculation and overhead irrigation.

Conclusions The material of *Mirabilis jalapa* used in this test was resistant to the *P. infestans* pathotype used. The *Datura stramonium* plants tested here showed a considerable degree of resistance to the late blight pathotype used. In *Datura stramonium*, two resistance mechanisms seem to operate: resistance to infection, and resistance by elimination of the fungus under conditions unfavourable to the late blight fungus. *Schizanthus pinnatus* showed to be susceptible.

3.3 METHODS OF ISOLATION AND CULTURE

Artificial media Many artificial media suitable for the cultivation of *P. infestans* are known. Some are completely synthetic (Wilde, 1961), others are partially composed of natural materials like rye, oats or lima beans (Snieszko et al., 1947; Hodgson & Grainger, 1964). On most of these *P. infestans* grows well. The fungus looses its pathogenicity after prolonged culture on artificial media (Jinks & Grindle, 1963) partly or completely. The use of artificial media to grow *P. infestans* for the purpose of pathogenicity tests should be discouraged.

Natural substrates Most of the late blight isolates were cultivated according to a method developed on the Institute for Phytopathological Research (IPO), Wageningen. Every week, non-sterilized slices of washed tubers of the potato cv. Bintje were inoculated, by picking up with a needle some mycelium from slices inoculated the week before, and passing this over the surface of fresh slices. The inoculated slices, stored in 9 cm petri-dishes, were kept at 15^0C in the dark. Some isolates pathogenic to tomatoes were growing poorly on potato slices, and did not sporulate at all. These isolates were grown on tomato leaflets stored in translucent shoe cases. The leaflets were irradiated by a single 40 W fluorescent tube (Philips TL 40 W/33) during 16 h per day. The leaflets were mounted in small 'bricks' of a spongy substance on urea-formaldehyd base manufactured for the purpose of floral arrangement (trademark 'Oasis', commercialized by V.L. Smithers A/S, Ganløse - Malmøve - Danmark). Every week a fresh leaflet was placed in close contact with the old one, whereupon the fungus invaded the new leaflet. The use of very young leaflets or leaflets of tomato plants younger than 6 weeks is not recommended, because of their susceptibility to bacterial rot.

Isolation The isolation of *P. infestans* from infected tissue was realized

by placing excised parts of the infected tissue under potato slices in petri-dishes. The petri-dishes were stored at 15°C in the dark. The fungus grew into the tuber tissue, and within a week it sporulated on the upper surface of the slices. When the inoculated fungus grew poorly on potato slices, a plug of the infected tuber tissue or a part of the original tissue (if still available) was used to inoculate a green tomato fruit. For that purpose, the infected tissue was transferred to a drop of deionized water, which was placed on the fracture resulting from the removal of the calyx. After 2 or 3 days, a small strip of the epidermis was removed from the fruit by means of a razor, to facilitate fructification. According to the pathogenicity of the isolate to tomatoes, sporulation could be observed within 5 to 12 days.

3.4 VARIABILITY IN PATHOGENICITY

3.4.1 Terminology

An attempt is made to apply and/or to adjust the terminology of Robinson (1969) to the available data on pathogenicity of *P. infestans* to various host species.

The deme system The deme system, devised by Gilmour & Heslop-Harrison (1954), is a system of classification to denote a set of individuals within a specified taxon having at least one characteristic in common. The suffix deme must be used with a prefix to describe this particular characteristic. For the purpose of disease resistance terminology, Robinson (1969) proposes to reserve the suffix deme for the host, and to use a parallel system with the suffix type for the pathogen. Both suffixes must be combinated with the prefix patho. In Robinson's terminology, a pathodeme is a population of a host in which all individuals have a particular character of resistance in common.

Unfortunately, the term 'host' may cause confusion, because this term sometimes refers to a susceptible individual or susceptible cultivar, and sometimes to a plant taxon (species, genus, or higher unit of classification). Furthermore, the term 'host' can be confusing in the combination 'host range', as a host range is little more than a set of cultivars or a set of species placed together according to a phytopathological criterium. Therefore, it is convenient to adhere closely to the original deme definition, and to define a pathodeme as a set of individuals within a specified taxon having at least one character of resistance in common. The pathodeme and corresponding patho-

Table 3. *Phytophthora infestans* on potatoes and tomatoes. Skeleton survey of pathodemes and pathotypes. Three theoretically possible pathodemes and corresponding pathotypes are represented. A late blight pathotype only pathogenic to tomatoes is not yet known.

Hosts	Taxon	
	Solanaceae	*P. infestans*
S. tuberosum	p-pathodeme	p-pathotype
L. esculentum	t-pathodeme	t-pathotype
S. tuberosum and *L. esculentum*	p/t-pathodeme	p/t-pathotype

p = potato
t = tomato

type can be described by a letter or combination of letters, denoting the host range (Table 3). Any desirable subdivision can be made for every pathodeme or pathotype, and indicated by an appropriate symbol (Table 4). For this purpose, the following terms are introduced:

Uniform (U) A 'uniform pathodeme' is characterized by 'uniform resistance'. 'Uniform resistance' is resistance, that acts equally to all pathotypes tested. The term 'uniform resistance' has been proposed by Van der Plank (1969) to replace the term 'horizontal resistance' introduced earlier (Van der Plank, 1963).

A 'uniform pathotype' is a pathotype characterized by 'uniform pathogenicity'. 'Uniform pathogenicity' acts equally to all pathodemes, characterized by 'uniform resistance'. The term 'uniform pathogenicity' replaces the term 'horizontal pathogenicity' introduced by Robinson (1969).

Differential (D) A 'differential pathodeme' is characterized by 'differential resistance'. 'Differential resistance' is resistance, that does not act equally to all pathotypes tested, but shows differential interaction. The term 'differential resistance' is introduced (Van der Plank, 1969) to replace the term 'vertical resistance' (Van der Plank, 1963).

A 'differential pathotype' is characterized by 'differential pathogenicity'. 'Differential pathogenicity' is pathogenicity, that does not act equally to pathodemes characterized by 'differential resistance'. The term 'differential pathogenicity' replaces the term 'vertical pathogenicity' introduced by Robinson (1969).

Table 4. *Phytophthora infestans* on tomatoes. Theoretical scheme of pathotypes characterized according to host range and type of resistance per host. The symbol + stands for pathogenicity to the host resistance type indicated in the respective column heads. Not all of these theoretically possible pathotypes have been found.

Hosts				Pathotypes
S. tuberosum		*L. esculentum*		
D	U	D	U	
+				D.p-pathotype
	+			U.p-pathotype
+	+			Two-dim.p-pathotype
+		+		D.p/D.t-pathotype
+			+	D.p/U.t-pathotype
+		+	+	D.p/Two-dim.t-pathotype
	+	+		U.p/D.t-pathotype
	+		+	U.p/U.t-pathotype
	+	+	+	U.p/Two-dim.t-pathotype
+	+	+		Two-dim.p/D.t-pathotype
+	+		+	Two-dim.p/U.t-pathotype
+	+	+	+	Two-dim.p/two-dim.t-pathotype

D - Differential
U - Uniform

Two-dimensional (Two-dim.) A 'two-dimensional pathodeme' is characterized by 'two-dimensional resistance'. The term 'two-dimensional resistance' was introduced by Zadoks (1972) to indicate the situation when differential and uniform resistance both are present in a single host genotype.

A 'two-dimensional pathotype' is characterized by 'two-dimensional pathogenicity'. There is a uniform action against uniform resistance, and a differential action against differential resistance.

3.4.2 *Differential pathogenicity to various hosts*

Potato The most striking observation on the variability in pathogenicity of *P. infestans* concerns the successive appearance of pathotypes capable to overcome the resistance of newly introduced potato cultivars. This resistance is usually characterized by a hypersensitive reaction, controlled by single dominant genes originating from *Solanum demissum*. These genes are called *R* genes. In 1920, German breeders started to utilize this type of resistance. In 1932, the first report was published on isolates that could attack a cultivar carry-

ing an *R* gene (Schick, 1932). From 1932 till now, many cultivars originally unaffected because of the presence of one or more *R* genes became attacked by *P. infestans* in the long run. To-day, 11 *R* genes have been identified. Pathotypes matching these 11 *R* genes singly or in combinations have been found (Malcolmson & Black, 1966). 2^{11} differential late blight pathotypes are possible, and the 1,2,3,4,5,6,7,8,9,10,11-p-pathotype can match all *R* genes known. This pathotype has not yet been found, but Malcolmson (1969) identified two late blight pathotypes with differential pathogenicity, 1,2,3,4,5, 6,7,8,9,10-p and 1,2,3,4,5,6,7,10,11-p respectively. Apparently, the continued use of *R* gene resistance is not the ultimate solution of the problem of late blight control.

Tomato Tomato breeders met with a similar problem after the introduction of the Ph_1 gene for hypersensitivity, that has been found in an ornamental tomato of the species *Lycopersicon pimpinellifolium*. This gene, originally called TR_1 (Gallegly & Marvel, 1955), provided resistance to late blight at the time of introduction into the breeding program. During the execution of the program, a new and compatible late blight pathotype appeared, and caused considerable damage (Conover & Walter, 1953; Gallegly & Marvel, 1955).

Tomato & potato Differential pathogenicity to tomatoes has been found to be independent of differential pathogenicity to potatoes as determined by means of pathodemes carrying the genes Ph_1, R_1, R_2, R_3, and R_4. (Wilson & Gallegly, 1955; Kedar et al., 1959; Kishy, 1962).

Other hosts There have been no investigations into the differential pathogenicity to other solanaceous species. A few observations are reported, however, on the existence of late blight isolates, collected from potatoes, which differed in the ability to infect plants of some species like *Solanum incanum* (Nattrass & Ryan, 1951), *S. villosum* and *Withiana somnifera* (Sztejnberg & Wahl, 1966).

3.4.3 Variability in pathogenicity to potatoes and tomatoes

In early publications, no reference has been made to a special relation between isolates of *P. infestans* from potatoes and those from tomatoes (Payen, 1847; Thaxter, 1891; Clinton, 1903; Smith, 1906). In later papers, references to a kind of biological specialization can be found (Wiltshire, 1915; Giddings

& Berg, 1919; Berg, 1926; Small, 1938). Isolates of *P. infestans* collected from potatoes were said to attack tomato leaves mildly at most. Isolates of *P. infestans* collected from tomatoes, on the contrary, severely infected tomato leaves. This difference between potato and tomato isolates with respect to the pathogenicity to tomatoes was found to be stable during at least one year, when isolates of *P. infestans* (pathogenic to tomatoes) were grown continuously in potato tubers (Berg, 1926; Röder, 1935; Small, 1938).

In 1940, Mills described a phenomenon which he called adaptative parasitism. Starting with monozoospore cultures of *P. infestans* originating from potatoes, he made several passages through tomato foliage. He noticed that the pathogenicity of isolates gradually increased during a sequence of passages. After 7 passages, no differences could be seen between the 'transfers' and the isolates of *P. infestans* originating from tomatoes. The change in pathogenicity proved to be stable during half a year of culturing in potato tubers. The pathogenicity to potato foliage was not affected by the successive passages through tomato leaves. Graham et al. (1961) obtained similar results. De Bruyn (1952), too, made passages through tomato foliage with monozoospore cultures of isolates originating from potatoes. She found that the acquired pathogenicity to tomatoes was stable, when she made passages with the changed isolates through leaves or tubers of clones of *Solanum tuberosum*. When she made passages through leaves or tubers of clones of resistant derivatives from the cross *S. demissum* × *S. tuberosum* or from their successive backcrosses to *S. tuberosum*, she noticed that the acquired pathogenicity to tomatoes could be lost. Conversely, the acquired pathogenicity to *R* gene resistant potato clones could be lost by successive passages through tomato foliage.

Kishy (1962) collected isolates of *P. infestans* from potato crops in the fields. He subdivided the isolates into three groups according to their pathogenicity to the tomato cv. Ponderosa: 'potato type', 'intermediate type', and 'tomato type' with respectively slight, moderate, and severe attack. By making passages through tomato leaves, he gave isolates, which were moderately pathogenic to tomatoes at the start, a level of pathogenicity equivalent to that of the tomato type. Kishy did not succeed in changing potato types into tomato types, as the potato type had declined after four passages.

It is evident, that the pathogenicity to tomatoes of *P. infestans* isolates, originating from potatoes, can be changed by means of passages through tomato foliage. Most reports on this phenomenon agree in two respects: the acquired pathogenicity to tomatoes is stable, and the pathogenicity to potatoes

is not affected. However, if the pathogenicity to potato is not affected, isolates of *P. infestans* pathogenic to tomatoes must have a selective advantage over isolates non-pathogenic to tomatoes. When this hypothesis is correct, p-pathotypes of *P. infestans* should have been replaced by p/t-pathotypes whenever tomatoes form part of the continuous chain of hosts. This conclusion is not confirmed by the present-day situation (Kedar et al., 1959; Ciccarone et al., 1961; Kishy, 1962). So, p-pathotypes must have something to withstand selection pressure in favour of p/t-pathotypes. As p-pathotypes and p/t-pathotypes compete on potatoes only, p-pathotypes must have a compensating selective advantage over p/t-pathotypes on potatoes. Consequently, p/t-pathotypes cannot be equally fit on potatoes as p-pathotypes, and pathogenicity to potatoes must be affected.

Hypothetically, the acquired pathogenicity to tomatoes can be explained by several mechanisms or combinations of mechanisms like mutation, selection, and adaptation to new substrates in the sense of 'dauer modifications'. Mills (1940), De Bruyn (1952), and Graham et al. (1961), who performed serial passages with monozoospore cultures, used to inoculate with large numbers of spores. They inoculated with populations of the fungus, populations that were intended to be genetically uniform, but as mutations can occur in the period needed to produce the mass inoculum, there is no certainty that these populations were still uniform at the moment of mass inoculation. Consequently, the observed changes in pathogenicity could have been changes at the population level. As no detailed investigations have been reported nothing is known of the frequency nor of the nature of change.

3.4.4 The appearance of a pathotype highly pathogenic to tomatoes in the field

Introduction In 1970, a field experiment was designed with the purpose to collect late blight isolates pathogenic to tomatoes. These pathotypes were needed for investigations on resistance of tomatoes against late blight.

Materials and methods Four small tomato plots were planted side by side to potato plots in an experimental field designed for the purpose of testing the field resistance of potato culticars against *P. infestans*. Three plots (1,5 × 4 m) were planted, each with 2 rows of 8 Moneymaker plants. The fourth plot (4 x 4 m) was planted with 16 Moneymaker plants, 8 plants of the line W.Va 700, and 4 plants of each of the following lines: Ottawa 30, Ottawa 31, Ottawa

33, New Hampshire Surecrop, Jamaica Cherry, Rockingham, and North Carolina 1951-315-N. The lines were distributed at random over the plot. Apart from Moneymaker, all these lines were characterized by resistance to 0-t-pathotypes of *P. infestans*.

On 70.07.07 (day zero), the potato plots and all 16 plants of one of the 3 smaller tomato plots were inoculated with a mixture of the pathotypes 1,2, 3-p, 1,3,4-p, and 1,4,10-p by spraying a suspension of 1000 zoospores per ml of each pathotype. Isolates were collected from tomatoes, whenever lesions suspected to be caused by *P. infestans* were present. The isolates were tested for pathogenicity to tomatoes by spraying a suspension of zoosporangia (10,000 per ml) over leaves of 8 to 10 weeks old Moneymaker plants. The leaves were inoculated as described in 4.4. The criterion for pathogenicity P was the percentage affected leaf area on day 7.

Results In the period from day 6 to 74, only small and necrotic lesions were found on the leaves of the cv. Moneymaker; the other tomatoes were apparently free from attack. Fruits of all tomato plants were seriously affected. Isolates collected from fruits and leaves were only slightly pathogenic to tomatoes, $P<10$. On day 75, 3 water-soaked lesions were found on leaves of a Moneymaker plant in the largest tomato plot; the next day the lesions and apparently unaffected surrounding tissues were covered with sporophores. Reisolates taken from one of these lesions and tested in the laboratory produced $P = 100$. These isolates belonged to the 2,4-p/1-t-pathotype. On day 82, two foci of heavy late blight attack were present in the largest tomato plot, and on day 112, the severity of attack on cv. Moneymaker was $S \geq 95$. From the other lines in this plot, Jamaica Cherry, New Hampshire Surecrop, and Rockingham, all carrying the Ph_1 gene for resistance to late blight, were nearly as seriously affected as Moneymaker. W.Va 700 and North Carolina were the most resistant; on day 112, $S \leq 50$. In the other tomato plots, no serious attack was observed. One isolate, collected from W.Va 700 (isolate T 7023) and belonging to the pathotype 2,4-p/1-t has been used for all resistance tests described in this paper.

Conclusion On day 112 of the experiment a reisolate was collected from a tomato plant, which was highly pathogenic to tomatoes with and without the Ph_1 gene. This reisolate, belonging to the 2,4-p/1-t-pathotype, differed from the initial pathotypes not only in pathogenicity to tomatoes, but differed also in differential pathogenicity to potatoes from all three initially introduced

pathotypes. It was remarkable that this new pathotype had not spread to the other plots of the experimental field.

Discussion The origin of the 2,4-p/1-t-pathotype is not clear. There are two possibilities: it came from elsewhere, or it originated in the experimental field. Concerning the first possibility, it must be remarked that (1), the 2,4-p-pathotype is always rare in the Netherlands, (2), in 1970 the weather conditions were unfavourable for late blight attack, and (3), no late blight on tomatoes was reported in 1970. Consequently, the chance, that the new pathotype came from elsewhere, is considered to be extremely small. The only reasonable conclusion is that the new pathotype originated in the field from the initial inoculum.

3.4.5 *A field experiment to demonstrate the increase in pathogenicity to tomatoes*

Introduction In laboratory tests, a gradual increase of the pathogenicity to tomatoes during serial passages experiments has been reported (Mills, 1940; de Bruyn, 1952; Graham et al., 1961; Kishy, 1962). It was supposed that the same gradual increase of pathogenicity takes place in the field. This hypothesis was tested in a field experiment.

Materials and methods In 1971, 3 tomato plots of 30 m^2 were grown next to 3 potato plots of 150 m^2. On 71.06.16, 80 plants per potato plot were inoculated with a suspension of 500 zoospores per ml of the 1,2,3-p-pathotype of *P. infestans*. In total 6500 ml suspension was applied to 240 plants by means of a knapsack mistblower. The pathotype used is seldom found in the Netherlands, and is supposed to have been virtually absent in 1971 because the weather conditions were unfavourable to late blight. Consequently, all reisolates collected from this experimental field and reacting like the 1,2,3-p-pathotype to potatoes are considered to have descended from the original inoculum.

After the first observation of sporophores on potato leaves, the tomato plots were examined daily for the presence of late blight. Once the late blight had appeared on the tomato plants, reisolates from tomato plants were collected at regular intervals. The pathotypes of the reisolation were determined in the laboratory using a differential set consisting of the potato differentials R_1, R_2, R_3, and R_4, and the tomato cv. Moneymaker. For each

test, two leaflets from each of the potato differentials and two leaves of 6 weeks old tomato plants were used. These detached leaves were inoculated by spraying a suspension of 5000 zoosporangia per ml till a continuous film of moisture was visible. The pathogenicity to tomatoes, *P*, of the reisolates was evaluated by estimating the percentage diseased leaf area on day 7 after inoculation.

Results On day 7 after inoculation of the potato plots, the first sporophores were noticed on the potato plants in the inoculated rows. On day 43, the late blight epidemic on potatoes came to a stop as hardly any potato foliage was left. On day 37, the first reisolates of *P. infestans* could be collected from the tomato plots, and on day 51 a small focus of late blight was noticed in one of the tomato plots. On day 63, a second focus was found, and, from that day onward, late blight spread more or less generally through the tomato plots.

Thirty of the reisolates collected from the tomato plots were tested for differential pathogenicity to potatoes; 29 belonged to the 1,2,3-p-pathotype, and one to the 0-p-pathotype. Thirty three resiolates collected from the tomato plots were tested for pathogenicity to tomatoes. With the advance of time the pathogenicity to tomatoes increased (Fig. 2). The variance in pathogenicity to tomatoes also increased with time. An isolate with P = 100 was found at about day 100. The single isolate of the 0-p-pathotype collected on day 94 was only weakly pathogenic to tomatoes (P = 25), and moderately pathogenic to potatoes (P = 60).

The reproducibility of the laboratory tests was checked. For this purpose, 5 isolates collected on different data were tested twice with a one-week interval between the first and second test for each isolate. This type

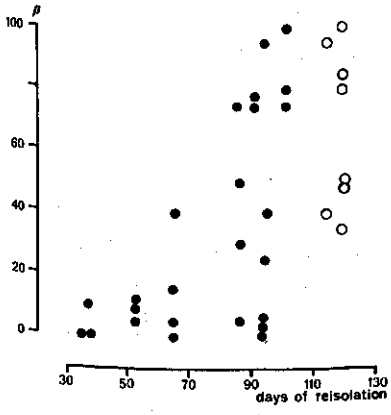

Fig. 2. *P. infestans* on tomatoes cv. Moneymaker in a field experiment. Reisolates from tomatoes were collected at regular intervals, and the pathogenicity *P* of these reisolates (ordinate) was assessed by means of a laboratory test (see text). The highest pathogenicity observed on successive reisolation days increases with the interval between field inoculation and the day of reisolation (abscissa; the day of field inoculation is day zero). Each dot represents one reisolate. The black dots represent reisolates from leaf tissues, the open dots reisolates from fruits.

Table 5. Assessment of the pathogenicity of late blight isolates, collected from tomato plants in the field, to tomato leaves in laboratory. For methods see text. The pathogenicity *P* is expressed as the percentage of leaf area affected by late blight 7 days after inoculation. Longitudinal replication.

Isolate number	Sampling dates	*P*	
		A	B
7108	71.08.06	3	0
7116	71.09.13	80	75
7122	71.09.16	5	5
7135	71.09.23	30	50
7143	71.10.11	100	100

A = First replication.
B = Second replication, one week after the first replication.

of replication might be called longitudinal replication, as contrasted to the usual simultaneous or cross-sectional replication (Zadoks, 1972). The difference between the results of the two test series was small and not significant ($p>0.20$; Tables 5 and 6).

Conclusion In spite of the large amount of inoculum building up in the potato plots from day 7 to day 43, no isolates of late blight could be collected from the tomato plots before day 37. Apparently, the 1,2,3-p-pathotype used for field inoculation, which originated from potatoes, was non-pathogenic or weakly pathogenic to tomatoes under field conditions.

As (1) the original 1,2,3-p-pathotype was at most weakly pathogenic to tomatoes, (2) the infection pressure of this 1,2,3-p-pathotype exerted by the massive presence in the potato plots ended on day 43, and (3) most reisolates highly pathogenic to tomatoes are from later date, it is concluded that all 1,2,3-p-reisolates more or less pathogenic to tomatoes have originated in the tomato plots.

Table 6. Analysis of variance of the data from Table 5.

Source of variance	Sum of squares	df	Variance	Variance ratio	Probability
Isolates	15,196	4	3,779	83	$p\leq 0.001$
Between tests	15	1	15	<1	$p>0.25$
Residual	182	4	45		
Total	15,394	9			

From day 37 onwards, there was a 65 days period during which an increase of the pathogenicity to tomatoes of the fungus population could be demonstrated. Like in the laboratory experiments this increase in pathogenicity was gradual. The mean pathogenicity of the population $\bar{P}$ increased with a rate of 0.07 logit units per day (see Section 3.4.6).

Discussion In 1971, no late blight on tomatoes has been reported in the Netherlands. Therefore, it can be concluded that the late blight reisolates collected from the field experiment and pathogenic to tomatoes, could not have come from sources within a radius of at least a 100 km. These isolates must have originated from the inoculum introduced into this experiment, unless a hitherto unknown long distance dispersal of *P. infestans* over more than a 100 km had occurred. Between the very first collection of a late blight reisolate from tomato plants, a reisolate that was weakly pathogenic to tomatoes, and the collection of a late blight reisolate, that was highly pathogenic to tomatoes, there was a period of 64 days. If the latent period in the field is estimated at 7 to 10 days, a 64 days' period allows for a number of 6 to 9 passages at most. This number is in accordance with the number needed in laboratory experiments to go from an isolate weakly pathogenic to tomatoes to an isolate, that is highly pathogenic to tomatoes.

If the increase in pathogenicity of the population would have been due to a single mutation, and, accordingly, the gradual increase in pathogenicity would have been due to the increase in frequency of the mutant, the following could be expected. After the mutation has taken place, a rapid multiplication of the mutant on the tomato plants can be expected. The reisolates from tomatoes should have either low or high pathogenicity to tomatoes but no intermediate values. There should be little or no variance in the pathogenicity of the highly pathogenic pathotypes. The relative frequency of the mutant should increase strongly at the expense of the original pathotype, and, consequently, the proportion of the weakly pathogenic reisolates per sampling day should decrease. This reasonable expectation has not been corroborated by the facts. The above hypothesis of the single mutation can be rejected, and the original hypothesis of a gradual increase of the pathogenicity to tomatoes can be accepted. The experiment does not provide a clue as to the underlying genetic mechanism of gradual increase in pathogenicity.

3.4.6 *The 'logistic' increase in pathogenicity to tomatoes in a field experiment*

In the experiment reported in Section 3.4.5, the test results per sampling day can be averaged into a value $\bar{P}$, and the function *logit* $(\bar{P})$ can be determined (Van der Plank, 1963). The lineair regression of *logit* $(\bar{P})$ on time t (in days) is given by the equation $\bar{P} = 0.07t - 6.40$ (Fig. 3).

For a single mutation causing increased pathogenicity, the 'logistic' increase of $\bar{P}$ can be demonstrated, when the following conditions are met:

1. the lesions are of short duration;
2. all lesions of one pathotype form the same quantity of effective spores per lesion under all conditions;
3. the ratio of the number of effective spores per lesion of one pathotype to that of another pathotype is constant (here, effective means causing a lesion).

The frequency of the pathotype at time t is proportional to the frequency at time $t-p$ (p = latent period) (van der Plank, 1963).

The frequency of the 'wild' pathotype is Fw, that of the 'mutant' pathotype is Fm. At time t the amount of the wild pathotype is xw_t, and that of the mutant pathotype is xm_t.

$$Fm = \frac{xm_t}{xm_t + xw_t} = \frac{1}{1 + \frac{xw_t}{xm_t}} \qquad (1)$$

Define a at time $t = 0$: $a = \frac{xw_0}{xm_0}$ (2)

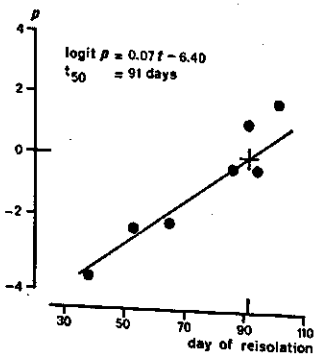

Fig. 3. *P. infestans* on tomatoes cv. Moneymaker in a field experiment. The regression on time of the mean pathogenicity $\bar{P}$ of reisolates collected on various days after field inoculation. For the laboratory assessment of $\bar{P}$ see text.
Abscissa: mean pathogenicity $\bar{P}$ of reisolates on various collection days, as assessed in a laboratory test, and expressed in logit units.

Define b in the logarithmic phase of the epidemic, where rm and rq are the 'logarithmic infection rates' of the mutant pathotype and the original pathotype, respectively:

$$b = rm - rq \mid rm > rw \tag{3}$$

In the logarithmic phase of the epidemic

$$\frac{xw_t}{xm_t} = \frac{xw_0 \times e^{rw\ t}}{xm_0 \times e^{rm\ t}} = a \cdot e^{-bt} \tag{4}$$

Combine (1) and (4):

$$Fm = \frac{1}{1 + \frac{xw_t}{xm_t}} = \frac{1}{1 + a \cdot e^{-bt}} \tag{5}$$

Equation (5) is a logistic equation, which after differentiation reads:

$$\frac{dFm}{dt} = b \cdot Fm\ (1 - Fm) \tag{6}$$

The mean pathogenicity of the population is $\bar{P}$. When Pw is the pathogenicity of the 'wild' pathotype, and Pm is the pathogenicity of the 'mutant' pathotype,

$$\bar{P} = \frac{Pw \cdot Fw + Pm \cdot Fm}{Fw + Fm} = Pw\ (1 - Fm) + Pm \cdot Fm =$$

$$= Pw + (Pm - Pw) \cdot Fm \tag{7}$$

When Fm increases logistically with time, $\bar{P}$ also increases logistically from its starting level Pw to its final level Pm.

Strictly spoken, the above reasoning is valid only in the logarithmic phase of the epidemic. The argument only serves to demonstrate that there is no conflict between epidemiological theory and the observed sigmoidal increase in pathogenicity with time. This conclusion also seems to hold true, when more mutants appear at different times.

3.4.7 *A laboratory experiment to demonstrate the increase in pathogenicity to tomatoes by serial passages*

Introduction The serial passages experiment was designed to investigate when and to what extent changes in pathogenicity could be observed on the level of small populations originating from single lesions. It is supposed that most of these single lesions were the result of monozoospore infections.

Materials and methods Single zoospore isolates made from the 1,4-p-pathotype of *P. infestans* originating from potatoes were tested for their pathogenicity to potatoes. One isolate, that was as pathogenic as the original culture, was selected for use in this experiment. This isolate was called the P_0-isolate. All isolates were grown on potato tuber disks.

Senescent leaves of the tomato cv. Moneymaker were used for the passages. The senescent leaves were taken from 10 weeks old plants of which 20 were grown in a single 10 cm diam. pot in a glasshouse. Tomato leaves of 6 weeks old 'Moneymaker' plants were used for the tests on pathogenicity to tomatoes; the test plants were grown in a walk-in growth chamber in order to provide test leaves of constant quality. Only leaves of the 4th, 5th, and 6th position above the cotyledones were used. The climatic conditions were: temperature 20°C; relative humidity 80%; day length 18 h; light intensity 27.5 J m^{-2} s^{-1}. Reisolates made during this experiment were tested for pathogenicity on leaflets of potato plants with and without the genes R_1, R_2, R_3, and R_4.

The serial passages experiment consisted of 7 successive passage cycles. Each passage cycle had 4 steps.

Step 1: Five detached senescent leaves of the tomato cv. Moneymaker were inoculated with a zoospore suspension (2000 spores per ml) of the P_n-isolate ($n = 0, 1, 2, 3, 4, 5, 6, 7$), and were incubated as described in Section 4.4. The suspension was applied by spraying with an atomizer until run-off.

Step 2: After a week, reisolations of the fungus were made from the senescent leaves. These reisolates were called $P_{n,m}$-isolates, $0 \leq n \leq 7$ being the number of the cycle passed through, and $1 \leq m \leq 12$ being the serial number of the isolate. For reisolation, 9 to 12 of the largest lesions were selected, and the affected tissue was treated as described in Section 4.4.

Step 3: For comparative tests of pathogenicity to tomatoes of the $P_{n,m}$-isolates

collected in step 2, leaflets of 6 weeks old tomato plants of the cv. Moneymaker were inoculated by immersing them in a suspension of sporangia (2000 spores per ml), and incubated as described in 4.6.6. The criterion for pathogenicity to tomatoes was the percentage diseased leaf area, see 3.4.5. The isolate, which caused the highest percentage diseased leaf area, was used as P_n-inoculum in the next passage cycle.

Step 4: Simultaneously with the next passage cycle, the isolate selected in step 3 was tested alone or together with another P-isolate for pathogenicity to tomato leaves of 6 weeks old 'Moneymaker' plants by means of a probit - log dosage analysis.

The first passage cycle differed from the later ones, because the P_0-isolate was tested for pathogenicity to tomatoes by means of a probit - log dosage analysis without being selected in a preceding step on the base of pathogenicity to tomatoes.

Results In step 3 of the first passage cycle, the 12 reisolates tested were not pathogenic to tomatoes. In step 3 of the second passage cycle, the reisolates $P_{2,8}$ and $P_{2,9}$ showed to be somewhat more pathogenic to tomato leaves than the other 7 reisolates tested. In step 2 of the third passage cycle, after inoculation with isolate $P_{2,9}$, 12 P_3-reisolates were collected of which 5 were not pathogenic to tomato leaves, 5 were slightly pathogenic to tomato leaves, and 2 were moderately pathogenic to tomato leaves according to the test results of step 3 of the third passage cycle. In the passages 4 to 7, all reisolates tested for pathogenicity to tomatoes in step 3 were moderately pathogenic.

The results of the tests for pathogenicity to tomato leaves of 6 weeks old plants of the cv. Moneymaker by means of probit - log dosage analyses executed in step 4 of the passage cycles are represented in Table 7 and Fig. 3. The calculated estimates for the ED_{50}-values derived from these probit analyses serve as a measure of pathogenicity to tomatoes.
The ED_{50}-values of the following reisolates or groups of reisolates differed significantly ($p \leq 0.05$):

1. $P_{2,9}$ versus $P_{2,8}$.
2. $P_{2,8}$ and $P_{2,9}$ versus P_0 and P_1.
3. $P_{2,9}$ and its descendents P_3 to P_7 versus P_0, P_1, and $P_{2,8}$.
4. Isolate 7023 (see Section 3.4.4) versus all reisolates P_0 to P_7.

Table 7. A serial passages experiment in the laboratory. Reisolates collected in a serial passages experiment were tested for pathogenicity to tomato leaves of 6 weeks old 'Moneymaker' plants by means of a probit - log dosage analysis. The regression equations are computed by means of the method of Finney (1952). The ED_{50}-values represent the mean concentration of zoospores per µl, at which 50% of the inoculations result in noticeable attack. The lower the ED_{50}, the higher the pathogenicity of the (re-)isolate. Tests with the same serial test number were performed simultaneously.

Serial test number	Isolate	Linear regression equation	ED_{50}	Fiducial limits $p \leq 0.05$	Variance of the regression coefficient
1	P_0	$y = 2.5x + 2.2$	13.4	10.7 - 16.6	0.1244
2	P_1	$y = 2.5x + 2.2$	13.3	11.1 - 15.8	0.0790
3	$P_{2,8}$	$y = 3.3x + 2.2$	7.0	6.5 - 7.8	0.0432
3	$P_{2,9}$	$y = 3.3x + 2.8$	4.6	4.1 - 5.0	0.0421
4	$P_{3,4}$	$y = 2.2x + 3.3$	6.3	5.4 - 7.3	0.0290
4	$P_{3,5}$	$y = 2.2x + 3.5$	4.9	4.1 - 5.6	0.0353
5	P_0	$y = 1.7x + 2.8$	18.3	14.6 - 23.9	0.0396
5	$P_{3,5}$	$y = 2.1x + 3.6$	4.5	3.8 - 5.3	0.0382
6	$P_{3,5}$	$y = 2.4x + 3.6$	3.8	3.2 - 4.7	0.0704
6	P_4	$y = 2.4x + 3.4$	4.4	3.7 - 5.7	0.0565
7	P_7	$y = 1.9x + 4.0$	3.2	3.0 - 3.5	0.0379
-	7023	$y = 2.5x + 6.4$	0.25	0.2 - 0.3	0.0724

The regression coefficients of the probit lines do not differ significantly from each other according to the Student test, except for the regression coefficients of the reisolates $P_{2,8}$ and $P_{2,9}$, which have a significantly higher value than the other regression coefficients. Reisolate P_7 reacted to potatoes like the 1,4-p-pathotype; P_7 did not differ notably from P_0 in its pathogenicity to potatoes.

Conclusion A pathotype markedly more pathogenic to tomato leaves of 6 weeks old 'Moneymaker' plants than the original P_0-isolate was reisolated in the second passage cycle. In the following passage cycles no further increase in pathogenicity to tomatoes could be demonstrated. The pathogenicity of the new pathotype, which was markedly less pathogenic to tomatoes than the 2,4-p/1-t-pathotype, isolate 7023, seemed to be stable.

Discussion In the course of serial passages experiments with *P. infestans* on potatoes reported up to now (Mills, 1940; de Bruyn, 1952; Graham et al., 1961; Kishy, 1962), a gradual increase in pathogenicity to tomatoes was observed, which resulted in stable pathotypes as pathogenic to tomatoes as t-

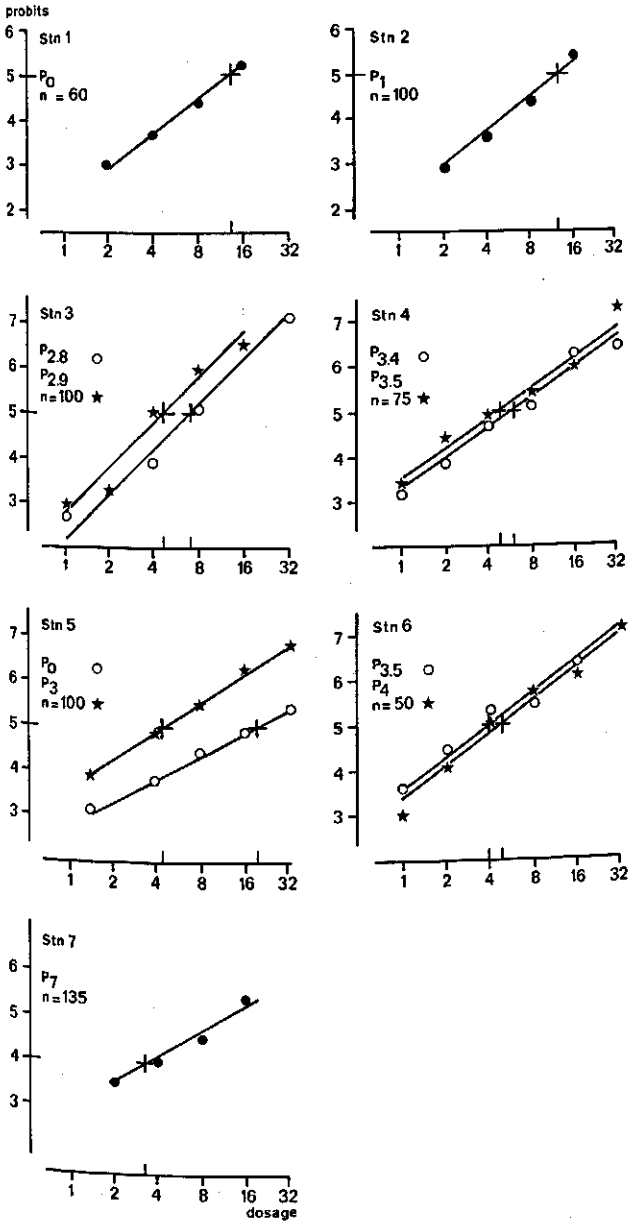

Fig. 4. Graphical representation of the results obtained by testing isolates, collected during a serial passages experiment, for pathogenicity to leaves of 6 weeks old tomato plants of the cv. Moneymaker by means of probit - log dosage analyses. Ordinates: Fraction of inoculations resulting in noticeable attack, expressed in probit units. Abscissae: Concentrations of the zoospore suspensions used for inoculations represented on a logarithmic scale. The numbers of the graphs correspond with the serial test numbers of Table 7.

n = number of inoculations made per dosage.

pathotypes collected from tomato field crops within 7 to 12 passages through tomato foliage. In the above-mentioned experiments, the inoculations and re-isolations were marked by large populations, and besides selection due to the selective medium tomato foliage, no other selection was operating. The above-mentioned results could be confirmed by the author in some preliminary experiments executed according to the 'classic' design.

In the present experiment, the increase in pathogenicity was abrupt, and did not reach the level of a t-pathotype collected from a tomato field crop. The deviant result may be related to a deviant design of the experiment. Contrary to the experiments reported earlier, an artificial selection pressure was applied on the base of one criterion for pathogenicity, lesion extension. The selection of lesions implied the selection of sub-populations, which had originated from single or few zoospores causing the selected lesions. Both, the selection criterion used and the small populations promote genetic fixation. At the same time, however, small populations contain little genetic variability, and thus counteract that accumulation of mutations needed to attain high levels of pathogenicity to tomatoes.

The increase in pathogenicity to tomatoes was limited in time and degree, and was easy to stabilize. Therefore, it is supposed that this increase in pathogenicity to tomatoes was due to a single mutation with a considerable effect on pathogenicity to tomatoes. No genetic proof for this hypothesis can be given.

According to Peto (1953), the slope of the probit - log dosage curve must have the value 2 at the ED_{50}-point (y = 5 probit units) in absence both of variability of resistance and synergism. Variation in resistance reduces this value; synergism increases this value. The interaction of both might result in any value between zero and infinity. The regression coefficients of the probit lines for the isolates $P_{2,8}$ and $P_{2,9}$ are high, thus giving the impression of the presence of synergism. In contrast with the isolates P_3 to P_7, which had much lower values for their regression coefficients, the $P_{2,9}$-re-isolate segregrated in the descendent P_3 populations for pathogenicity to tomatoes. One explanation is, that $P_{2,9}$ is a mixture of the original pathotype and a new one, and that there was synergism between zoospores of the old and the new type.

3.4.8 *A laboratory experiment to demonstrate the increase in pathogenicity to Solanum dulcamara by serial passages*

Introduction *P. infestans* is reported to sporulate on foliage of *S. dulcamara* plants growing in the neighbourhood of affected potato crops during late blight epidemics (de Bary, 1876; Hirst & Stedman, 1960). In a laboratory test, inoculation of leaves of *S. dulcamara* by the 1,4,10-p-pathotype failed to induce symptoms.

As the pathogenicity of late blight isolates, initially non-pathogenic to tomatoes was increased by passages through either senescent tomato leaves or leaves of young tomato plants (Mills, 1940; de Bruyn, 1952; Graham et al., 1961; Kishy, 1962), two serial passages experiments were designed to find out wether the pathogenicity to *S. dulcamara* of the 1,4,10-p-pathotype of *P. infestans* could be increased by passages through senescent leaves or through foliage of *S. dulcamara*.

Materials and methods Plants of *S. dulcamara* were grown in a glasshouse from cuttings taken from a plant found in the neighbourhood of Wageningen. Senescent leaves were selected, and mounted on a piece of 'Oasis'. The leaves were inoculated by spraying a zoospore suspension (2000 spores per ml), placed in a closed translucent plastic box, and incubated by putting them during one day at 15°C in dark, and thereafter in a walk-in growth chamber with 18 h light per day and a temperature of 15°C by day and 12°C at night. When present, lesions were excised on day 8 after inoculation, placed under potato slices in petri dishes, and stored at 15°C in dark. After a week, the sporangia formed at the surface of the potato slices were collected by washing, and a new passage cycle was started. When there was sporulation on the leaves, the next passage cycle was started with sporangia washed from the leaves. Simultaneously with the inoculation of the senescent leaves, fresh leaves were inoculated with the same suspension to test the pathogenicity of the reisolates. Young leaves were obtained by cutting the tops with the upper 3 leaves from the plants. These young top leaves were treated in the same way as the senescent leaves.

Results: Senescent leaves On day 8 after inoculation, lesions with diameters ranging from 1 to 4 mm were present on the senescent leaves. The lesions were necrotic, and no sporulation was observed. The second passage, which was started with a mixture of reisolates originating from 4 lesions, resulted in a slight sporulation on the senescent leaves on day 7 after inoculation; no reaction

was noticeable on the young leaves. The third passage, started with the sporangia collected from the senescent leaves, resulted in a complete infection of both the senescent and young leaves, followed by sporulation on day 5 after inoculation. No further increase in pathogenicity to *S. dulcamara* was observed during the following 3 passages.

Results: Young leaves Three successive inoculations did not succeed in starting the first passage cycle; no infection was observed. At the fourth attempt, one leaf with a broken midrib was also inoculated. On day 11 after inoculation, some sporulation was observed on the distal part of the leaf. A second passage, started with a zoospore suspension (500 per ml) originating from the broken leaf, resulted in many slowly extending water-soaked lesions. On day 5 after inoculation some sporulation was observed on the necrotizing centres of the lesions, and on day 11 sporulation occurred on the chlorotic tissue surrounding the water-soaked tissue. The third passage resulted in abundant sporulation on the day 5 after inoculation both on young foliage and full-grown leaves. In the following three passages no further increase in pathogenicity was observed.

Conclusion *Solanum dulcamara* was not susceptible to the original isolate of *P. infestans*, but the pathogenicity was increased by passages through senescent or damaged leaves. Whenever *P. infestans* has an opportunity to infect the host tissue, the increase in pathogenicity is effectuated in only a few passages. These experiments give no clue as to the underlying genetical and physiological phenomena.

3.5 CONCLUSIONS

Chapter 3 discusses a.o. the subjects 'host range', in particular hosts with survival value to the late blight fungus, and 'variability in pathogenicity', especially the relation between 'tomato races' and 'potato races'. The results presented in this chapter will be used here to answer the questions mentioned in the last paragraph of the introduction (Chapter 1). The import of these questions and of the answers, is restricted to the situation in the Netherlands.

1 *Which other hosts are important?* There is no evidence, that apart from potatoes and tomatoes, other hosts are important to late blight development on tomatoes.

2 *What is the relation of late blight on tomatoes to late blight on potatoes?* There are three relations. The first relation is a climatic one: there will be a late blight epidemic on potatoes and tomatoes only, when the weather conditions are favourable to the development of a severe epidemic of late blight on potatoes. The second relation is a epidemiological one: late blight on potatoes is a permanent source of inoculum to tomato crops. The third relation is a fysiological one: late blight pathotypes highly pathogenic to tomatoes and potatoes (p/t-pathotypes) originate from pathotypes only pathogenic to potatoes (p-pathotypes), as can be demonstrated by means of serial passage experiments in the laboratory, and in field experiments.

3 *How do tomato races of late blight overcome unfavourable periods?* There is no evidence, that tomato races of late blight overwinter in glasshouse crops. An alternative could be the overwintering of tomato races in potato tuber tissues, with subsequently infection tomato crops. When present, this mode must be rare, as late blight on tomatoes is a disease found in late summer and autumn mainly and the growth of potato seed, the next year's crop, comes to an end before August. The normal situation seems to be a permanent reservoir of p-pathotypes, overwintering in potatoes, from which p/t-pathotypes originate under favourable conditions by a proces not unlike that seen in the serial passages experiments.

4 The assessment of resistance

4.1 MONOCYCLIC AND POLYCYCLIC TESTS

Tests on resistance can be classified as either 'monocyclic' or 'polycyclic' tests (Zadoks, 1972). Cycle stands for infection cycle, which is the sequence of processes from one generation of spores through infection, latency etc. to the next generation of spores. In 'monocyclic' tests, only one infection cycle is involved, and mostly this type of test is executed in the laboratory. In 'polycyclic' tests, more than one infection cycle is involved; many field experiments belong to this type of test. In the polycyclic test, the inoculum for the next infection cycle is produced by the plant or crop tested. The test permits to estimate the effect of partial resistance on the growth rate of epidemics. When, however, most of the inoculum originates from outside the plant or crop tested, e.g. from nearby fields or from spreaders planted to that purpose, the effect of partial resistance will be under-estimated. This type of test had been called a 'continuous monocyclic' test (Zadoks, 1972).

4.2 PARTIAL RESISTANCE

A type of resistance well-known to breeders is resistance based on hypersensitivity, which protects the crop completely. In segregrating offspring of crosses between susceptible and resistant parents, the individuals are easily classified as either resistant or susceptible. No special methods for the measurement of resistance are needed. The ratio of resistant to susceptible individuals in segregrating offspring agrees with expectations based on the 'Mendelean way of inheritance' of one or a few genes causing resistance. This type of inheritance is called qualitative inheritance; the resistance involved is considered to be a qualitatively inheriting character, and is shortly called qualitative resistance. The hope, that this type of resistance will ever lead to a permanent solution of the late blight problem of potatoes and tomatoes (see Section 3.4.2), is decreasing nowadays. Attention is being focussed more

and more on partial resistance. The philosophy behind this growing interest in partial resistance is the expectation that partial resistance will last longer than resistance based on hypersensitivity. Also, there is a fear that the pool of genes for hypersensitivity might become exhausted. In some instances, genes for hypersensitivity are rare, as is the case in the genus *Lycopersicon* with respect to *P. infestans*.

Partial resistance is not so easy to handle in plant breeding. The inheritance of partial resistance is not always in accordance with expectations based on the segregration of one or a few genes causing resistance as in the 'Mendelean way of inheritance'. In segregrating offspring, an individual may have any level of resistance within the range limited by the two parents, and - when there is transgression - even outside this range. No clearly distinct classes of resistance can be seen, and special methods for the assessment of resistance are needed. This type of inheritance is called quantitative inheritance; the resistance involved is a quantitatively inheriting resistance, or shortly quantitative resistance.

In this section various aspects of partial resistance will be discussed, using the term 'partial resistance' only as a phenomenological description without any genetical connotation. A genetical interpretation of the data presented will be given in Chapter 7.

4.3 NUMERICAL VALUE OF PARTIAL RESISTANCE

The numerical value of partial resistance RES of a test plant can have any value from zero to one: $0 \leq RES \leq 1$ (Zadoks, 1972). When RES = 0, the test plant is as diseased as the most susceptible standard. When RES = 1, no disease is noticeable, and resistance is complete. So, partial resistance is that resistance which has a numerical value between zero and one: $0 < RES < 1$. Actually, not the resistance but the disease rating DIS(T) of the test plant and the disease rating DIS(S) of the most susceptible standard are assessed. The resistance of the test plant can be related to that of the susceptible standard by means of the equation (Zadoks, 1972):

$$RES = 1 - \frac{DIS(T)}{DIS(S)}$$

DIS(T) can have any values from zero to one, relative to the value of the most susceptible standard which is given the disease rating 1.
As $DIS(T) \leq DIS(S)$, RES is again a value between zero and one.

4.4 COMPONENTS OF PARTIAL RESISTANCE

Partial resistance is subdivided in components, which can be assessed by typological and/or quantitative methods. The typological method uses a descriptive key to characterize two or more classes of reactions denoted by figures or symbols; the quantitative method uses characteristics, which can be measured or counted (Zadoks, 1972). The resistance components described in this section have been studied extensively in potatoes infected by late blight. Differences in resistance to infection have been noticed by Lapwood & McKee (1966) and Umaerus (1969a,b), differences in resistance by prolongation of the latent period have been found by Lapwood (1961), and differences in resistance to sporulation are obvious (van der Zaag, 1956; Lapwood, 1961b). For late blight on tomatoes, only studies on spore production are known Grümmer & Eggert, 1968). The following characters were chosen:

1. *Percentage successful inoculations, S* the number of resulting lesions expressed as a percentage of the number of drop inoculations, using drops of a specified volume and of a specified zoospore suspension.
2. *Lesion extension, E* the linear lesion growth within a specified time interval.
3. *Sporulation intensity, I* the density of sporophores, classified by means of a nine class scale (Table 8).

The first 2 characters are assessed by means of quantitative methods, the third is assessed by means of a typological method.

For each character the corresponding 'component of resistance' can be defined:

a. *Resistance to infection* the ability of the plant to reduce the 'percentage successful inoculations'. The measure used is the number of zoospore per µl of that dosage, that causes 50% successful inoculations when 4.2 µl droplets are applied (see Section 4.5.1). This dosage, the so-called effective dosage 50 (ED_{50}), is estimated by means of a probit - log dosage analysis (see Section 4.4.1). The 'resistance to infection' of a test plant is related to that of the susceptible standard by the equation:

$$RES(S) = 1 - \frac{ED_{50}(S)}{ED_{50}(T)}$$

in which $ED_{50}(S)$ is the ED_{50} of the susceptible standard, and $ED_{50}(T)$ is the ED_{50} of the test plant.

b. *Resistance to extension* the ability of the plant to impede lesion growth. The measure used is the mean value in mm of the longest diameters of lesions

Table 8. Sporulation density. The estimate used is the number of sporophores per unit area, relative to the number of sporophores per unit area of sporulating lesions on the susceptible standard 'Moneymaker'. The sporophore density on the susceptible standard is taken to be 100%. For convenience, a 9 class subdivision has been made.

Sporulation intensity (sporophore density) in %	0	5	10	25	50	75	90	95	100
Sporulation classification number	0	1	2	3	4	5	6	7	8

measured parallel to the large veins originating from the midribs. The 'resistance to extension' of a test plant is related to that of the susceptible standard by the equation

$$RES(E) = 1 - \frac{E(T)}{E(S)}$$

in which E(T) is the lesion extension on the test plant, and E(S) is the lesion extension on the susceptible standard.

c. *Resistance to sporulation* the ability of the plant to reduce sporophore density. The measure used is the number of sporophores per unit area, relative to the number of sporophores per unit area of sporulating lesions on a susceptible standard (Table 8), applying standardized techniques (see Section 4.6). The 'resistance to sporulation' of a test plant is related to that of the susceptible standard by the equation

$$RES(I) = 1 - \frac{I(T)}{I(S)}$$

in which I(T) is the number of the sporophore density class of the test plant, and I(S) is that of the susceptible standard.

The resistance components thus assessed can be combined into a combined index of relative resistance (Zadoks, 1972). In the experiments described in this chapter the combined index for partial resistance $RES(E,I) = 1 - E(T)/E(S) \times \times I(T)/I(S)$ is sometimes used. As the ratios E(T)/E(S) and I(T)/I(S) both are ≤ 1, the product of both ratios also will be ≤ 1, and consequently $0 \leq RES(E,I) \leq 1$.

Note, that a distinction has been made between the following concepts: character, measure, and 'component of resistance'.

A *character* is a phytopathological or epidemiological aspect of the disease, that can be indicated by a technical term like percentage successful inoculations, lesion extension, or sporulation intensity.

A *measure* is a specific property of a character, that is actually counted, measured, or ranked, like the ED_{50}, the longest lesion diameter, or the spo-

rulation classification number.
A *'component of resistance'* is the complement of the character seen from the breeder's point of view. It can be calculated using the measure of a character. The corresponding 'components of resistance' are: 'resistance to infection', 'resistance to extension', and 'resistance to sporulation'.

4.5 MATHEMATICAL TECHNIQUES

4.5.1 Probit - log dosage analysis

The inoculation with a droplet of a zoospore suspension is called 'drop inoculation'. A drop inoculation is successful, when it results in a lesion. Success depends a.o. on the number of infective zoospores per drop. The number of zoospores per drop is a function of the concentration (more correctly: density) of the zoospore suspension and the size of the drop. The infectivity of the zoospores is a function of the pathogenicity of the isolate, the treatment of the zoospores, and the resistance of the test plant. A drop inoculation is either successful or not. This type of reaction is called a quantal response. For the statistical analysis of quantal responses in bio-assays, various methods have been introduced, all of which make use of transformations of the observed data. The most common transformations are the probit transformation (Bliss, 1934), the logistic transformation (Berkson, 1944), and the angle transformation (Knudsen & Curtis, 1947). Here, the probit transformation, a linear metric transformation of the normal distribution, has been chosen. This choise is based on the assumption, that the tolerance of the inoculated leaf area to log dosage has a normal distribution. Finney's (1952) method of probit - log dosage analysis will be used. The method implies the estimation of the linear regression of the probit value corresponding with the percentage successful inoculations on log dosage. The regression line, represented by the equation $y = bx - a$, is called 'probit line'. The fiducial limits of the linear regression coefficient can be determined. In addition, estimates for the ED_{50} (see Section 4.4) and its fiducial limits can be calculated. When the probit lines of two (groups of) plants are parallel, the estimate for the relative potency can be calculated. The antilog of the relative potency ARP is the distance between these probit lines, measured parallel to the abscissa. The ARP-value indicates how many times one plant (or group of plants) is more or less resistant than the other plant (or group of plants) of the pair under comparison.

The experimental data presented in this paper show that the number of

zoospores per drop was low (1 - 256), especially when the susceptible tomato cv. Moneymaker was inoculated with the 2,4-p/1-t-pathotype. Nevertheless, Finney's probit - log dosage method appeared to be fully applicable to results from experiments executed with low dosage drop inoculations.

4.5.2 *Variance analysis*

Apart from variance analysis with equally filled classes of data, variance analysis had to be executed with unequally filled classes of data. Unequal classes of test data originated from experiments using drop inoculations with different dosages, when lesion diameters had to be measured or sporulation intensities had to be estimated on resulting lesions. When the differences between and within inoculated leaves are small, and the variance is only due to a normal distribution of the variables observed, the pooled within dosage variance can be considered as an estimate of the residual variance. An estimate of the variance due to dosage effects can be obtained by substracting the pooled within dosage variance from the total variance (over all dosages). The significance of between dosages effects can be determined by means of a Fisher test.

4.6 MATERIALS AND METHODS

4.6.1 *Introduction*

In testing resistance of the hypersensitive type, small variations in environmental conditions or dosage of inoculum do not markedly affect the test results. Results of tests for partial resistance depend, however, to a large extent on environmental conditions and inoculum dosage. Therefore, tests of partial resistance require environmental control and high precision of dosage. In order to reduce environmental variance to a minimum, most of the test plants were grown and all tests were performed in the walk-in growth chambers of the Department of Phytopathology, State Agricultural University, Wageningen. Much attention was paid to the experimental design with a view to the statistical analysis of the test results.

4.6.2 The growing of the test plants

The test plants were grown in a walk-in growth chamber under fluorescent tubes (Philips TL-MF 40W/33RS) with a radiation intensity of 27.5 J m^{-2} s^{-1} (9500 lx) at soil level. The other climatic conditions were: temperature 20°C ± 0.2; relative humidity 90% ± 3%; day length 16 h and night length 8 h. When the lamps were on, the temperature within the canopy was raised by ca. 1.5°C. Eight seedlings were grown in 13 cm diameter pots of sterilized compost (Trio special 17), and transplanted after 4 weeks to a water culture system. Three water culture units were available. Each consisted of a 100 l tank, two troughs in which the plants were grown, and a circulation system with a pump. Each trough (120 × 14 × 14 cm) was covered by a board with holes to accomodate the plants. In each unit, a hundred liters of a nutrient solution was circulating. The nutrient solution has been prepared after recipes for macro-elements by Steiner (1968), spore-elements by Steiner (1970), and 'iron', applied in the form of FeEDTA, by Steiner & van Winden (1970). The recommended quality of FeEDTA had to be doubled in order to prevent the chlorosis observed in preliminary experiments.

4.6.3 The preparation of the test leaves

The test leaves were detached, and the petioles were fitted into a piece of water-soaked foam on urea-formaldehyd base manufactured for the purpose of floral arrangement (trademark 'Oasis', commercialized by V.L. Smithers A/S, Ganløse - Malmøve - Danmark). These leaves with 'Oasis' were placed in plastic trays. The trays, measuring 36 × 26 × 5.5 cm, were lined with a sheet of thoroughly wetted filter paper. A small-mesh pvc netting was mounted in a wooden frame, and placed in the trays 0.5 cm above the filter paper, this netting preventing the leaves from contact with the wet paper. One tray gave room to 2 leaves with 5 leaflets each, or to one leaf with 7 leaflets.

4.6.4 The preparation of the zoospore suspension

Sporangia of *P. infestans* were grown on leaflets of the tomato cv. Moneymaker (see Section 3.3.2) and were harvested by immersing 3 leaflets during half a minute in 50 ml deionized water. The suspension was sieved through polyamid filtering tissue (polyamid 1383, manufactered by Vereinigten Seidenwebereien AG, Krefeld, Germany). Small sporangia and bacteria passed through

this tissue. The filter was rinsed 5 times with 20 ml deionized water to make sure that most of the bacteria had gone through the sieve. The sieve residue including the large zoosporangia was resuspended in 50 ml deionized water, to which 10 ml potato tuber extract per liter was added (McKee, 1964). This suspension was kept at 10°C for 2.5 h to induce zoospore liberation. Subsequently, the suspension was filtered again, to separate the smaller zoospores from empty and non-germinated sporangia, which do not pass the filtering tissue. The zoospore suspension was stored at 5°C for 2 h at most. Ten ml of the suspension was mixed with 10 ml formol 8% for killing and fixation of the zoospores. The concentration of the zoospores in the suspension was determined by means of a haemocytometer (Fuchs-Rosenthal, 0.200 mm). Counts were repeated until the total number of zoospores counted surpassed 1000, or until 20 counts were made. The concentrations required for the tests were prepared by dilution of the original zoospore suspensions using deionized water with potato tuber extract.

4.6.5 *The application of the inoculum*

The inoculum was applied in the form of droplets of a zoospore suspension (Section 4.5.4). Reproducibility of the droplet size was assured by using a repeating dispenser, constructed after a design of Dr. J.W. Seinhorst (IPO, unpublished). The dispenser consists of a 2 ml syringe (Socorex, standard 50), provided with a mechanism to discharge droplets of 4.2 µl at the press of a button. The droplets were placed on the abaxial side of the leaflets. To prevent damage of the leaf tissues, a 30 cm teflon needle (Hamilton, KF30TF, 0.033 mm ϕ) was used.

When 4 dosages were used, the 2 lower dosages were applied to the right-hand leaflets of the leaf, and the 2 higher dosages to the left-hand leaflets. Usually, the apical leaflet was not inoculated, but when this was done, the lowest dosage was applied to the right-hand side and the highest dosage to the left-hand side. On each leaflet, 5 droplets of the lowest dosage were applied to the right-hand side, and 5 droplets of the other dosage to the left-hand side.

When 8 dosages had to be applied, numbered 1 through 8 from the lowest to the highest dosage, the dosages 1, 3, 5, and 8 were applied to leaves with an even leaf position number. The remaining dosages were applied to the leaves with an odd leaf position number. Leaf positions were numbered from bottom to top, the cotyledones being indicated by the number 0 (zero).

For each group of dosages, the same distribution of the dosages over the leaflets per leaf was used as described above for inoculation with 4 dosages.

When 6 dosages were applied, the dosages 1, 2, 5, and 6 were applied to leaves with an even leaf position number, and the dosages 1, 3, 4, and 6 were applied to leaves with an odd leaf position number.

When the differential pathogenicity of late blight isolates to a set of differentials had to be determined, the inoculation was performed by spraying a spore suspension over the abaxial side of the leaves until run-off.

4.6.6 The incubation of the inoculated leaves

After inoculation, the trays were covered with another pvc netting, again mounted in a wooden frame, to protect the inoculated leaves from contact with an enclosing bag. A colourless polythene bag was put around each tray and closed, in order to guarantee a high moisture level in the trays conducive to the development of symptoms. Then the trays were stored for 24 h at 15^0C in the dark. After this initial dark phase, the trays were placed in a growth chamber at 18^0C. The leaves were irradiated by means of fluorescent tubes (Philips TL-MF 40W/33RS), the light intensity at leaf level was 10.6 J m^{-2} s^{-1} (3,650 lx), the light period lasted 16 h and the dark period 8 h per day. Irradiation by the lamps raised the temperature in the trays ca 1.5^0C. Three days after inoculation, the sheets of filter paper and the bricks of 'Oasis' were wetted again.

5 Results: Monocyclic tests in the laboratory

5.1 INTRODUCTION

The monocyclic tests in the laboratory were designed to develop suitable techniques for measuring partial resistance, making use of detached leaves of plants grown in growth-chambers or greenhouses. Within-plant variances have been studied. Special attention has been given to the effect of age of the host plant on the expression of partial resistance. The possibility of a dosage dependent response to inoculation has also been considered.

5.2 WITHIN PLANTS VARIABILITY OF LESION EXTENSION

5.2.1 *Six weeks old 'Moneymaker' plants*

Materials and methods Four 'Moneymaker' plants were grown as described in 4.6.2. At the age of 6 weeks after seeding, the leaves with positions 4 to 8 were harvested, inoculated, and incubated as described in 4.6.3 to 4.6.6. Four drops per leaflet were applied of a suspension of 10 zoospores per µl of the 2,4-p/1-t-pathotype of *P. infestans*. Five days after inoculation, the lesion extension was measured.

Table 9. Tomato cv. Moneymaker. 'Resistance to extension' of 6 weeks old plants against a 2,4-p/1-t-pathotype of *P. infestans*. Analysis of variance of the within-plant variability of the character lesion extension.

Source of variance	Sum of squares	df	Mean squares	Variance ratio	Probability
Plants	838	3	279	4.83	$p \leq 0.05$
Leaf position	272	4	68	1.17	$p > 0.2$
Within leaflets	694	12	58		
Total	1804	19			

Table 10. Tomato cv. Moneymaker. 'Resistance to extension' of 8 weeks old plants against a 2,4-p/1-t-pathotype of *P. infestans*. Analysis of variance of the within-plant variability of the character lesion extension.

Source of variance	Sum of squares	df	Mean squares	Variance ratio	Probability
Plants	660	1	660	6.7	$p \leq 0.05$
Leaf positions	3445	8	431	4.4	$p \leq 0.05$
Leaflets positions	49	3	16	< 1	$p > 0.2$
Residual	5863	59	99		
Total	10017	71			

Results and conclusions The test results have been used in an analysis of variance (Table 9). The mean lesion extension m = 31.4 mm, with a standard deviation s = 3.1 mm, and the coefficient of variation m/s = 0.10. There is a small but significant effect between plants at $p \leq 0.05$ (the coefficient of variation m/s = 0.06). There was no significant effect of leaf position on lesion extension, and consequently on 'resistance to extension'. The within leaflet variance includes the variance due to the differences in leaflet positions per leaf, and the variance due to different locations of lesions per leaflet. As all of them were not significant at $p > 0.2$, they are pooled with the residual variance to obtain the within leaflets variance.

5.2.2 *Eight weeks old 'Moneymaker' plants*

From 2 'Moneymaker' plants, treated as the plants described in 5.2.1, the leaves with positions 4 to 12 were inoculated.

The test results have been used in a variance analysis (Table 10). The mean lesion extension m = 26.8 mm with a standard deviation s = 1.8 mm, and a coefficient of variance m/s = 0.07. There is a small effect of leaf position on lesion extension, and consequently on 'resistance to extension'. Fig. 4 suggests, that younger and older leaves are more 'resistant to extention' than the leaves of the positions 6 to 8. For experimental purposes, the observed differences are negligible as long as leaves from the optimum positions are used. There is no demonstrable effect of leaflet position.

The influence of leaf position on 'resistance to infection' has also been studied. In an experiment with 6 weeks old 'Moneymaker' plants, of which the leaf positions 2 to 9 were inoculated with drops of suspension with 1 zoospore per µl resulting in 76% successful inoculations, no significant effect between leaves within plants could be demonstrated. The coefficient of variation was 0.05. A small but significant between plants effect at $p \leq 0.05$ was present.

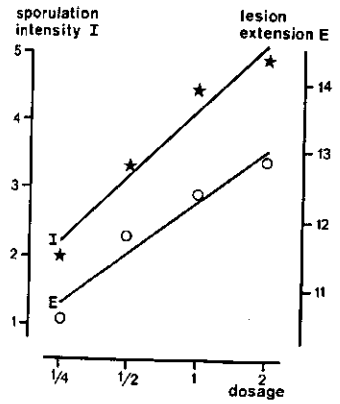

Fig. 5. Tomato cv. Moneymaker. The effect of dosage on sporulation intensity I and lesion extension E, after inoculation with a 2,4-p/1-t-pathotype of *P. infestans*. The dosage is expressed as the number of zoospores per µl on a log scale.
I = sporulation classification number (Table 9)
E = lesion extension in mm

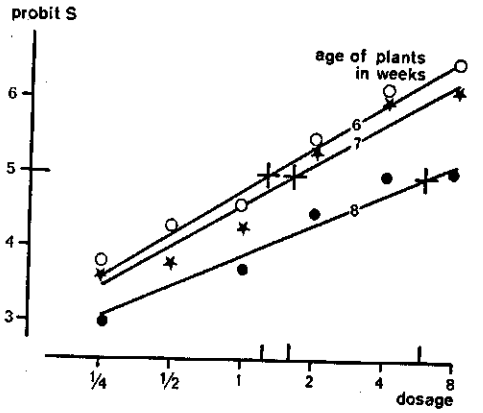

Fig. 6. Tomato W.Va 700. The effect of age on 'resistance to infection'. Leaves of 6, 7, and 8 weeks old plants were tested for 'resistance to infection' against a 2,4-p/1-t-pathotype of *P. infestans*. The test results are presented in the form of probit S (ordinate) on log dosage, expressed as number of zoospores per µl (abscissa).
S = 'percentage successful inoculations'
○ = test results of 6 weeks old plants
★ = test results of 7 weeks old plants
● = test results of 8 weeks old plants

5.3 THE EFFECT OF DOSAGE ON LESION EXTENSION AND SPORULATION INTENSITY OF SOME TOMATO LINES

Materials and methods Two plants of the cv. Moneymaker were grown, harvested, inoculated, and incubated as the W.Va 700 plants used in the test described in 5.4. All plants were 8 weeks old. For this test, leaves of the positions 5 to 9 were inoculated with 4 suspensions of a 2,4-p/1-t-pathotype of *P. infestans* with respectively ¼, ½, 1, and 2 zoospores per µl.

Results and conclusions The results are presented in Table 11 and Fig. 5. Within the range of dosage tested, lesion extension increased with increasing dosage according to the regression equation $y = 3.2 \log_{10} x + 13.6$; y is expressed in mm, and x as the number of zoospores per µl. When the dosage was doubled, the lesion extension was increased with 1 mm. The sporulation intensity I increased with increasing dosage according to the equation $y = 2.5 \log_{10} x + 2.8$; y is the mean sporulation classification number (see Table 9) x is the number of zoospores per µl. When the dosage was doubled, the sporulation classification number increased with 0.75 point. An analysis of variance

Table 11. Tomato cv. Moneymaker. The effect of dosage on sporulation intensity and lesion extension, after inoculation with a 2,4-p/1-t-pathotype of *P. infestans*. The test results are expressed as mean values per treatment. Dosage is expressed as the number of zoospores per µl.

	Dosage			
	¼	½	1	2
Lesion extension E	11.5	12.8	13.9	14.3
Sporulation intensity I	1.1	2.3	2.9	3.4

(see Section 4.5.2) was used to estimate the within dosages variance from the variance per dosage. According to this analysis, the variance due to dosages was significant at $p \leq 0.01$ (Table 12).

In the susceptible cv. Moneymaker, lesion extension and sporulation intensity showed to be dosage dependent. When the cv. Moneymaker is used as the susceptible standard, the effect of dosage dependency must be taken into consideration.

5.4 THE 'RESISTANCE TO INFECTION' OF 6, 7, AND 8 WEEKS OLD W.VA 700 PLANTS

Introduction Wilson & Gallegly (1962) noticed, that the resistance of tomato seedlings belonging to a susceptible cultivar, two accessions with 'dominant resistance', and two accessions with 'multigenic resistance', increased with age from 7 to 39 days after transplanting. It was important to find out to what age and to what extent resistance increases.To this purpose, experiments have been done using the resistant tomato accession W.Va 700 and the susceptible cv. Moneymaker. The criterion for 'resistance to infection' chosen in these experiments was the 'percentage successful inoculations' (see Section 4.4).

Table 12. Tomato cv. Moneymaker. Analysis of variance of lesion extension E.

Source of variance	Sum of squares	df	Mean squares	Variance ratio	Probability
Within dosages	2145	190	11.1		
Between dosages	164	3	54.7	4.93	$p \leq 0.01$
Total	2309	193			

Table 13. Tomato accession W.Va 700. Results of tests for 'resistance to infection' against a 2,4-p/1-t-pathotype of *P. infestans* in 6, 7, and 8 weeks old plants. The measure of 'resistance to infection' is S, the 'percentage successful inoculations'. The results are analysed by the probit - log dosage method.

Linear regression probit - log dosage	ED_{50}	Fiducial limits ($p \leq 0.001$)	Variance of the regression coefficient
$y_6 = 2.0x + 4.8$	1.25	1.1 - 1.4	0.018
$y_7 = 1.9x + 4.6$	1.70	1.5 - 1.9	0.010
$y_8 = 1.4x + 3.9$	6.12	4.3 - 7.4	0.028

y = response in probits of the tested plants; the subscript indicates the plant age in weeks after seeding
x = $\log_{10}$ dosage (dosage is measured as number of zoospores per µl)
ED_{50} = dosage, expressed in number of zoospores per µl, causing 50% successful inoculations

Materials and methods Plants of the accession West Virginia 700 (W.Va 700) were grown on water cultures (see Section 4.6.2). On 72.07.06, leaves of 6, 7, and 8 weeks old plants were harvested, inoculated, and incubated with the 2,4-p/1-t-pathotype of *P. infestans* as described in 4.6.3 to 4.6.6. Suspensions with concentrations of $\frac{1}{4}$, $\frac{1}{2}$, 2 and 8 zoospores per µl were prepared, and 110 drops per suspension were placed on leaves with an even leaf position number (4.6.5). 110 drops of suspension with concentrations of $\frac{1}{4}$, 1, 4, and 8 zoospores per µl were applied to leaves with an odd number. For each of the 3 age groups 2 plants were used, and from every plant the leaves of the positions 4 to 8 were inoculated. On day 4 and 5 after inoculation, the numbers of successful inoculations were scored for all treatments.

Results The results, expressed as the 'percentage successful inoculations' S, were transformed into probits, and analysed according to Finney's (1952) method of probit - log dosage analysis as described in 4.5.1 (Table 13, Fig. 6). The ED_{50}, this is the estimated dosage effectuating 50% successful inoculations, and its fiducial limits were calculated. The ED_{50}-values of different age groups differed significantly at $p \leq 0.001$; the value of 6 weeks old plants was 1.3 times, and the value of 8 weeks old plants 4.9 times the value of 6 weeks old plants. The regression coefficients decreased with increasing plant age from 6 to 8 weeks after seeding. The regression coefficients of 6 and 7 weeks old plants did not differ significantly, but both differed significantly at $p \leq 0.05$ from the regression coefficient of the 8 weeks old plants (Table 14). The probit values have been subjected to an analysis of variance, to test

Table 14. Tomato accession W.Va 700. The regression lines of Table 11 are tested for parallelism by means of Student's test.

Pairs of regression coefficients	$t_n = \frac{d_b}{\sqrt{\text{Var } d_b}}$	Probability
b_6 and b_7	$t_{10} = 0.60$	$p > 0.60$
b_6 and b_8	$t_9 = 2.65$	$p \leq 0.05$
b_7 and b_8	$t_9 = 2.43$	$p \leq 0.05$

b = regression coefficient; the subscript indicates the age of the plants in weeks
d_b = difference between the regression coefficients of any pair
n = number of degrees of freedom

Table 15. Tomato accession W.Va 700. Results of tests for 'resistance to infection' against a 2,4-p/1-t-pathotype of *P. infestans* of 6 and 7 weeks old plants. The test results, scored as the percentage successful inoculations S, are transformed into probits, and tabulated.

Age in weeks	Plant number	Dosage: number of zoospores per µl							
		¼	½	1	2	4	8	Σ	Σ
6	1	3.9	4.3	4.6	5.7	6.3	6.9	31.7	61.7
	2	3.5	4.3	4.5	5.2	6.2	6.3	30.0	
7	3	3.5	3.8	4.4	5.2	5.8	6.2	28.9	58.3
	4	3.7	3.8	4.2	5.3	6.2	6.2	29.4	
Σ		14.6	16.2	17.7	21.4	24.5	25.6	120.0	

Table 16. Tomato accession W.Va 700. Analysis of variance of the data from Table 15.

Source of variance	Sum of squares	df	Variance	Variance ratio	Probability
Dosage	25.62	5	5.12	206.6	$p \leq 0.001$
Age	0.48	1	0.48	19.4	$p \leq 0.001$
Plants within age groups	0.25	2	0.13	5.1	$p \leq 0.05$
Residual	0.37	15	0.025		
Total	26.72	23			

the significance of differences between plants within age groups of 6 and 7 weeks (Table 15, Table 16). The differences were small but significant at $p \leq 0.05$.

Conclusions The resistance to infection of the W.Va 700 plants tested increases with the age of the plants, in the test situation described. Eight weeks old plants are about 5 times as resistant to infection against the 2,4-p/1-t-pathotype used as 6 weeks old plants. The regression coefficient of the probit - log dosage relation decreases with increasing age of the test plants. In other words, the variance of the percentage successful inoculations, var S, increases with increasing age of the plants. There is little reason to expect that the increased variance was of experimental origin. The increase in var S could indicate that the resistance to infection of the various parts of the leaves does not increase in the same way or to the same extent.

5.5 THE 'RESISTANCE TO INFECTION' OF 6 AND 9 WEEKS OLD 'MONEYMAKER' PLANTS

Introduction This experiment was designed to find out to what extent the resistance of the susceptible tomato cv. Moneymaker increased during the interval from week 6 to week 9 after seeding.

Materials and methods Three plants from both the age groups of 6 and 9 weeks old tomato plants of the cv. Moneymaker were grown, harvested and incubated in the same way as the W.Va 700 plants used in the test described in 5.4. For this test, leaves of the positions 4 to 7 above the cotyledones of the 9 weeks old plants were used. Four suspensions with respectively $\frac{1}{2}$, 1, 2, and 4 zoospores of the 2,4-p/1-t-pathotype per µl were applied as described in 4.6.5.

Results and conclusions The results are analysed statistically as in 5.4 (Table 17, Fig. 7). The ED_{50}-values and the relative potency have been calculated. The relative potency is the ratio of the 2 dosages causing equal effects. Here, the relative potency is a measure for the relative increase in resistance in the interval from 6 to 9 weeks after seeding. The ED_{50}-values of the two probit lines differ significantly at $p \leq 0.001$; the antilog of their relative potency is 1.7 with fiducial limits 1.15 and 2.40 ($p \leq 0.001$). Consequently, it can be concluded that the 'resistance to infection' by a 2,4-p/1-t-pathotype increases significantly in the interval from 6 to 9 weeks after seeding.

5.6 THE 'RESISTANCE TO INFECTION' OF 9 AND 11 WEEKS OLD 'MONEYMAKER' PLANTS

Introduction This experiment is a follow-up of the experiment described in 5.5, designed to test the change in 'resistance to infection' of 'Moneymaker' plants from 9 to 11 weeks after seeding.

Materials and methods Three plants of each age group of 9 to 11 weeks old tomato plants of the cv. Moneymaker were treated as the 9 weeks old 'Moneymaker' plants described in 5.5. Six suspensions with respectively ¼, ½, 1, 2, 4, and 8 zoospores per µl of the 2,4-p/1-t-pathotype were applied as described in 4.6.5. For the statistical analysis of the results see 4.5.1.

Results and conclusions The differences between the ED_{50}-values and between the slopes of the two lines representing the two ages are not significant at $p \leq 0.05$. ARP, the antilog of the relative potency is 1.00 (Table 18, Fig. 8). Consequently, there has been no noticable increase in 'resistance to infection' in the interval between 9 and 11 weeks after seeding.

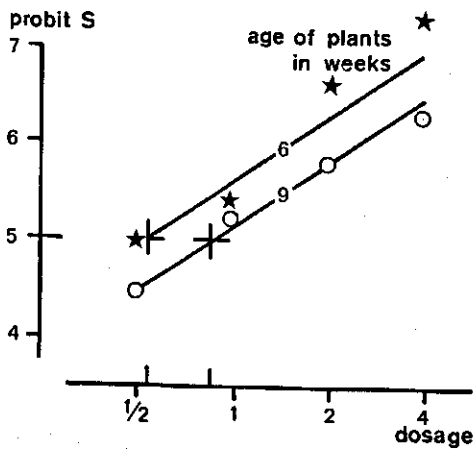

Fig. 7. Tomato cv. Moneymaker. The effect of age on 'resistance to infection' against a 2,4-p/1-t-pathotype of *P. infestans*. The test results are presented in the form of regression lines of probit S (ordinate), on log dosage, expressed as number of zoospores per µl (abscissa)
S = percentage successful inoculations
★ = test results of 6 weeks old plants
○ = test results of 9 weeks old plants

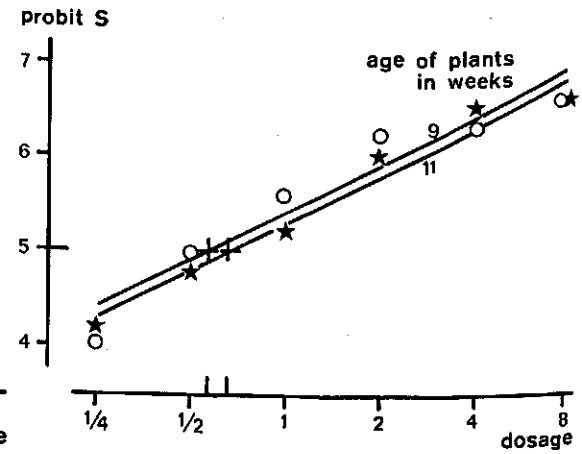

Fig. 8. Tomato cv. Moneymaker. The effect of age on 'resistance to infection' against a 2,4-p/1-t-pathotype of *P. infestans*. The test results are presented in the form of regression lines of probit S (ordinate), on log dosage, expressed as number of zoospores per µl (abscissa).
S = percentage successful inoculations
⊙ = test results of 9 weeks old plants
★ = test results of 11 weeks old plants

Table 17. Tomato cv. Moneymaker. Results of tests for 'resistance to infection' against a 2,4-p/1-t-pathotype of *P. infestans* in 6 to 9 weeks old plants. The measure of resistance is S, the 'percentage successful inoculations'. The results are analysed by the probit - log dosage method.

Linear regression probit - log dosage	ED_{50}	Fiducial limits ($p \leq 0.001$)	Chi square tests	df	Probability
$y_6 = 2.2x + 5.6$	0.53	0.45 - 0.63	par. 0.01	1	$p > 0.9$
$y_9 = 2.2x + 5.1$	0.90	0.80 - 1.01	lin. 4.42	4	$p > 0.9$
	ARP = 1.66 (Fiducial limits: 1.15 - 2.40, $p \leq 0.001$)				

y = response in probits of the tested plants; the subscript indicates the age of the plants in weeks
x = $\log_{10}$ dosage (dosage is measured as number of zoospores per µl)
par. = parallelism
lin. = linearity
df = degrees of freedom
ED_{50} = dosage, expressed in mean number of zoospores per µl, causing 50% successful inoculations
ARP = antilog of the relative potency indicating how many times the 'resistance to infection' increased

Table 18. Tomato cv. Moneymaker. Results of tests for 'resistance to infection' against a 2,4-p/1-t-pathotype of *P. infestans* in 9 to 11 weeks old 'Moneymaker' plants. The measure of resistance is S, the 'percentage successful inoculations'. The results are analysed by the probit - log dosage method.

Linear regression probit - log dosage	ED_{50}	Fiducial limits ($p \leq 0.05$)	Chi square tests	df	Probability
$y_9 = 1.7x + 5.4$	0.60	0.53 - 0.68	par. 0.01	1	$p > 0.9$
$y_{11} = 1.7x + 5.3$	0.68	0.61 - 0.76	lin. 0.55	8	$p > 0.9$
	ARP = 1.00 (Fiducial limits: 1.00 - 1.00, $p \leq 0.05$)				

y = response in probits of the tested plants; the subscript indicates the age of the plants in weeks
x = $\log_{10}$ dosage (dosage is measured as number of zoospores per µl)
par. = parallelism
lin. = linearity
df = degrees of freedom
ED_{50} = dosage, expressed in mean number of zoospores per µl, causing 50% successful inoculations
ARP = antilog of the relative potency indicating how many times 'resistance to infection' increased

5.7 THE 'RESISTANCE TO INFECTION' OF 6, 7, AND 8 WEEKS OLD 'MONEYMAKER' PLANTS

Introduction This section, where the effect of age on 'resistance to infection' of 'Moneymaker' plants is estimated, differs from the two preceding sections because the test results have not been obtained from a single experiment designed for this purpose, but have originated from various experiments, in which plants of the cv. Moneymaker were used as a susceptible standard.

Materials and methods In various experiments, leaves of the tomato cv. Moneymaker were harvested, inoculated, and incubated in the way described in 4.6. Only the test results for the dosages of $\frac{1}{4}$, $\frac{1}{2}$, 1, and 2 zoospores per µl will be considered, because these dosages were applied to all plants tested. The results of 6 weeks old plants are from a single test with 3 plants; the results of the 7 and 8 weeks old plants are from 3 experiments for each age group, in which 2 plants per experiment were tested. The overall percentage successful inoculations S was calculated, for each dosage and age group, and transformed into probit S.

Table 19. Tomato cv. Moneymaker. Results of tests for 'resistance to infection' against a 2,4-p/1-t-pathotype of *P. infestans* in 6, 7, and 8 weeks old plants. The measure for resistance is the 'percentage successful inoculations'. The results are analysed by the probit - log dosage method.

Linear regression probit - log dosage	ED_{50}	Fiducial limits ($p \leq 0.001$)	Chi square tests	df	Probability
$y_6 = 2.5x + 6.4$	0.25	0.23 - 0.29	par. 3.53	1	$p \leq 0.05$
$y_7 = 2.5x + 5.9$	0.45	0.42 - 0.47	lin. 15.16	8	$p \leq 0.05$
$y_8 = 2.5x + 5.5$	0.61	0.58 - 0.65			

$ARP_{6.7} = 1.6$ (Fiducial limits: 1.4 - 2.0, $p \leq 0.05$)
$ARP_{6.8} = 2.3$ (Fiducial limits: 1.9 - 2.7, $p \leq 0.05$)
$ARP_{7.8} = 1.4$ (Fiducial limits: 1.2 - 1.5, $p \leq 0.05$)

y = response in probits of the tested plants; the subscript indicates the plant age in weeks after seeding
x = $\log_{10}$ dosage (dosage is measured as number of zoospores per µl)
par. = parallelism
lin. = linearity
df = degrees of freedom
ED_{50} = dosage, expressed in number of zoospores per µl, causing 50% successful inoculations
ARP = antilog of the relative potency indicating how many times the 'resistance to infection' increased with age; the subscripts indicate the age of the pairs of age groups compared

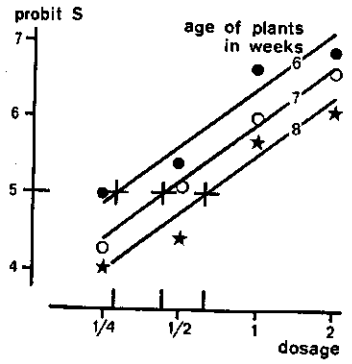

Fig. 9. Tomato cv. Moneymaker. The effect of age on 'resistance to infection' against a 2,4-p/1-t-pathotype of *P. infestans*. The test results are presented in the form of regression lines of probit S (ordinate), on log dosage, expressed as number of zoospores per µl (abscissa)
S = percentage successful inoculations
● = test results of 6 weeks old plants
○ = test results of 7 weeks old plants
★ = test results of 8 weeks old plants

Results and conclusions The results are analysed statistically as in 5.6 and 5.5 (Table 19, Fig. 9). The ED_{50}-values and the relative potencies have been calculated. The ED_{50}-values of all age groups differ significantly at $p \leq 0.001$. Consequently, it is concluded that the 'resistance to infection' increased in the intervals from week 6 to 7 and from week 7 to 8 after seeding. According to the relative potencies, the 'resistance to infection' increased about 1.6 times from week 6 to 7 after seeding, and about 1.4 times from week 7 to 8. In total the 'resistance to infection' increased about 2.3 times.

5.8 THE RESISTANCE OF 6 AND 8 WEEKS OLD PLANTS OF THE F_2: MONEYMAKER x W.VA 700

Introduction This experiment was designed to determine the extent of the change in resistance of F_2-individuals from the cross Moneymaker × W.Va 700 in the period from 6 to 8 weeks after seeding, and the effect of this change on the frequency distribution of partial resistance in the F_2-population.

Tests on 'resistance to infection' by means of the probit - log dosage analysis using an adequate number of plants required too much space in the growth chamber. Instead, the characters lesion extension E and sporulation intensity I were assessed. It was assumed that the within-plant differences between leaves of the positions 4 and 5 are small in comparison with differences between leaves of different plants or differences due to age of the plants (see Section 5.2).

Materials and methods Three plants of the cv. Moneymaker and 27 plants of the F_2: Moneymaker × W.Va 700 were grown in a glasshouse on compost (Trio 17 special) in 2 liter pots. The temperature was 20 ± 2°C; the plants were irradiated

by fluorescent tubes (Philips TL-MF 140W/33RS) during 18 h per day. The irradiation intensity at soil level was 40.6 J m^{-2} s^{-1}.

The leaves of the positions 4 and 5 were harvested, inoculated, and incubated as described in 4.6 in the 6th and 8th week after seeding, respectively. Each leaf was inoculated with ten drops of a suspension of 20 zoospores per µl of the 2,4-p/1-t-pathotype of *P. infestans*. On day 5 after inoculation, the lesion extension E was measured, and the sporulation intensity I estimated.

Results and conclusions For each individual F_2-plant, the test results were expressed as its resistance relative to cv. Moneymaker using the equation for relative resistance (see 4.4):

$$RES(E,I) = 1 - \frac{E(T)}{E(S)} \times \frac{I(T)}{I(S)}$$

E = lesion extension in mm

I = sporulation intensity; sporulation classification number (Table 9)

T = test plant

S = susceptible standard.

The RES(E,I)-values are presented in Fig. 10. The mean extension $\bar{E}$ of the 3 'Moneymaker' plants 6 and 8 weeks old was 30.6 and 19.6 mm respectively. The mean sporulation intensity $\bar{I}$ was 8 and 6 respectively. In all F_2-plants, resistance increased considerably in the period from 6 to 8 weeks after seeding. The 6 weeks old 'population' did not show a noticable differentiation of the resistance distribution pattern; all classes were more or less equally filled ($\chi^2_5 = 2.06$; $p < 0.8$). The same 'populations', but 8 weeks old, showed a differentiation into a 'resistant' group and a 'moderately susceptible' group; a test on equally filled classes gave $\chi^2_4 = 19.85$; $p < 0.01$. There is a centrifugal shift with age into a 'susceptible' and a 'resistant' group in a 1 : 3 ratio.

In Fig. 10, the ranking number of the F_2-plants are shown in order of increasing RES(E,I)-value assigned when the plants were 6 weeks old. The 9 plants, which were most susceptible at the age of 6 weeks, were still the most susceptible at the age of 8 weeks, with only one exception (plant 6). F_2-plants, that showed a resistant reaction in the 6 weeks test, also showed a resistant reaction in the 8 weeks test, but the number of resistant individuals increased at the expense of the intermediate group.

The genetical implications of these test results will be discussed in 7.3.4.

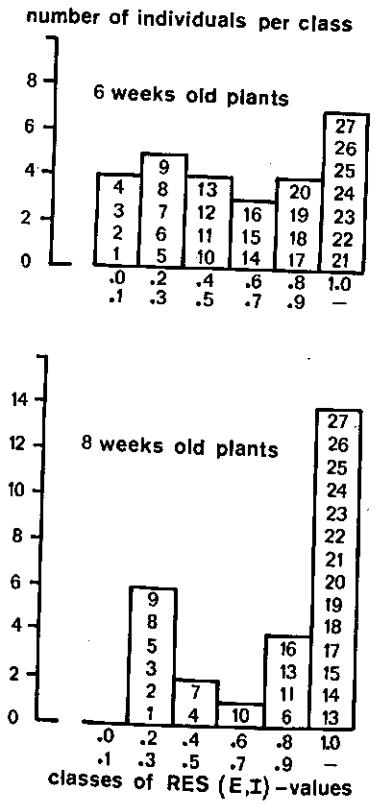

Fig. 10. Tomato F_2: Moneymaker × W.Va 700. Frequency distributions of relative resistance against *P. infestans* in a F_2 population, tested at the ages of 6 weeks (upper diagram) and 8 weeks (lower diagram). The relative resistance RES(E,I) is a combined estimate of 'resistance to extension' and 'resistance to sporulation'. For details see text 4.4 and 5.8. The figures in the columns represent ranking numbers assigned to the F_2-plants in the order of increasing RES(E,I)-value when the plants were 6 weeks old.

5.9 RESISTANCE OF 6 AND 8 WEEKS OLD PLANTS OF THE FIRST BACK CROSS B_1: MONEYMAKER x (MONEYMAKER x W.VA 700)

Materials and methods Four plants of Moneymaker, W.Va 700, and F_1: Moneymaker × W.Va 700, and 16 plants of the first back cross B_1: Moneymaker × (Moneymaker × W.Va 700) were grown in a glasshouse on compost (Trio 17 special) in 2 litre pots. The temperature was 24 $\pm$ 2°C by day, and 20 $\pm$ 2°C at night. The plants were irradiated by fluorescent tubes (Philips TL-MF 140W/33RS) during 18 h per day. The radiation intensity was 40.6 J m^{-2} s^{-1}. The leaves were harvested, inoculated and incubated as in the experiment described in 5.8. On day 4 after inoculation, the lesions were measured and the sporulation was estimated.

Results and conclusions The test results, analysed in the same way as in 5.8, are presented in Fig. 11. The mean lesion extension $\bar{E}$ of the 6 weeks old 'Moneymaker' plants was 19.0 mm, and of the 8 weeks old plants 14.5 mm. The

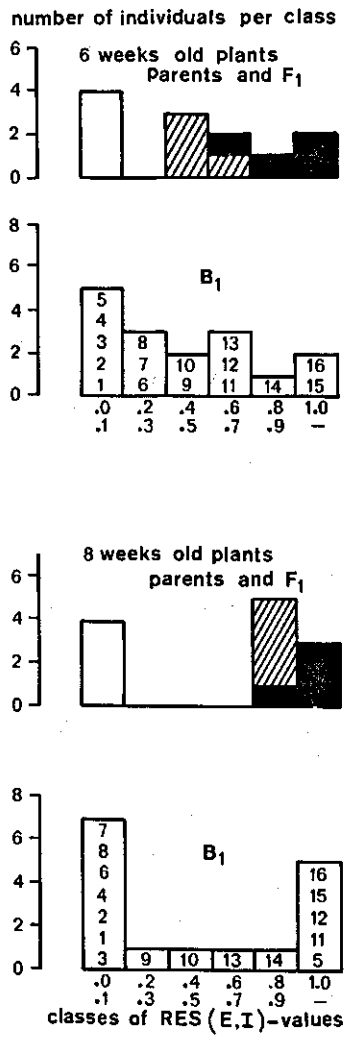

Fig. 11. Tomato B_1: Moneymaker × (Moneymaker × W.Va 700) and parents. Frequency distributions of relative resistance against *P. infestans* RES (E,I) in a B_1 population and in the parents, tested at the ages of 6 weeks (upper 2 diagrams) and 8 weeks (lower diagrams). The relative resistance RES (E,I) is a combined estimate of 'resistance to extension' and 'resistance to sporulation'. For details see text 4.4 and 5.9. The figures in the columns represent the ranking numbers assigned to the B_1 plants in the order of increasing RES(E,I)-value when the plants were 6 weeks old.
Parents and F_1:
White columns - 'Moneymaker' plants
Black columns - W.Va 700
Shaded columns - F : Moneymaker × W.Va 700

mean sporulation intensities $\bar{I}$ were 6.3 and 4.5 respectively. The combined values of 'resistance to extension' and 'resistance to sporulation' RES(E,I) of 6 weeks old F_1-plants is intermediate between that of both parents at the same age. The 8 weeks old F_1-plants are about as resistant as the W.Va 700 plants at the same age. The population of 6 weeks old B_1 plants did not show a noticable differentiation between susceptible and resistant groups, but the population of 8 weeks old B_1 plants suggests a differentiation into 2 about equally sized groups, one group as susceptible as 'Moneymaker', the other group as resistant as W.Va 700. The ranking order of the B_1 plants was not

much affected, plant 5 excepted. Like in the F_2 population described in 5.8 the shift in resistance is restricted largely to plants with an intermediate position.

5.10 CONCLUSIONS

It was demonstrated, that the 'resistance to infection' of the susceptible 'Moneymaker' and of the resistant W.Va 700 increases considerably in the period from 6 to 8 weeks after seeding. After this period, the increase in resistance of the 'Moneymaker' was small and not significant. The resistance of plants of W.Va 700 older than 8 weeks had not been assessed, but the large increase in resistance in the 8th week suggests that the resistance to infection might still increase after the 8th week.

The 'resistance to extension' and 'resistance to sporulation' of parents, F_1, and F_2 of the cross Moneymaker × W.Va 700, and of the B_1: Moneymaker × (Moneymaker × W.Va 700), also increased in the period from 6 to 8 weeks after seeding. The rate and amount of increase differed for individual F_2 and B_1-plants. Six weeks old plants of the F_2 and B_1 could not be differentiated in distinct RES(E,I) groups. Eight weeks old plants showed differentiation in two distinct groups; one about as susceptible as 'Moneymaker', the other about as resistant as W.Va 700. The resistance of F_1 plants increased from intermediate between both parents to about as resistant as the W.Va 700 plants during the period from 6 to 8 weeks after seeding.

It is concluded that plants younger than 8 weeks should not be used in studies on the genetics of partial resistance. Both, lesion extension and sporulation intensity were found to be dosage dependent in the susceptible 'Moneymaker'.

6 Results: Continuous monocyclic tests in the field

6.1 INTRODUCTION

The effect of partial resistance on the growth of an epidemic is a 'slowing down' of the rate at which the epidemic grows. The higher the resistance, the lower the growth rate. In reverse, the growth rate of an epidemic can be considered as a measure for partial resistance. The rate of growth of an epidemic can be calculated from disease assessments made at regular intervals, e.g. by calculating the regression coefficient of the fraction diseased foliage on time. Van der Plank (1963) introduced the term 'apparent infection rate' r (see Section 2.3.1), which is the the regression coefficient of the fraction diseased foliage, expressed in logits, on time. The value r can be determined for individual plants as well as for populations of plants. When individual plants within a population differ in resistance, as can be the case in F_2-populations, the effect of partial resistance can be assessed for each plant separately, when these plants are uniformly exposed to an epidemic.

All field tests reported in this section were artificially infected. The initial inoculum was applied to spreader rows, which in their turn infected the test plants. The test plants differed markedly in resistance level. Therefore, the test situation is considered to be that of a 'continuous monocyclic test' (see Section 4.1). The more resistant the test plant, the more likely it is that most of the inoculum for the next infection cycle comes from surrounding and more susceptible plants, and the more likely it is that the resistance of the test plant will be under-estimated. In this context, reference should be made to Van der Plank's (1963) 'cryptic error in field experiments'.

6.2 PARTIAL RESISTANCE OF THE CV. MONEYMAKER, THE LINE W.VA 700, THEIR F_1 AND THEIR F_2 IN A FIELD EXPERIMENT, 1972

Introduction This experiment was designed to try a method of testing individual plants in the field, and to determine their level of resistance against late blight for the purpose of genetical analysis. The measure of resistance

was the apparent infection rate r.

Materials and methods Two eight-plant plots of each of the cv. Moneymaker, the line W.Va 700, and the F_1: Moneymaker × W.Va 700, and 2 sixteen-plant plots of the F_2: Moneymaker × W.Va 700 were grown in an experimental field, that was situated in a corn field. A path divided this field (20 x 16 m) into 2 equal parts, and each part consisted of 9 plots (7 x 1.5 m). Each plot contained 2 rows of 8 plants at a row distance of 0.5 m and a plant spacing within the rows of 75 cm. The 9 plots at one side of the path were subdivided into 18 eight-plant plots. This experimental field was surrounded by a row of 98 'Moneymaker' plants, serving as spreaders, which had been inoculated on 72.08.08 by placing on each plant one 10 µl drop of a suspension containing 4 zoosporangia per µl of the 2,4-p/1-t-pathotype of *P. infestans*.

The field was irrigated by a sprinkling installation provided with an irrigation control device. The device consisted of a micro-switch activated by the reaction of a 1.10 m hemp kite-line. The thread shrunk when wetted, and turned back to normal length when drying. By means of this device and a manual switch, the plants were kept wet during one 40 h period per week at a minimum of water usage. The plants were pruned weekly, and they were topped after having reached a height of 1.20 m. In this way, excessive growth of the foliage was prevented. Every 4th day after inoculation of the spreader rows, the late blight severity of every plant was assessed separately with the help of a special disease assessment key (see Fig. 14).

Results The 'Moneymaker' plants tested showed large lesions on the stems, some of which exceeded 15 cm in length. The W.Va 700 plants did not show such large stem lesions at all; a few necrotic lesions were found with a $\phi \leq 4$ mm. The plants of the F_1: Moneymaker × W.Va 700 only showed a few stem lesions with a $\phi \leq 4$ mm. From the 32 F_2 plants, there were 7 plants on which the typical large lesions were found. The other F_2 plants were free from lesions exceeding a ϕ of 4 mm.

The 'apparent infection rate', averaged over 4 'Moneymaker' plants, was r = 0.43. For 7 W.Va 700 plants and 8 F_1 plants the r-values calculated were 0.15 and 0.18, respectively. The 32 plants of the F_2: Moneymaker × W.Va 700 varied in resistance, and had r-values between 0.15 and 0.42 (see Fig. 12). The 7 F_2 plants with the large stem lesions were at the same time the 7 most susceptible plants of the F_2 population.

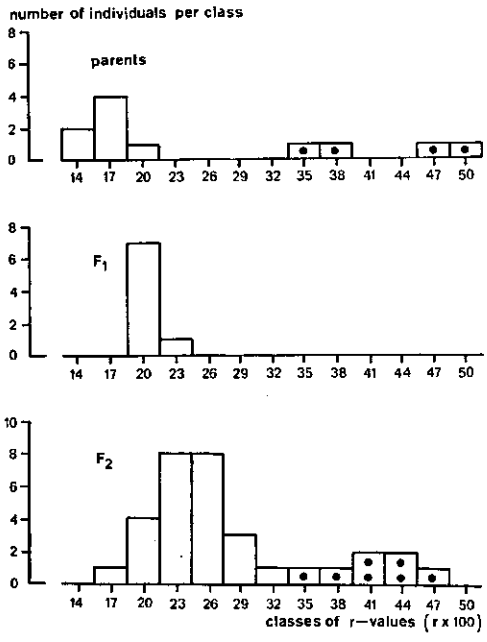

Fig. 12. Parents, F_1 and F_2 of the cross Moneymaker × W.Va 700. Frequency distributions of r, the apparent infection rate, expressed in logit units per day for the 2 parents (upper diagram), the F_1 (middle diagram), and the F_2 (lower diagram). The black dots indicate the plants marked by large stem lesions; in the upper diagram these are the 'Moneymaker' plants.

Conclusions W.Va 700 showed a considerable degree of partial resistance against the late blight pathotype used, and the F_1: Moneymaker × W.Va 700 was about as resistant as W.Va 700. The plants of the F_2: Moneymaker × W.Va 700 varied in resistance from the highest to the lowest parental value; no transgression was observed.

Conclusions about the genetics of the partial resistance carried by W.Va 700, which can be drawn from the results of this experiment, will be presented in Section 7.3.3.

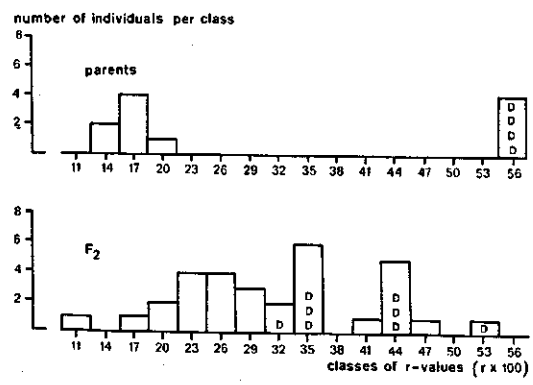

Fig. 13. Parents and F_2 of the cross 'Atom' × W.Va 700. Frequency distributions of r, the apparent infection rate, expressed in logit units per day for the 2 parents (upper diagram), and the F_2 (lower diagram). The D's in the columns indicate the determinant plants; in the upper diagram these are the 'Atom' plants.

6.3 PARTIAL RESISTANCE OF THE CV. ATOM AND OF THE F_2: ATOM x W.VA 700

Materials and methods This experiment was designed for the same purpose, and executed in the same way as the experiment described in 6.2. The cv. Atom is a determinate type (bushy type), which did not exceed a plant height of 40 cm in the field experiments. The cv. Atom has an outstanding 'resistance to sporulation' (Grümmer & Eggert, 1968; author, unpublished). Two plots with 4 plants of the cv. Atom and 2 plots with 16 plants of the F_2: Atom × W.Va 700 were grown.

Results and conclusions One F_2 plant died before the inoculation of the spreader rows. The r-values calculated as usual are presented in Fig. 13. In spite of its characteristic 'resistance to sporulation', the cv. Atom reacted 'susceptible' in the field experiment. The 31 F_2 plants showed a broad spectrum of r-values. Eight of these 31 plants belonged to the determinant type, and these 8 were among the 12 most susceptible plants. One F_2 plant was more resistant than the resistant parent; all other plants were intermediate between the 2 parents.

The genetic interpretation of the test results will be discussed in Section 7.3.4.

Discussion The very susceptible reaction of the cv. Atom is probably due to its determinate and compact way of growing, and to the fact that the design of the test, a continuous monocyclic test, weighs 'resistance to infection' and 'resistance to extension' much heavier than 'resistance to sporulation'.

6.4 THE PARTIAL RESISTANCE OF SOME TOMATO LINES IN THE 1972 FIELD EXPERIMENT

Materials and methods The tomato lines discussed in this section were all grown on the experimental field described in 6.2, and treated as indicated there. Two four-plant plots of each of the cultivars Atom, Moneymaker, Verclabrid, and W.Va 63, and 2 eight-plant plots of the tomato lines PI 198674, PI 224675, W.Va 700, F_1: Moneymaker × W.Va 700, and F_1: PI 224675 × W.Va 700 had been planted. All of these plants have at least some partial resistance to late blight, 'Moneymaker' excepted.

Results and conclusions In Table 20, the tomato lines are ranked according to their level of partial resistance as assessed in the field experiment. The

Table 20. Tomato lines tested for partial resistance in a field experiment, Wageningen. The measure of disease was the 'apparent infection rate' r, expressed in logit units per day. T is the period of time during which the fraction diseased foliage increased from 0.01 to 0.50. RES(r) is the partial resistance relative to the susceptible standard 'Moneymaker'.

Tomato lines	n	r	T	RES(r)	Sign.
Atom	4	0.58	8	-	a
Moneymaker	4	0.43	11	0.00	a
Verclabrid	2	0.28	16	0.34	a b
W.Va 63	4	0.24	19	0.44	b
F_1: Moneymaker × W.Va 700	8	0.21	23	0.51	b c
PI 198674	2	0.17	27	0.59	c d
W.Va 700	7	0.17	27	0.59	c d
PI 224675	4	0.16	29	0.62	c d
F_1: PI 224675 × W.Va 700	4	0.13	35	0.69	d

n = the number of plants assessed
Sign. = lines with different letters differ significantly in r at $p \leq 0.05$

partial resistance relative to the cv. Moneymaker has been calculated by means of the equation:

$$RES(r) = 1 - \frac{r(T)}{r(S)}$$

r(T) is the 'apparent infection rate' of the test plant
r(S) is the 'apparent infection rate' of the susceptible standard 'Moneymaker'

The r-values of the F_1: Moneymaker × W.Va 700, PI 198674, PI 224675, and F_1: PI 224675 × W.Va 700 did not differ significantly at $p \leq 0.05$ from W.Va 700. The line PI 198674 and W.Va 700 differed neither in type and level of resistance, nor in morphology. PI 224675, though about as resistant as W.Va 700, was marked by the presence of sporulating lesions, which were seldom found on W.Va 700. It is concluded, that W.Va 700 and PI 198674 are identical lines, and that the resistance of PI 224675 is different from that of W.Va 700.

Discussion W.Va 63 is closely related to W.Va 700, but was found to be significantly ($p \leq 0.05$) less resistant than W.Va 700 in this test. In contrast to W.Va 700, the cv. W.Va 63 had a compact growth type, not exceeding a height of 80 cm. Both compactness and low height might be held responsible for a comparatively high r-value, because compactness leads to a micro-climate within the plant canopy, that is relatively favourable to late blight; moreover, the micro-climate is more favourable to late blight at lower levels than that at higher

levels of the crop.

6.5 CONCLUSIONS

The method described above, which implies the assessment of the fraction diseased foliage at regular intervals, the calculation of the 'apparent infection rate' r, and the use of r as a measure of resistance, has given useful results.

The tomato lines W.Va 700 and F_1: Moneymaker × W.Va 700 showed a considerable level of resistance in this experiment, but 'Atom', characterized by bushy growth and 'resistance to sporulation' was found to be highly susceptible according to this test.

The F_2: Moneymaker × W.Va 700 and F_2: Atom × W.Va 700 showed a spectrum of r-values within the ranges limited by the corresponding parents.

The continuous monocyclic test is a good test for the evaluation of differences in partial resistance, when this is due to 'resistance to infection' and/or 'resistance to extension'. The continuous monocyclic test cannot be used for the evaluation of 'resistance to sporulation'.

7 Genetic analysis of resistance against Phytophthora infestans

7.1 INTRODUCTION

Most of the experiments reported in this paper have been done with the tomato accession West Virginia 700 *(L. pimpinellifolium)*. According to Gallegly (1964), W.Va 700 bears the Ph_1-gene for resistance by hypersensitivity against pathotype 0-t of *P. infestans*, in addition to a high level of 'multiple gene resistance' against pathotype 1-t. In view of its high level of supposedly polygenic resistance, W.Va 700 has been chosen for the genetic analysis of partial resistance against an isolate of pathotype 2,4-p/1-t of *P. infestans*. The tomato cv. Atom, characterized by a high level of 'resistance to sporulation' (Grümmer & Eggert, 1968), and bushy growth type, and the F_2: Atom × W.Va 700 have also been tested. Unfortunately, no F_1 plant of the cross Atom × W.Va 700 could be tested, because of the failure to obtain enough F_1 seeds.

7.2 RESISTANCE OF THE STEMS

7.2.1 Field test data of the cross Moneymaker × W.Va 700

Introduction All plants of 'Moneymaker' grown in the field experiment described in Section 6.2 reacted to late blight with characteristic large lesions on the stems. Lesions >4 mm were never observed on stems of plants of W.Va 700 or F_1: Moneymaker × W.Va 700. The F_2 population of the cross Moneymaker × W.Va 700 contained 7 plants with and 25 plants without large stem lesions, caused by the late blight fungus. These results suggest the segregration of one dominant gene preventing large stem lesions.

The hypothesis, that a single dominant gene prevents large stem lesions, will be tested in the following experiment.

Materials and methods F_3 seeds were harvested from all 16 F_2 plants of the cross Moneymaker × W.Va 700 grown on one plot of the field experiment de-

scribed in 6.2. Three of these F_2 plants were characterized by the presence of large stem lesions caused by 2,4-p/1-t-pathotype of *P. infestans*; the other 13 F_2 plants were free of stem lesions with a ϕ >4 mm. From each F_3 line 12 to 16 plants were grown on an experimental field (18 x 14 m) without irrigation. The lines were grown in rows at a distance of 1 m and a plant spacing in the rows of 0.75 m. The field was surrounded by a row of 66 'Moneymaker' plants. On 73.07.27, all plants were inoculated by means of a knapsack mist-blower. Two liter of a suspension of 2 zoosporangia per µl of an isolate of the 2,4-p/1-t-pathotype were applied; the age of the plants was 12 weeks. On 73.08.03, the F_3 plants were inspected for stem lesions.

Results and conclusions Large stem lesions were present on all 46 plants of the 3 F_3 lines, which descended from the 3 F_2 plants characterized by large stem lesions in the experiment described in 6.2. Seven of the 13 F_3 lines descending from F_2 plants free of stem lesions in that experiment, were marked by the presence of 1 to 6 plants with large stem lesions. The plants of the other F_3 lines were free of stem lesions. In the F_3, the observed ratio agrees with a 1:2:1 ratio ($\chi^2_2 = 1.38$; $p > 0.5$) based on the expected segregration of a single, dominant gene.

The segregrating F_3 lines contained 98 plants, of which 23 were characterized by large stem lesions and 75 were free of stem lesions. The observed ratio between both types of plants agrees with a 1:3 ratio ($\chi^2_1 = 0.12$; $p > 0.7$) as caused by the segregration of a single, dominant gene. All evidence supports the hypothesis, that W.Va 700 carries a dominant gene for resistance of the stems to the 1-t-pathotype of *P. infestans*.

Discussion The gene, that protects the stems against pathotype 1-t of *P. infestans* is different from the gene Ph_1, because tomatoes resistant to the 0-t-pathotype only show large stem lesions when attacked by the 2,4-p/1-t-pathotype of *P. infestans*. It is proposed to name this new gene Ph_2.

7.3 RESISTANCE OF THE LEAVES

7.3.1 Statistical analysis

In general, the phenotypic expression of partial resistance depends more on environmental conditions than that of resistance by hypersensitivity. Neglecting interaction, phenotypic variance can be represented by:

$$VAR(P) = VAR(G) + VAR(E)$$

in which VAR(G) is variance due to genotypic sources and VAR(E) variance due to environmental sources. When VAR(G) is absent, as is the case in pure lines and F_1 hybrids of these lines, the observed variance of any measure of resistance will be of environmental origin. Conversely, pure lines and hybrids can be used to obtain estimates of environmental variance.

As cultivated and currant tomatoes are mainly inbreeding crops, tomato lines and F_1 hybrids usually consist of genetically identical individuals. By subjecting the test results of these lines to an analysis of variance, the within line variance can be calculated, and this variance is an estimate of the environmental variance.

7.3.2 Environmental variance of partial resistance

Materials and methods The field results of 4 plants of each of the tomato lines 'Moneymaker', W.Va 700, PI 224675, F_1: Moneymaker × W.Va 700, and F_1: PI 224675 × W.Va 700 were used in a variance analysis. The plants were chosen in such a way, that of each 4 plants tested, 2 were grown in a border row and the other 2 somewhere in the centre of the field, to see if there was an effect due to the position of the plant in the field. Plants in border rows are supposed to belong to block A, and the other ones to block B.

Results and conclusions The results are shown in Table 21 and 22. Variance between lines is highly significant ($p \leq 0.001$), but variance due to blocks or blocks × lines is not significant. Apparently, there is no significant

Table 21. Partial resistance of tomato against *P. infestans*. Lines tested in a field experiment (see 4.7.4). The test results are presented in the form of values of r, 'the apparent infection rate' expressed in logit units per day, for the purpose of a variance analysis. The entries to the table are r × 100.

Lines (genotypes)	Block A		Block B		Σ
Moneymaker	46.1	50.5	38.0	35.4	170.0
W.Va 700	13.9	16.5	18.3	19.7	68.4
PI 224675	16.2	19.1	12.2	18.0	65.5
F_1: Moneymaker × W.Va 700	21.2	20.3	20.6	19.4	81.5
F_1: PI 224675 × W.Va 700	10.4	10.2	13.9	17.1	51.6
Σ	224.4		212.6		437.0

Table 22. Analysis of variance of the data in Table 21.

Source of variance	Sum of squares	df	Variance	Variance ratio	Probability
lines	2241.3	4	560.0	43.4	$p \leq 0.001$
blocks	7.1	1	7.1	<1	$p > 0.2$
blocks × lines	92.2	4	23.1	1.79	$p > 0.2$
residual	128.6	10	12.9		
total	2469.2				
within lines	227.9	15	15.2		

The within lines variance is the period variance of blocks, blocks × lines, and residual

difference between plants grown in the border rows and those in the centre of the field. As the variances due to the sources blocks and blocks × lines do not differ significantly from the residual variance, they can be pooled with the residual variance to obtain the within lines variance. The within lines variance, which in this experiment is an estimate of the environmental variance VAR(E), has a value 15.2 ($\times 10^{-4}.r^2$), and 15 degrees of freedom. The coefficient of variation s/m is $\sqrt{15.2/21.9} = 0.18$.

7.3.3 Field data of the cross Moneymaker × W.Va 700

The field results of the cross Moneymaker × W.Va 700, described in section 6.2, will be interpretated genetically. After a subdivision of the F_2 plants into 2 groups on the basis of absence or presence of stem lesions (see Section 7.2), a variance analysis of the r-values of the F_2 groups has been made (Table 23). The 2 groups, which had mean r-values of 0.24 and 0.41 respectively, differed significantly at $p \leq 0.01$. The variances of the F_2 plants,

Table 23. F_2: Moneymaker × W.Va 700. Inheritance of partial resistance against *P. infestans*. Comparison of variances calculated from F_2 plants with stem lesions (Group A) and without stem lesions (Group B) to the environmental variance VAR(E), calculated in 7.3.2, by means of an F-test.

	Variance	df	Variance ratio	Probability
Group A	14.5	6	$F_{24}^{6} = 1.27$	$p > 0.2$
Group B	11.4	24	$F_{15}^{6} = 0.96$	$p > 0.2$
Var (E)	15.2	15	$F_{24}^{15} = 1.33$	$p > 0.2$

11.4 and 14.5 ($\times 10^{-4}.r^2$), did not differ significantly from the estimate of VAR(E) = 15.2 ($\times 10^{-4}.r^2$; see Section 7.3.2) at $p \leq 0.2$. Consequently, within the 2 groups no other sources of variance seem to be present than the environmental source, VAR(G) being small or absent. The difference in partial resistance between the F_2 groups could be due to the dominant gene Ph_2 (see Section 7.2), that protects stems against the 1-t-pathotype of *P. infestans*, or to a gene linked with it. The hypothesis, that partial resistance of W.Va 700 is monogenic, has been tested at a larger scale in an experiment, reported in Appendix 1.

7.3.4 Genetic analysis of the laboratory data of the cross Moneymaker × W.Va 700

F_1: Moneymaker × W.Va 700 The resistance of 6 weeks old F_1 plants is intermediate between the resistance of 'Moneymaker' and that of W.Va 700 of the same age. The resistance of 8 weeks old plants is nearly equal to the resistance of the W.Va 700 plants of the same age (see Fig. 11).

F_2: Moneymaker × W.Va 700 The distribution of the RES(E,I)-values of 6 weeks old plants was continuous, and all classes of RES-values were more or less equally filled (Fig. 10). Such data could lead to the conclusion, that the partial resistance of W.Va 700 inherites quantitatively. These data give no clue to the genetic basis of the resistance involved.

The frequency distribution of the RES(E,I)-values of 8 weeks old plants suggests a differentiation of the plants into 1 group as resistant as W.Va 700, and another group as susceptible as 'Moneymaker', in a 3:1 ratio (Fig. 10). These data indicate that the partial resistance of the leaves of W.Va 700 is controlled by a single gene.

B_1: Moneymaker × (Moneymaker × W.Va 700) The data from the B_1 test (see Section 5.10) support the conclusion that the resistance of W.Va 700 is due to a single gene for partial resistance. In the 8 weeks old plants of this back cross, there is a differentiation, which suggests the presence of 2 equally filled classes (Fig. 11), one as susceptible as 'Moneymaker', the other as resistant as W.Va 700.

7.3.5 Field data of the cross Atom × W.Va 700

In the cross Atom × W.Va 700, the phenotypic expression of partial resistance is influenced by 3 genetic systems. First, there is the gene for partial resistance of W.Va 700 named Ph_2 (see Section 7.2 and 7.3.3). Second, there is the determinate growth due to a single gene. Third, there is the 'resistance to sporulation' of unknown genetic nature carried by the cv. Atom. When the effect of 'resistance to sporulation' is considered to be small (as can be seen from the data of cv. Atom, Table 20), two monogenic systems with a considerable effect on the expression of resistance are left. All determinate plants belong to the susceptible part of the population (Fig. 13). When the determinate plants are excluded, there are 23 plants left, out of which 7 are as susceptible as the 7 F_2 plants with large stem lesions descending from the cross Moneymaker × W.Va 700 (Fig. 12). These 7 plants had an 'apparent infection rate' $r \geq 0.35$. The ratio 7:16 agrees with a 1:3 ratio at $p > 0.5$; $\chi^2_1 = 0.36$. When the single plant with $r = 0.32$ is also accepted as susceptible, $p > 0.2$; $\chi^2_1 = 1.17$.

In conclusion, there is evidence that, apart from the gene for determinancy, the Ph_2 gene is the only gene with a considerable effect on the expression of resistance in F_2 plants of the F_2: Atom × W.Va 700. No proof can be given, because the subdivision into resistant and susceptible plants is an arbitrary one.

7.4 THE 'ATOM' PARADOX

In the F_2 population of the cross Atom × W.Va 700, no determinate plants were found in the resistant group. The chance, that all 8 determinate F_2 plants were susceptible because of the absence of the Ph_2 gene is very small ($p = 15.10^{-6}$). So, there must have been determinate plants with the Ph_2 gene present. In the test situation described (see Section 6.3), determinate growth seemed to cause epistatic susceptibility over resistance by the Ph_2 gene. This epistatic susceptibility can be due to absence of the resistance mechanism (genetically epistatic) or due to that fact that the determinate plants grow under conditions more favourable to late blight development (environmentally epistatic). There are indications, that the environmental conditions are the decisive ones, as is also the case with the plants of 'Atom', which reacted as highly susceptible in spite of their 'resistance to sporulation' (see Section 6.4).

7.5 DISCUSSION

There is convincing evidence that the resistance of the stems in W.Va 700 against the late blight pathotype used is controlled by a single dominant gene, here called Ph_2 (see Section 7.2). In the field trial with F_2 plants, it was demonstrated that the variance of partial resistance in the leaves was determined by the genotypic effect of Ph_2 together with environmental effects, and that no other sources of variance could be demonstrated (7.3.3). The laboratory tests with the cross Moneymaker × W.Va 700 give evidence of the presence of a single gene, that controls partial resistance in the leaves (see Section 7.3.4). Obviously, this gene for partial resistance of the leaves is the same as the gene Ph_2 for resistance of the stems.

Partial resistance of W.Va 700 increases until at least 8 weeks after seeding (see Section 5.4). Six weeks old plants of the F_2: Moneymaker × W.Va 700 and of the B_1: Moneymaker × (Moneymaker × W.Va 700) are not accessible to genetic analysis. The results from 6 weeks old plants can only lead to comments like: partial resistance of W.Va 700 may inherite quantitatively. It is not justified to use the term 'multigenic resistance' on the base of such vague information.

For the purpose of selection, 6 weeks old plants might be more suitable than 8 weeks plants. There is evidence, that the Ph_2ph_2 genotype is more distinct from the Ph_2Ph_2 genotype at the age of 6 weeks than at the age of 8 weeks, because the resistance of the heterozygote F_2 plant is intermediate at the age of 6 weeks, but nearly as high as that of the resistant parent at the age of 8 weeks.

The high level of resistance of the F_1: PI 224675 × W.Va 700, and the fact, that the reaction of PI 224675 to late blight was different from W.Va 700, might indicate that in W.Va 700 and PI 224675 different genes for late blight resistance are present, possibly with additive effects.

The 27 top leaflets of the leaves of the 27 F_2 plants and 4 top leaflets of the leaves of the 4 'Moneymaker' plants tested at an age of 6 weeks in the experiment described in 5.8 were inoculated by spraying a zoospore suspension of the 1,2,3-p/0-t-pathotype of *P. infestans* until run-off. After incubation (see Section 4.6), the leaflets were observed for late blight attack. All 4 'Moneymaker' leaflets and 1 F_2 leaflet (F_2 plant 3) were seriously affected. The other 26 F_2 leaflets were apparently free from infection. The hypothesis, that Ph_2 gene does not act against 0-t-pathotype of *P. infestans* can be re-

jected ($\chi^2_1 = 6.45$; $p < 0.02$), as in this case 6.7 susceptible F_2 plants were expected.

7.6 CONCLUSION

The resistance of the stems in W.Va 700 against the 2,4-p/1-t-pathotype *P. infestans* used is controlled by a single dominant gene, which is named Ph_2. The partial resistance of the leaves in W.Va 700 is due to the same gene, or to a gene closely linked with Ph_2.

In the continuous monocyclic test, the determinate F_2 plants of the cross Atom × W.Va 700 were epistatically susceptible over resistance by the Ph_2 gene.

8 Comparison of testing methods with special reference to their application in commercial breeding programs

8.1 A COMPARISON OF TESTING METHODS

Introduction During a 3 years' period methods have been developed and tested for the assessment of partial resistance. A reliable and easy method for the assessment of partial resistance opens the way to the application of partial resistance in commercial breeding programs. In this section, the various methods will be compared, and their applicability in commercial breeding programs will be considered.

In an attempt to evaluate the various testing methods, the coefficient of variation of certain measures has been chosen as a means of comparison. The coefficient of variance CV used here is the standard deviation s expressed as a fraction of the average m (CV = s/m). As the standard deviation depends on the number of observations, the ideal is to compare CV values based on equal numbers of observations. This was, alas, not always possible.

The methods The following three methods are compared here:
a. Probit - log dosage method (see Sections 4.5.1 and 5.4),
b. Components analysis method (see Section 5.8),
c. Field method (see Section 6.2).
Strictly speaking, the probit - log dosage method is only a complicated variant of the usual method to estimate the infection ratio. Accordingly, it could be considered as a part of the components analysis method, but because of its complexity it is considered as a separate method here.

Results The probit - log dosage method is the most precise method. The $CV(ED_{50})$ varies between 0.03 (see Section 5.4) and 0.06. However, this method demands a high labour input, and it requires expensive facilities like growth chambers and a well equiped laboratory. The labour needed is roughly estimated at 1 man-hour per plant. Consequently, it is a very costly method.

The components analysis method was less precise than the probit - log dosage method. The data of the experiment described in Section 5.9 yielded

CV(E) = 0.11. The labour required is about 0.2 man-hour per plant. The duration of a single experiment from seeding to testing is about 2 months. Other characters, like latent period and sporulation intensity can be assessed too, using the same materials. The experiments can be designed on a year-round base. The disadvantages are the high costs of the laboratory and glasshouse facilities, and the peak work-loads during inoculation and measuring.

The field method was the least precise one. It can be calculated that CV(r) = 0.18 (see Section 5.4). When CV(r) is calculated for the same lines and the same number of plants as the above mentioned CV(E), 4 plants of each of the lines Moneymaker, W.Va 700, and F_1: Moneymaker × W.Va 700, a figure of 0.18 is found again. The estimated labour investment is 0.1 man-hour per plant. Additional advantages of the field method are its relative simplicity, the possibility to observe other characters of importance to the breeder, and the absence of high peak work-loads. Disadvantages of this type of test (continuous monocyclic test) are the under-estimation of partial resistance in general, and the failure to assess the value of some types of resistance, e.g. the 'resistance to sporulation' carried by 'Atom'. The method is season dependent; in the Netherlands only 1 experiment per year can be done. The test has a duration of 4 months, from seeding to harvest.

Conclusions

1. The probit - log dosage method is a rapid and precise method, but is also a costly method, and it does not satisfy the practical requirement of applicability to large numbers of plants.
2. The components analysis method is a rapid and rather precise method, applicable to large numbers of plants, but is not a cheap method.
3. The field method is the least precise one, but it is an adequate method. The method is simple and cheap, season dependent, and there is no limitation to the numbers of plants tested.

8.2 A SIMPLIFIED RECIPE FOR THE BREEDER

The method of disease assessment by estimating the fraction diseased foliage, and subsequent transformation of the fraction estimated into logit units, followed by the calculation of the regression line, can be simplified. A method is given, in which the fraction diseased foliage is estimated by means of a 21 class scale (Fig. 14). The scale values have been called transformation-values or shortly TR-values. These values were chosen, because they were

convenient to use in the field. They can easily be transformed into logit units, as the TR-values are proportional to the logit values within the range from 0.01 to 0.99. Within this range, the following equation is valid:

$$y = 1.16x - 5.80$$

in which y stands for logit units and x for TR-values. The calculation of the 'apparent infection rate' r can be simplified by using a graphical method to estimate r. For this purpose the observed TR-values are plotted against time (Fig. 14), and the regression line is drawn by eye-fitting. The tangent of the angle between regression line and abscissa is an estimate of r, expressed in TR-values per day. An estimate of the 'apparent infection rate' r in logit units per day is obtained by multiplying the value found by 1.16.

As an example, the results from two different plants are given (Fig. 15). The calculated r-values are 0.36 and 0.20 logit units per day, respectively. The r-values obtained by the graphical method are 0.32 and 0.20 logit units per day, respectively. The results obtained by the 2 methods are in good agreement.

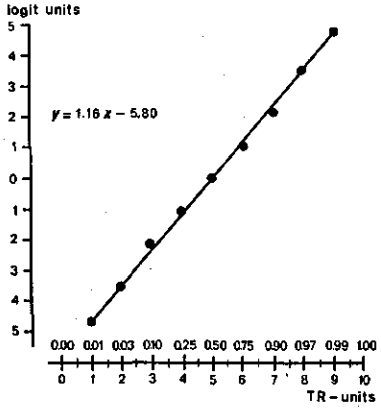

Fig. 14. The relation between a logit units-scale and the TR-units, used in the field experiments to classify the fraction diseased foliage.
Abscissa: upper row of figures present the percentage 'fraction diseased foliage'; lower row of figures present the according TR-units.
Ordinate: logit units.

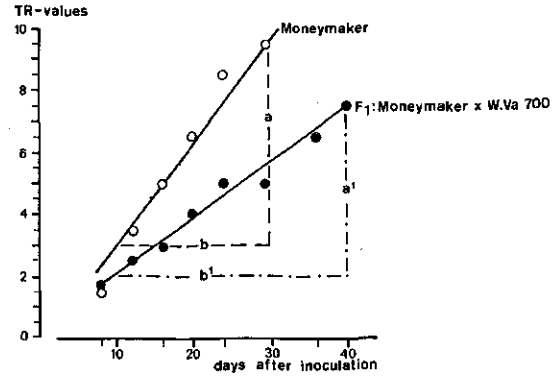

Fig. 15. *P. infestans* on tomatoes in a field experiment. Tomatoes assessed for resistance by means of the field method (see text). Linear regression of fraction diseased foliage (expressed in TR-values) on time. Graphical method to estimate r ($r = a/b$, $r^1 = a^1/b^1$).

Summary

Chapter 1 In the period from 1965 to 1968, *P. infestans* caused epidemics on tomatoes in unheated and slightly heated glasshouses in the Netherlands. Before this period, late blight was hardly known as a disease of tomatoes under glass. Apart from the climatic conditions conducive to the development of late blight, the prominence of the disease was attributed to the growing of tomato cultivars resistant against tomato leaf mould *(Cladosporium fulvum)*. As a consequence of the use of *C. fulvum* resistant cultivars, treatment by fungicides was omitted, thus giving *P. infestans* a chance to develop without hindrance.

As treatment with fungicides is not always effective and sometimes undesirable because of spray residues, resistance to late blight became imperative. However, sources of resistance are rare, resistance is often incomplete or 'partial', the level of resistance being too low to protect the crop without the use of fungicides. One solution of the problem could be the accumulation of partial resistance by adequate genetic methods. Partial resistance, however, is difficult to handle by breeders, as its genetic nature is often unknown. Its phenotypic expression depends on environmental conditions.

Chapter 2 In the Netherlands, the disease is at present not a serious problem, but in those parts of Europe where tomatoes are grown in the open, tomato late blight is considered to be the most important disease of outdoor tomatoes. The opinion prevails, that incidence and severity of the disease increased during the last decade. The year-round cropping system is responsible for this increase, as can be demonstrated by the experiences in Bulgaria and Hungary before and after 1965. Along with the large scale introduction of spring and autumn crops in glasshouses in 1965, tomato late blight rose in status from an unimportant disease to a very serious one.

Chapter 3 Studies were made on the host range of tomato late blight in the Netherlands. A new host, *Phacelia tanacetifolia*, could be added to the list. In the Netherlands, there is no evidence for other sources of tomato late

blight than the late blight on potatoes. Laboratory experiments with serial passages through tomato leaves have shown, that the pathogenicity to tomatoes of late blight isolates collected from potatoes, which at the start were not or slightly pathogenic to tomatoes, could be raised considerably. Similar results were obtained in a field trial.

Chapters 4 and 5 Investigations were made on partial resistance. The approach was twofold, i.e. the development of laboratory methods for the analysis of 'components of resistance', and the development of a field method to evaluate differences in partial resistance of individual plants outdoors. The assessment of 'components of resistance' required high precision of the dosage of inoculum, and low environmental variance. To reduce environmental variance to a minimum, the test plants were grown on water cultures in the walk-in growth chambers of the Department of Phytopathology, State Agricultural University, Wageningen. In most of the experiments on partial resistance 2 lines have been used, the susceptible cv. Moneymaker and the line W.Va 700 characterized by a high level of partial resistance. 'Resistance to infection', 'resistance to extension', and 'resistance to sporulation' increase with age of the plant up to 8 weeks after seeding or later.

Chapter 6 The field method, in which after inoculation of the spreader rows the plants were kept wet by means of an automatic sprinkling installation during one 40 h period each week, was suitable for the evaluation of differences in partial resistance between individual plants. The character used in the field experiments was r, the 'apparent infection rate', expressed in logit units per day.

Chapter 7 Results from 6 weeks old plants of the F_2: Moneymaker × W.Va 700 and the B_1: Moneymaker × (Moneymaker × W.Va 700) were not accessible to conclusive genetic analysis, whereas the results from the same plants at the age of 8 weeks showed Mendelean segregration. From both field and laboratory tests, it was concluded that the partial resistance of W.Va 700 against the 1-t-pathotype of *P. infestans* is controlled by a single gene, which gives complete resistance to the stems and partial resistance to the leaves. This new gene has been called the Ph_2-gene.

Chapter 8 The three methods used for testing resistance, the probit log dosage analysis, the components analysis, and the field method, are compared and

their applicability to practical tomato breeding are considered. The probit - log dosage analysis is the most precise and most expensive method. The components analysis method is somewhat less precise, applicable to large numbers of plants, but it is again a costly method. The field method is not so precise, but it is an acceptable method. For breeding purposes a simplified method is indicated.

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Appendix

Resistance of the tomato lines Moneymaker, W.Va 700, F_1: Moneymaker × W.Va 700, F_2: Moneymaker × W.Va 700, and the B_1: Moneymaker × (Moneymaker × W.Va 700)

INTRODUCTION

In 1973, an experiment was designed to verify the conclusion, that resistance of W.Va 700 against the 1-t-pathotype of *P. infestans* is controlled by a single gene which determines complete resistance of the stems and partial resistance of the leaves, a conclusion based on earlier experiments (see Section 7.3.3).

MATERIALS AND METHODS

Three field plots (12 x 12 m), situated in a corn field, were organized as follows:

a. 5 rows of 11 plants of the F_2: Moneymaker × W.Va 700,
b. 3 rows of 13 plants of the B_1: Moneymaker × (Moneymaker × W.Va 700), and
c. 2 rows of 11 plants of a randomized mixture of 22 'Moneymaker' plants, 22 W.Va 700 plants, and 44 plants of the F_1: Moneymaker × W.Va 700.

The rows were randomized. The row distances were 0.5 and 1 m in turn, and the plant spacing within the rows was 0.75 m. Each field was surrounded by 48 'Moneymaker' plants, which were inoculated on 73.07.28 as described in 6.2. The test plants were treated and classified for resistance as described in 6.2.

RESULTS

Resistance of the stems From the 66 plants of the mixed population (sub c), only the 'Moneymaker' plants showed stem lesions. Among the 166 F_2 plants (sub a; including one extra plant), there were 39 plants with and 127 plants without stem lesions. From the 117 B_1 plants (sub b) tested, there were 57 plants with and 60 plants without stem lesions. The ratios of the numbers

of plants with and without stem lesions of the F_2 and B_1 agree with the expected ratios 1:3 and 1:1, based on the segregration of a single dominant gene for resistance at $p > 0.5$ ($X_1^2 = 0.20$) and $p > 0.8$ ($X_1^2 = 0.08$), respectively. These results corroborate the conclusion in 7.2.1: the resistance of the line W.Va 700 against the 2,4-p/1-t-pathotype of *P. infestans* is due to a single dominant gene (called the Ph_2 gene).

Resistance of the leaves From day 20 to 23 of this experiment, no increase in the fraction diseased foliage was observed, high temperatures having temporarily stopped late blight development. As these days were meaningless for the development of the epidemic on the tomatoes, they were excluded from the calculation of the 'apparent infection rate' r.

The mean r-values of the F_2 plants with and without stem lesions were 0.24 and 0.14, respectively. The mean values of the B_1 plants with and without stem lesions were 0.26 and 0.16, respectively. These values are considerably lower than the values obtained in the 1972 field experiment (see Section 6.2). High temperature and dry conditions are responsible for the slow late blight development in the 1973 field experiment. The somewhat higher mean r-values of the B_1-plants compared with the corresponding F_2 plants might be due to the fact that in the B_1 population twice as much susceptible plants were present as in the F_2 population, resulting in more inoculum for the nearest neighbours, and thus in more lesions.

The mean differences between r-values of plants with and without stem lesions are 'probably significant' at $p < 0.1$ for both the F_2 and the B_1 population. The mean differences between r-values of corresponding groups of plants of the F_2 population and the B_1 population are not significant at $p > 0.2$.

An estimate of the environmental variance Var(E) of r is obtained by pooling the variance calculated for each line mentioned sub c per field (see Section 7.3.1), its value being 16.34 ($\times 10^{-4}.r^2$). The variance within fields was calculated for both the F_2 plants with and the F_2 plants without stem lesions, the results being 18.08 and 16.02 ($\times 10^{-4}.r^2$), respectively. The variances within fields for the B_1 plants with and those without stem lesions were 13.07 and 18.05 ($\times 10^{-4}.r^2$), respectively. These values do not differ significantly at $p > 0.2$ from the estimate of the environmental variance, and they do not differ significantly from each other. The same result was found for the variance ratios per field. These results confirm the conclusion drawn in 7.3.3, that within both groups of plants, those with and those without

stem lesions, no other source of variance can be demonstrated than the environmental source.

CONCLUSION

The evidence derived from the mean values of the apparent infection rates is not conclusive. Nevertheless, the results from the 1973 experiment reported in this appendix corroborate the conclusion that the partial resistance of the leaves of W.Va 700 is based on the gene, which also protects stems against late blight attack of the 2,4-p/1-t-pathotype of *P. infestans* (or on a gene closely linked with it).