BACTERIAL BLIGHT (*PSEUDOMONAS PISI* SACKETT) OF PEAS IN SOUTH AFRICA, WITH SPECIAL REFERENCE TO FROST AS A PREDISPOSING FACTOR

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635.656:632.35Pseudomonas pisi Sackett.095.15

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PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD
VAN DOCTOR IN DE LANDBOUWWETENSCHAPPEN,
OP GEZAG VAN DE RECTOR MAGNIFICUS,
PROF. DR. IR. H. A. LENIGER,
HOOGLERAAR IN DE TECHNOLOGIE,
IN HET OPENBAAR TE VERDEDIGEN OP
VRIJDAG 29 SEPTEMBER 1972 TE 16.00 UUR
IN DE AULA VAN DE LANDBOUWHOGESCHOOL
TE WAGENINGEN

H. VEENMAN & ZONEN N.V. – WAGENINGEN 1972

STELLINGEN

I

Een radicale verandering van de taxonomie van phytopathogene bacteriën is bij de huidige stand van het onderzoek niet gewenst.

KIRALY, Z. (Ed.) Methods in plant pathology. Akademiai kiado, Boedapest, 1970, 125–126.

II

Het derde postulaat van KOCH, n.l. datgene dat betrekking heeft op de herisolatie, dient in de phytobacteriologie vervangen te worden door het volgende: aangetoond moet kunnen worden dat de bacterie zich in de geïnoculeerde plant kan vermeerderen.

III

De door *Pseudomonas pisi* geproduceerde caseïnolytische proteasen spelen geen rol als virulentiefactor.

Dit proefschrift.

IV

ERCOLANI heeft niet bewezen dat bacteriën, 'carrying the pathogenicity factor only', d.w.z. virulente, phytopathogene bacteriën in een niet-passende waardplant, zich niet blijvend kunnen hechten aan de vermeerderingsloci binnen in de plant.

ERCOLANI, G. L. Phytopathologia Mediterranea 9 (1970), 145–150.

V

Bij de uitleg van spuitproeven wordt de wenselijkheid om onbehandelde controleveldjes in de proef op te nemen bepaald door het doel van de proef en de aard van het fungicide.

V. D. PLANK, J. E. Plant Pathology, epidemics and control. Academic Press, New York en Londen, 1963.

VI

Salmonella typhi beweegt zich voort door middel van een golfbeweging van de cel en niet door middel van flagella.

PIJPER, A. Bacterial Flagella and Motility. Ergebn. Mikrobiol. ImmunForsch. exp. Ther. 30 (1957), 37–95.

Dissertatie B. H. BOELEMA
Wageningen, 29 september 1972.

VII

Bij de selectie van tomaten op resistentie tegen *Corynebacterium michiganense* kan uit de relatie tussen de logaritme van de proportie gezond gebleven planten en de concentratie van het inoculum, waarmee deze ingespoten zijn, afgeleid worden of het uitgangsmateriaal met betrekking tot resistentie tegen de bacterie homogeen is.

VIII

Het is onwaarschijnlijk dat de tegen meeldauw (*Oidium tuckeri*) resistente druivecultivars, die in Zuid Afrika verkregen zijn door kruising van gevestigde *Vitis vinifera* rassen met *V. labrusca*, in de afzienbare toekomst hun resistentie tegen meeldauw zullen verliezen.

IX

De mening dat de belangstelling van het Oud-Testamentische volk Israël voor insekten geheel bepaald werd door utiliteitsmotieven is onjuist.

BRAUN, H. Geschichte der Phytomedizin. In: SORAUER, P. Handbuch der Pflanzenkrankheiten I.1. 7de druk. Paul Parey, Berlijn en Hamburg, 1965.

X

De in het Afrikaans algemeen gebruikte woorden navorsing en beplan(nen) behoren ook in het Nederlands taalgebruik een plaats te krijgen.

... en daar by geoordeelt hebbe, dat soodanige kleyne Dierkens met haar duysent Milioenen te samen, niet meer lighaams souden uytmaken als een grof sand groot is, als voor desen hebbe geseyt; en als wy dan daar nevens gedenken, uyt wat saaken de werktuygen van het lighaam van soo een Dierke bestaan, waar mede het sig beweegt en verplaatst, en daar benevens, wat een groot geheym is soo een Dierke kan gehuysvest zijn, soo moeten wy als verbaast staan, als niet komende beseffen, de hoe kleynheid der deelen, waar uyt de schepsels sijn te samen gevoegt, en seggen in ons selven, Hoe ondergrondelijk is de diepte der wijsheid!

ANTONI VAN LEEUWENHOEK

117de Missive
Geschreven aan de Hoogedele Heeren
die van de Koninklijke Societeit in Londen
Delft in Holland den 23. Juny 1699

VOORWOORD

Bij het verschijnen van mijn proefschrift wil ik graag mijn dank betuigen aan allen die tot mijn wetenschappelijke vorming hebben bijgedragen.

Hooggeleerde OORT, ik ben U zeer erkentelijk dat U mij bij het onderzoek leiding hebt willen geven. Uw voortdurende belangstelling en waardevolle suggesties zijn het proefschrift zeer ten goede gekomen.

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1. INTRODUCTION

Green peas (*Pisum sativum* L.) are grown in South Africa for marketing, canning and seed, only in certain regions of the Republic. They are also grown in home gardens, not in specific regions but throughout the whole country. Those for the fresh markets and for the canning factories are produced (a) in the Cape Province in the area between Wellington, Malmesbury, Worcester and Tulbagh and at Oudtshoorn and Uniondale, and (b) in the Transvaal near Ermelo, Bethal, Delmas and Groblersdal. Seed is produced mainly at Vaalhartz, Upington, Groblersdal, Lydenburg and Oudtshoorn; in these places the peas are grown under irrigation. They are planted from April to June and the seed is harvested in September, October or November; the cool, dry weather in winter makes these areas eminently suitable for seed production.

In the early 1950's, however, bacterial blight caused by *Pseudomonas pisi* Sackett, made its appearance in these areas. It had first been found in 1915 by SACKETT in Colorado, U.S.A. (SACKETT, 1916) and it was later reported from many other American states and also from Canada, Bermuda, Uruguay, Japan, Australia, Tasmania, New Zealand, Germany, France, Bulgaria, Hungary and Morocco (ANONYMOUS, 1952). More recently it has been observed in the Argentine (GOLDBERG and VON DER PAHLEN, 1960), the Ukraine (GORLENKO, 1965), in Greece (THANASSOULOPOULOS, 1965) and in Lebanon (SAAD and NIENHAUS, 1969). It also occurs, but only sporadically, in the Netherlands (VAN POETEREN, 1923; DE TEMPE, 1954). In 1929 OGILVIE (1930) found a bacterial disease on peas in England and thought it was probably identical with that caused by *Ps. pisi* in the U.S.A., but in 1949 DOWSON mentioned that bacterial blight had so far not appeared in Britain. In Africa south of the Sahara, the disease was found in Tanganyika and South Africa. In Tanganyika it was observed for the first time in 1951 (WALLACE and WALLACE, 1951, 1952), but it was not recorded in the years 1953 to 1958 (ANONYMOUS, 1958). It has never been found in Rhodesia (BATES, 1954). It is impossible to find out when the disease was first reported in South Africa. As early as 1921 'bacterial diseases of peas, beans and tobacco' were mentioned in a review of plant diseases occurring in South Africa, compiled by the Division of Botany (ANONYMOUS, 1921). No bacterial disease of peas was mentioned in the list of plant diseases published by DOIDGE in 1924. In a revised list (DOIDGE and BOTTOMLEY, 1931) bacterial blight was reported to have completely destroyed a crop of peas near East London. LOEST (1953) mentioned that a bacterial blight of peas had been known for years to occur sporadically in the Transvaal Lowveld, but the identity of the causal organism was never established. Bacterial blight was reported by the Seed Inspection Service from Groblersdal and Lydenburg in 1951, and in 1952 a severe outbreak on the Vaalhartz Irrigation Scheme caused great concern to seed growers, as well as to the Department of Agriculture. It is highly probable that the disease was introduced with seed from the Groblersdal-

Lydenburg area, because much of the pea seed planted at Vaalhartz in 1952 was from that area. In 1953 the disease was noticed for the first time at Oudtshoorn, Upington and Pretoria and a year later also at Cradock. Since 1956 it has never been of great economic importance in South Africa.

In the present study the causal and conditioning factors of bacterial blight were investigated. In the second chapter the symptoms, which develop under different weather conditions, are described and chapter 3 deals with the identification of the pathogen. Ever since the first severe outbreak of the blight in 1951 it has always been obvious that weather conditions, particularly frost, have a great influence on the disease. A study of the effect of conditional factors is recorded in chapters 4 and 5. Experiments on the origin and spread of the disease are described in chapter 6 and control measures are discussed in chapter 7. In the final chapter experiments are described which were done to investigate the basic causes of the effect of frost on the disease.

This study was undertaken at the Pretoria Horticultural Research Institute, situated at a geographic position of 25° 35′ south and 28° 21′ east and at an altitude of 1087 m.

2. SYMPTOMATOLOGY

2.1. SYMPTOMS

The symptoms of bacterial blight are dependent on the weather that prevails during and after infection: symptoms that develop in dry weather with occasional frost are different from those found under rainy conditions. In the Transvaal the first mentioned are the most common. Thus when the disease assumed epidemic proportions during the cold winters in the beginning of the fifties, growers generally ascribed the symptoms to frost injury. For comparison a description of frost damage is given later in this chapter.

2.1.1. *Symptoms in dry weather with occasional frost*

Stems. The infected portion of the stem is at first water-soaked, but soon becomes olive-green and finally purplish-brown. The epidermis can at this stage easily be stripped off, exposing the brown tissue underneath.

Stipules. The stipules are usually infected from the stem. The principal veins become brown to black starting at the base. The interveinal tissue is first water-soaked, then ochre-yellow to rust-brown or purplish; it dies later and becomes papery. The spread of the infection in the stipules is limited by the tiny veins. Hence a fan-like pattern, which is very characteristic for the disease,

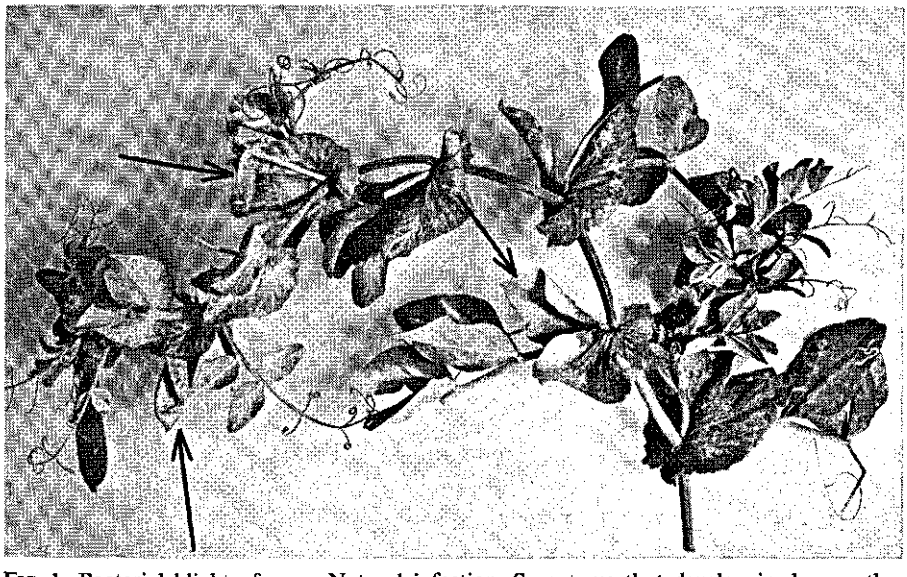

FIG. 1. Bacterial blight of peas. Natural infection. Symptoms that develop in dry weather with occasional frost. Notice frost damage to pods and leaves (arrows) and the apparently healthy vine that grows from the lowest axil.

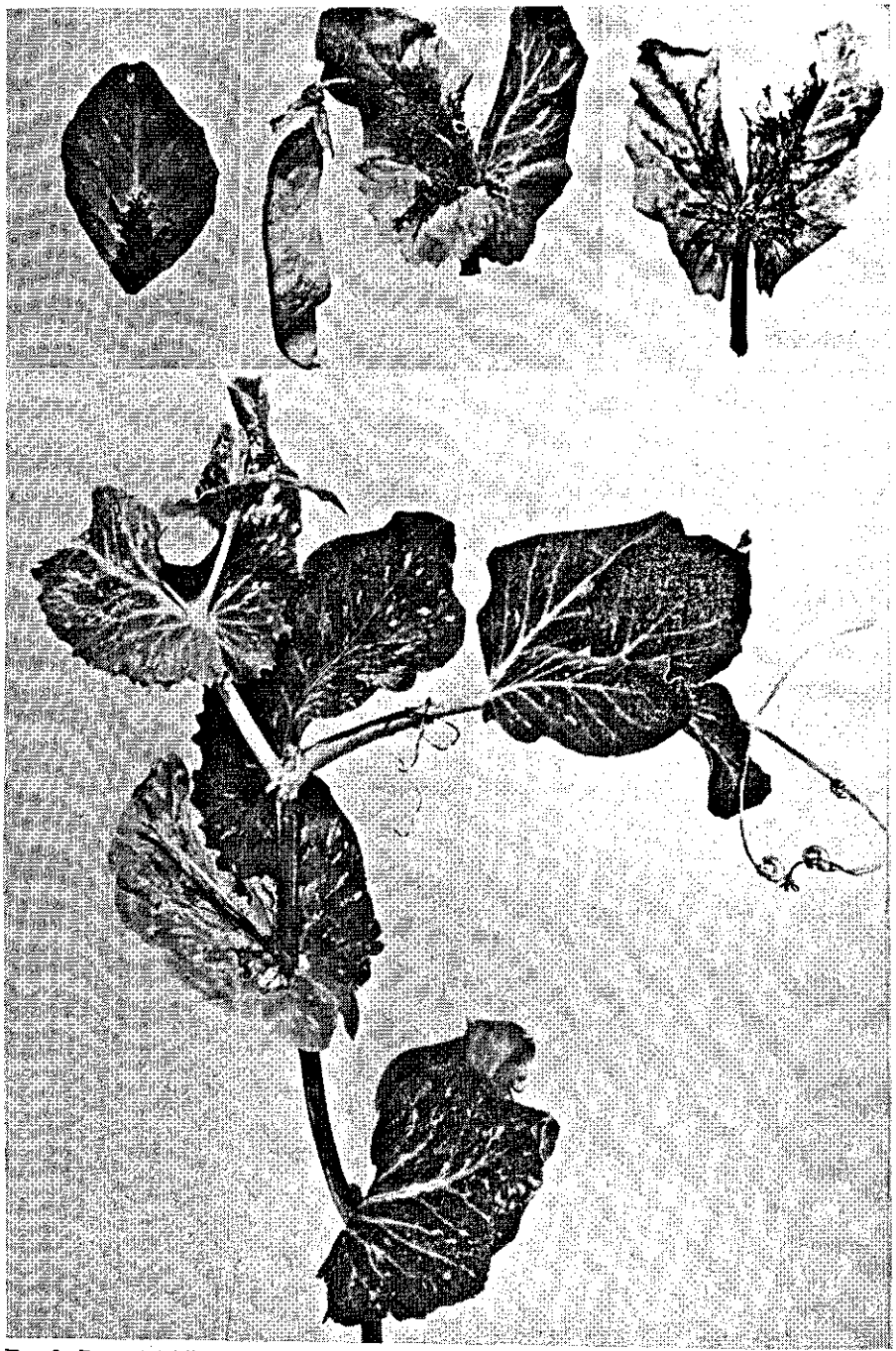

FIG. 2. Bacterial blight of peas. Natural infection. Symptoms that develop in dry weather with occasional frost.

develops in the stipules. At a later stage the infected stipules die. Occasionally only one stipule of a plant is infected, the rest of the plant being healthy (Fig. 1 and 2).

Petioles and leaflets. Symptoms similar to those on the stems and stipules may be found on petioles and leaflets respectively, but on the leaflets typical syptoms are seldom seen.

Peduncles and flowers. The peduncle, arising from the axil of an infected stipule, is usually also infected and the symptoms are similar to those on the stems (Fig. 2). The flower or young pod on an infected peduncle seldom develops further but usually shrivels and dies.

Pods and seeds. Pod infection is not common in South Africa. Infected pods were occasionally observed in the fields, but the following description is mainly based on symptoms that developed after artificial inoculation. The lesions on the pods are sunken, water-soaked, purplish-brown, sharply defined, round to irregular, with a diameter of one to four millimeter. The spots may coalesce and cover a considerable part of the pod. Sometimes the infection is limited to a narrow band along the dorsal and ventral sutures. On mature pods the lesions

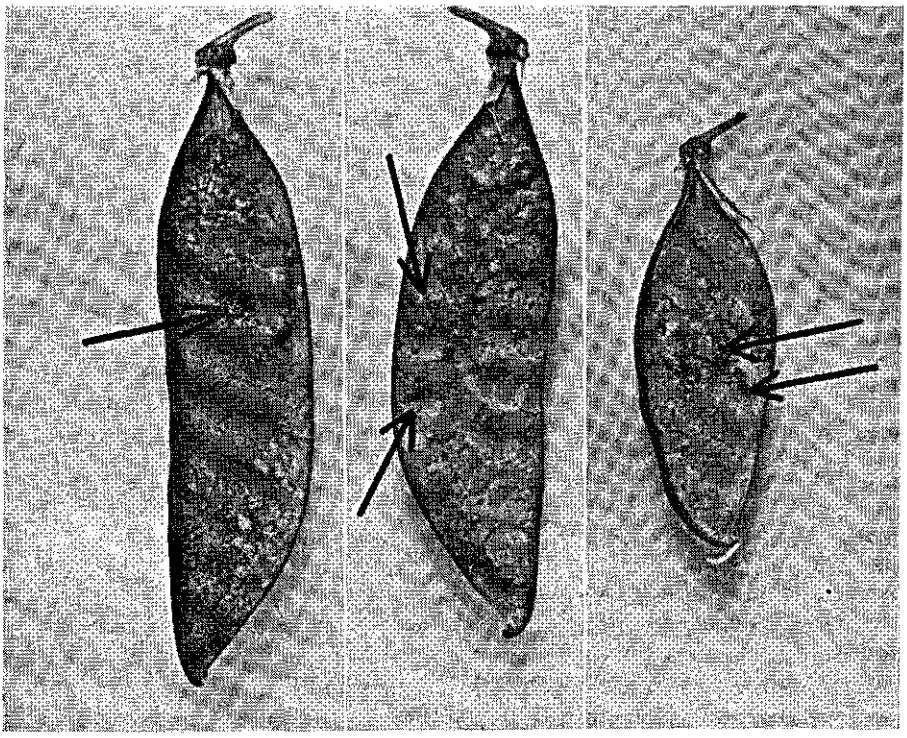

FIG. 3. Pea pods with symptoms of frost injury and bacterial blight (arrows).

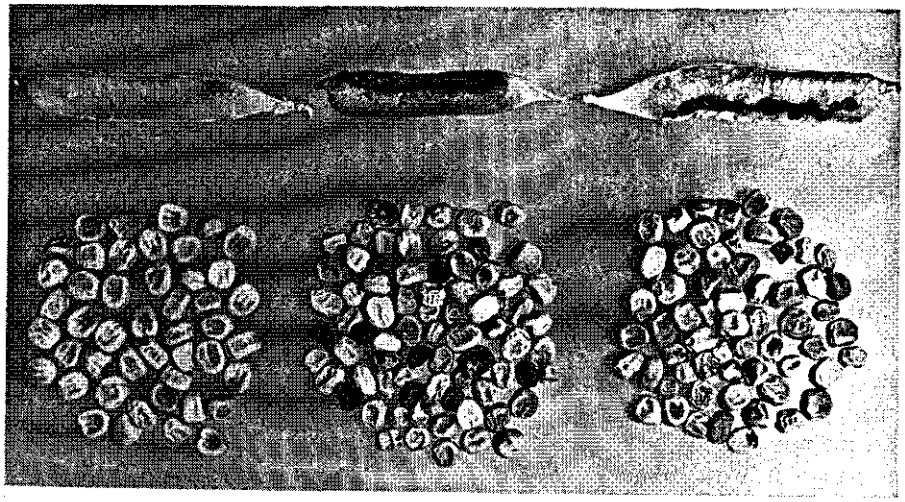

FIG. 4. Mature pea pods and seeds artificially inoculated with *Pseudomonas pisi*. Left: control.

are green to brown contrasting with the normal yellow colour of a ripe pod (Fig. 3 and 4). Seeds are water-soaked and olive-green to brown-yellow. Some are small and shrivelled (Fig. 4).

Roots. No symptoms have ever been seen on the roots.

Slime. No slimy exudate has been observed on infected plants.

The disease usually starts at the base of the stems a few inches above the soil, at a point where a stipule is attached. The infection spreads upwards inside the stem to the leaves and stipules; when it reaches the top, the stem withers and dies. If the infection remains restricted to the base of the stem the top may stay green for a long time and the stem may even produce flowers and pods. New vines usually grow out of the stem base below the infected portion. Thus, if the main stem becomes infected and dies when it is still young, the healthy side shoots can take over and the plant then appears to be healthy and looks as if it had never been infected. If young plants die before they form vines, they become overgrown by neighbouring healthy plants. In severe cases the new side shoots are also infected and before long the whole crop is brown and withered.

2.1.2. *Symptoms under rainy conditions*

Although the winters in the Transvaal are practically always dry (see Table 1), the rainfall in 1957 was exceptionally high; 123.2 mm was recorded from June to August and the following symptoms were observed: scattered rust-brown spots a few millimeter in diameter, more or less round or angular and sharply defined by the veins, developed on the stipules and leaflets. They were at first water-soaked but later turned papery-brown and often showed a lighter coloured centre (Fig. 5). Spots were also found on the stems.

TABLE 1. Averages of climatological data taken at the Horticultural Research Institute from 1953 to 1968.

Month	Average monthly temperatures in °C			Average monthly rainfall in mm
	Maximum	Minimum	Average	
January	29.0	16.6	22.9	119.5
February	29.0	16.5	22.9	90.8
March	28.0	14.5	21.3	61.7
April	25.4	11.2	18.3	55.3
May	22.9	6.0	14.4	26.2
June	20.2	2.9	11.9	9.1
July	20.4	2.8	11.6	4.5
August	23.3	4.6	14.0	4.4
September	26.6	9.0	17.8	17.2
October	28.6	13.2	21.0	60.6
November	28.2	15.2	21.7	113.1
December	28.4	16.0	22.0	98.9

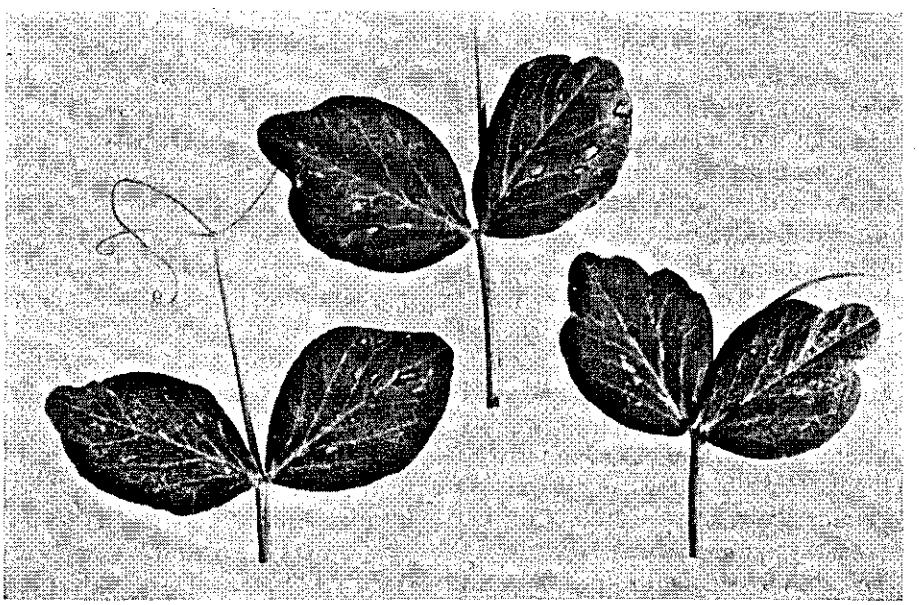

FIG. 5. Bacterial blight of peas. Symptoms that develop under rainy conditions.

Stems, stipules and leaves. The symptoms, described by SACKETT (1916) in the first publication on bacterial blight, are very similar to those that developed in the Transvaal in dry weather with frost. This type of weather apparently prevails also during the growing season in the San Luis Valley, where SACKETT made his observations. Peas are grown under irrigation in this area and SACKETT mentions that frost can be expected any time of the year. There is a distinct difference between the symptoms described by SACKETT and those described by other American authors. According to HARTER et al. (1945) and SCHROEDER (1953) the disease is characterized by scattered spots on stems, leaves and stipules. These symptoms were also described in Tanganyika (WALLACE and WALLACE, 1951), Hungary (KLEMENT and LEHOCZKY, 1960) and New Zealand (BRIEN et al., 1955). They are very similar to the symptoms described under 2.1.2 and they also developed under moist conditions. In a later publication from New Zealand, that specially refers to Canterbury, not only scattered leaf spots are mentioned as symptoms of bacterial blight, but also fan-like lesions on stipules and leaflets (YOUNG et al., 1969). In this publication the authors mention both rainy conditions and frost as predisposing factors.

In New South Wales (ANONYMOUS, 1939) infected areas on the stems are watery and greenish-brown, whereas those on stipules and leaflets have a watery and bruised appearance. They may start either on the stem near ground level and extend upwards to the stipules and leaves, or they may begin as numerous small spots scattered over the lower part of the plant on the stem, stipules and leaflets. It thus appears that both syndromes (described under 2.1.1 and 2.1.2) are found in New South Wales. The author does not mention weather conditions that are favourable for the disease, though he does state that rain is a means of spread. According to WARK (1954), however, either frost or high humidity is necessary for the development of symptoms in Canberra, which is situated within the boundaries of New South Wales. BROWN and EVANS (1937) also distinguish between symptoms that (a) start on the stem near the soil and extend upwards to the stipules and leaves and (b) start as scattered spots on stems, stipules and leaves. They mention that late spring frosts favour blight and that the disease spreads easily under moist conditions.

In Italy the disease, both in the field and in the glasshouse, is characterized by scattered leaf spots and by a necrosis of the leaf veins (CIRULLI and ERCOLANI, 1969). The vein necrosis results in the death of the adjoining tissue, but the pattern that develops is apparently different from that described by SACKETT (1916).

Flowers. WALLACE and WALLACE (1951) mention brown spots on the flowers.

Pods. Symptoms on pods were described for the first time by LUDWIG in 1926. They are very similar to those described under 2.1.1. The same symptoms were described by several authors in different countries (BROWN and EVANS, 1937;

STAPP, 1937; HARTER et al., 1945; KLEMENT and LEHOCZKY, 1960; YOUNG et al., 1969).

Seed. BROWN and EVANS (1937) found that young seeds may be either killed by bacterial blight or, if they mature, show small water-soaked spots; they may be coated with bacteria. BRIEN et al. (1955) and WALLACE and WALLACE (1951) found that in humid weather the seed becomes covered with a bacterial slime, which either dries on the seed or invades the coat, causing the seed to be discoloured and blotchy. STAPP (1937) and SKORIC (1927) also mention these symptoms and add that the spots are mainly found near the hilum.

Roots. No author has ever mentioned symptoms on the roots.

Wilting. SACKETT (1916) and STAPP (1937) have pointed out that infected plants did not show a true wilting caused by a decreased turgor pressure. Wilting was, however, observed by SKORIC (1927) and BROWN and EVANS (1937) after the bacteria had invaded the vascular system.

Slime. Bacterial slime was found on stems, stipules and pods, particularly under moist conditions (SACKETT, 1916; STAPP, 1937; HARTER et al., 1945; KLEMENT and LEHOCZKY, 1960).

2.3. FROST INJURY OF PEA PLANTS

Though the symptoms described under 2.1.1 are associated with frost, symptoms of frost injury as such are distinctly different from those of bacterial blight. The following description is based on observations made in the Transvaal Middleveld.

Stems and stipules that have been exposed to frost, become water-soaked and translucent, but usually they appear to return to normal within a few hours. After a day or two permanent injury may become visible. The upper leaflets of erectly growing plants, which have been slightly affected by frost, are pale yellow and withered and sometimes show dark-blue margins. If the frost is more severe the older leaves and stipules are damaged as well, while the stems are flaccid for some time.

If the plants are bent over and the tops touch the soil surface, the middle leaves and stipules are more severely damaged than those at the tops or the bases. In other words, those plant parts that are highest above the soil line suffer most from frost injury. Stems of plants that have toppled over, may become light-brown to purplish, probably as a result of subsequent sunscald. This can be distinguished from bacterial blight because a) it occurs only on that side of the stem that is facing the sun, b) the injury does not go deeper than a few cell layers, and c) it does not spread to the stipules.

Frost may cause brown, necrotic spots to develop on the stipules and leaflets. They develop between the veins in a symmetric pattern, usually two or three on either side of the middle vein. They are first seen on the under side of the leaves as small tears in the epidermis. The leaflets and stipules that develop after exposure to frost are sometimes misshapen. If the tops of young plants are killed

by the frost, new vines may grow out of the stem bases and may take the place of the main stems. Young plants are not injured at all by light frost, but those that are at the flowering or pod forming stage are affected most severely; the flowers and young pods are killed outright. When older pods are injured they become mottled white and green and the young seeds do not develop (Fig. 3).

In a vigorously growing pea crop it is often found that the epidermis of the stem is torn longitudinally just under the points of attachment of the stipules; these are not a result of frost: frost causes rifts that go deeper into the stem.

In the literature a detailed description of frost injury to peas can be found in a publication by WALKER (1939), who describes it in pea crops planted in spring. These crops are injured by late frost when the plants are still fairly young. If the growing point is killed, the lower buds are stimulated to grow and a new main stem develops, usually with longer internodes than normal. The irregular growth interferes seriously with the elimination of off-types in seed crops. WALKER describes malformation of leaflets and stipules and mentions that interveinal necrotic spots could easily be mistaken for bacterial blight, were it not for their regular form and position on the leaf lamina and their lack of translucency. Stem cankers are commonly associated with frost injury: some of the epidermal and cortical cells are killed in a narrow band running parallel to the long axis of the internode. Since the remaining cells are not affected, growth and expansion continue, causing a rift in the superficial dead tissue.

KERLING (1952) investigated these rifts microscopically and found that a wound-cork layer develops and rejects the dead parts of the cortex.

2.4. DISCUSSION

In the description of the disease symptoms on stems, stipules and leaves a distinction is made between those that develop a) in dry weather with occasional frost (Fig. 1 and 2) and b) under rainy conditions (Fig. 5). The same association between expression of disease symptoms and prevailing weather conditions can be found elsewhere in the literature, but is not mentioned explicitly. Another bacterial disease that shows a distinct relationship between symptoms and weather conditions is blossom blight of pears (*Pseudomonas syringae*). Two types of symptoms could be distinguished: a) necrosis of the calyx cup, which is wide-spread and endemic and b) blight, which is caused by a severe and extensive infection of the receptacles and pedicels, is predisposed by frost and results in a complete destruction of the flower trusses (PANAGOPOULOS and CROSSE, 1964a and b).

The differences between the symptoms of bacterial blight of peas on the one hand and those of sunscald and frost injury on the other, have been mentioned under 2.3. In the Transvaal spotted wilt is sometimes wrongly diagnosed as bacterial blight, because of the purplish streaks that develop on the stems of virus infected pea plants, but the twisted tops and the numerous very small spots on the leaves distinguish it from bacterial blight.

3. ISOLATION, DESCRIPTION AND IDENTITY OF THE CAUSAL ORGANISM

Several isolations were made both from diseased pea plants with symptoms that develop in dry weather with frost and from those with symptoms that develop under rainy conditions. Their pathogenicity was tested according to different methods. Two isolates of the pathogen were described. Moreover their virulence towards beans was tested, because this is an important criterion to distinguish between *Ps. pisi* and *Ps. syringae.*

3.1. ISOLATION TECHNIQUE AND PATHOGENICITY TESTS

The causal organism was isolated according to standard methods (DOWSON, 1957; STAPP, 1958). The isolation was fairly easy provided the diseased plants were fresh and the infections not too old. The following pathogenicity tests were used:

a) *Tests on pods.* Pods of healthy pea plants, cv. Little Marvel, were washed in running water and put in sterile petri dishes, two in each dish. They were pricked at two different places with the needle of a hypodermic syringe containing a suspension in sterile water of the bacterium to be tested. Before the inoculation the syringe was boiled for ten minutes in distilled water or disinfected in alcohol 70%. The pods used as controls were pricked with a hypodermic syringe filled with sterile water only. After the inoculation the petri dishes with pods were incubated at 24–25°C. If sunken water-soaked, green to brown spots developed around the punctures on the inoculated pods the results were taken as positive.

b) *Tests on stems.* Stems of pea plants grown in a glasshouse were injected under the epidermis with a water suspension of the bacterium to be tested. The needle of the syringe was stuck into the axil of a stipule and the suspension was injected downwards into the stem. The control plants were injected with sterile water. The injected portions of the stems became water-soaked, but this condition soon disappeared. If after a few days they became water-soaked again and subsequently brown to purplish-brown, the result was considered as positive.

c) *Tests with seeds.* Pea seeds were disinfected for five minutes in a 0.1% aqueous solution of mercuric chloride after they had been dipped for a few seconds in alcohol 96% to drive off the air from the surface. They were rinsed in tap-water and planted in trays containing vermiculite, which had been sterilized in an autoclave for half an hour at 121°C. After the trays had been in an incubator at 25°C for two or three days the germinated seeds were inoculated by soaking them for three hours at 25°C in a suspension of the bacterium to be tested. The control seeds were soaked in sterile water under the same conditions. After these treatments the seeds were planted in steam sterilized soil. In tests with

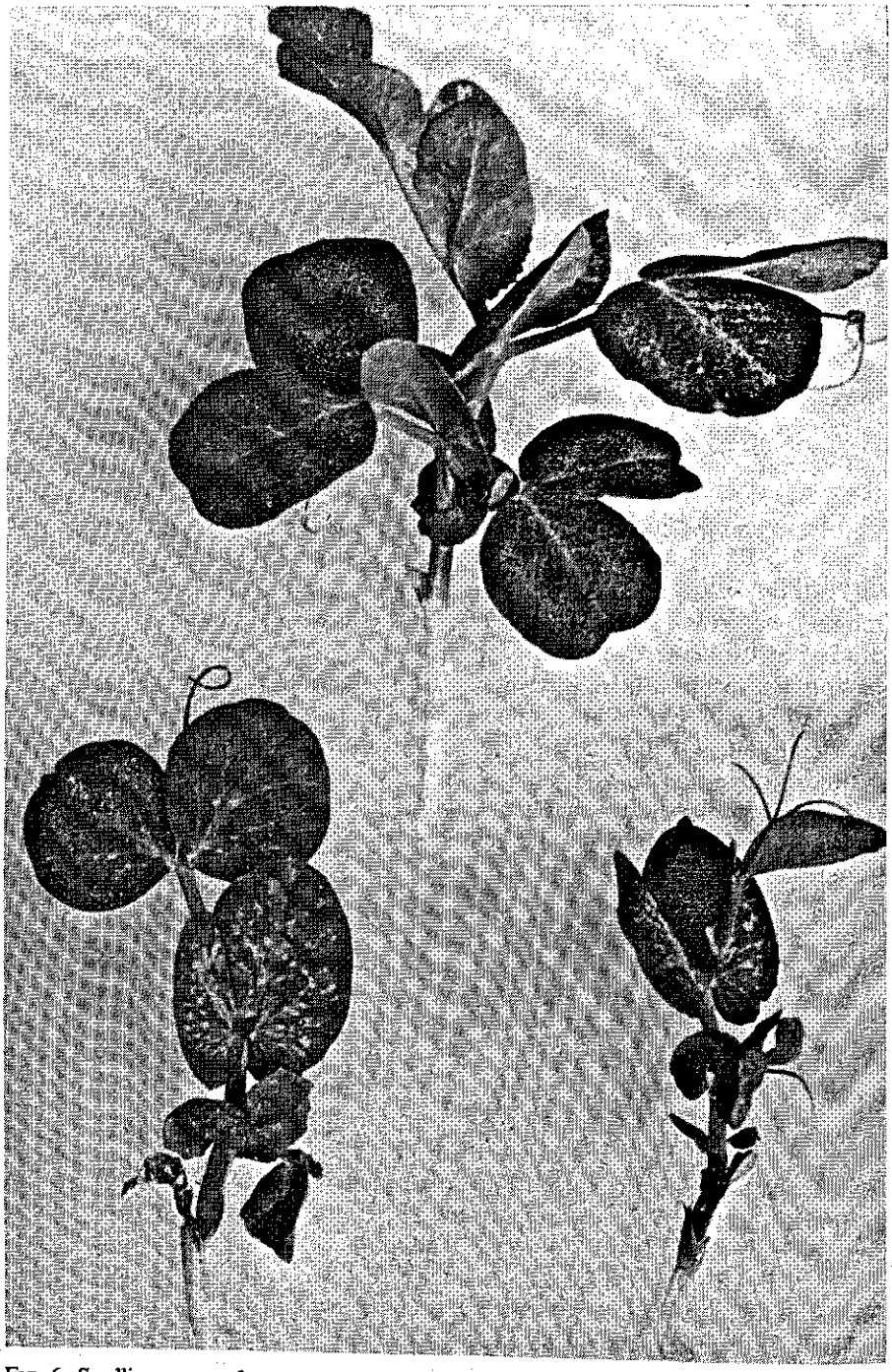

FIG. 6. Seedlings grown from seed artificially inoculated with *Pseudomonas pisi*. Top: control.

positive results brown, water-soaked spots developed on the stems and the lower stipules; sometimes the growing point was killed and laterals developed from the stem base (Fig. 6).

3.2. Isolates and culture media

In Table 2 details are given of the isolates that were used in the experiments described in this publication. They were all made from pea plants grown at the Horticultural Research Institute. Most of the experiments were done with isolate III. This isolate is kept in the National Collection of Plant Pathogenic Bacteria under number 1653.

The isolates were mostly grown on or in the following Difco media: Bacto Nutrient Agar (NA), Bacto Tryptone Glucose Extract Agar (TGA) and Bacto Nutrient Broth (NB). The pH of these media is about 6.8 after autoclaving.

Table 2. Details of isolates of *Pseudomonas pisi*.

Isolate No.	Date of isolation	Pathogenicity tests
I	June 1954	a, b**
Ia*	April 1955	a
II	August 1957	a, b, c
III	August 1961	a, b, c

* Ia is a reisolate from seeds inoculated with isolate I in September 1954.
** a, b and c mean successful pathogenicity tests on pods, stems and seeds respectively.

3.3. Description of the pathogen

A study was made of morphological, cultural, biochemical and physiological characteristics of isolates II and III.

3.3.1. *Morphological and cultural characteristics*

Material and methods. The flagella staining was done according to Zettnow (Stapp, 1958); the Gram staining according to Nyfeldt (Dowson, 1957); the acid-fast staining according to Ziehl Neelsen (Dowson, 1957); the volutin staining according to Stapp (1958) and the fat staining according to Burdon (1946). The media were autoclaved for 15 min at 121°C, except Uschinsky's and Fermi's media which were steamed for $\frac{1}{2}$ to 1 hr on three successive working days. The pH of the medium was, if necessary, adjusted with 5% Na_2CO_3 or with 4% or 40% NaOH. The pH values mentioned in the description were measured with an electric pH meter after the media had been autoclaved or steamed. As a rule the cultures were incubated at 25°C. Only those grown on potato cylinders and those in Uschinsky's and Fermi's media were incubated at 27°C. In order to measure the size of the cells water suspensions were made from 24 hr old cultures on TGA and the living cells were slightly stained with fuchsin.

Unless otherwise stated no important differences between the two isolates were observed.

Description. The pathogen is a rod with rounded ends, occurring singly or in pairs. In 24- and 48-hr old cultures on TGA chains and filaments were found. The cells of isolate II grown on TGA were 2.1 μ (1.4–2.8 μ) × 0.9 μ and those of isolate III were 2.3 μ (1.9–3.7 μ) × 0.9 μ in size. The flagella of isolate III were mono- or bipolar, 1–5 flagella per pole. Isolate II had no flagella at the time when the staining was done. The pathogen is Gram-negative, not acid-fast and no prominent sudanophilic inclusions were found after one day on HAYWARD's (1960) medium (pH 7.5). Very little volutin was found in cells from 2-days old cultures on TGA. Spores were never observed.

After 24 hr on TGA slants the growth was filiform, glistening, grayish white, slightly raised, transculent and smooth. After 48 hr the medium was slightly green. The growth was moderate after 24 hr and became abundant by the third day. Cultures on NA slants looked the same, but the growth was not so vigorous. After 72 hr on TGA plates the colonies were circular with undulate edges, convex, transculent, grayish white, but in transmitted light bluish with a cream coloured centre; diameter 3–4 mm. The colonies on NA plates looked the same, but were somewhat smaller: 2–3 mm in diameter.

On potato cylinders the growth was cream coloured and after five days the cylinders had turned gray. Nutrient Broth (Difco) became turbid after one day and after two days a slight sediment had formed. The growth on Uschinsky's and Fermi's agar media (pH 6.7) was good. Isolate III grew well in the liquids (pH 6.7) too; isolate II grew well in Uschinsky's liquid, but slowly in Fermi's liquid medium.

3.3.2. *Biochemical and physiological characteristics*

Material and methods. Techniques described by the following authors were used: i. DOWSON (1957), ii. DYE (1960), iii. HAYNES (1951), but with sodium-gluconate instead of potassium gluconate, iv. HUGH and LEIFSON (1953), v. KLEMENT (1963), vi. KOVACS (1956), vii. LE COSQUINO DE BUSSY (1936), viii. LELLIOTT et al. (1966), ix. SNEATH (1956), x. STAPP (1958) and xi. THORNLEY (1960). The method (xii) used to test the presence of tyrosinase was as follows. The pathogen was grown on a medium consisting of (L)-Tyrosine 0.1 g, glucose 0.1 g, Bacto Nutrient Agar 2.3 g in 100 ml distilled water (pH 6.6). The production of a reddish-brown pigment was assumed to be the result of the action of tyrosinase. In the above tests, including those described by LELLIOTT et al. (1966), Difco media were used. For pH adjustment see 3.3.1. Details with regard to incubation temperatures and the sterilization of the media are given in Table 3. The final results of the tests were read after 7 days with the exception of H_2S and NH_3 production (16 days), growth in litmus milk (2 months), acid production from carbon compounds (28 days), the tobacco-hypersensitivity test (1, 2 and 3 days), the determination of the temperature maximum (28 days) and that of the temperature minimum (14 days).

The pH range was assessed according to SACKS (1956). The pH was determined with 'Universal Indikatorpapier Riedel-de Haën'.

Unless otherwise stated no important differences between the two isolates were observed.

Results. The results are summarized in Table 3, with the exception of the pH range. The results of this test were as follows. The optimum growth after one day was between pH 6.5 and 7.5. After 3 days it was found that the pathogen had grown well in the pH range $5\frac{1}{2}$ to 8, but not beyond these limits.

TABLE 3. Biochemical and physiological characteristics of *Pseudomonas pisi* as recorded by various authors.

Test	Method*	Results	SACKETT, 1916	STAPP, 1937	KLEMENT LEHOCZKY, 1960	CIRULLI ERCOLANI, 1969
Metabolism (glucose)	iv S 27 °C	oxidative				oxidative
Fluorescence	vii S 27 °C	+				+
LOPAT and subsidiary tests						
levan	viii S 27 °C	+				+
oxidase	vi	−				−
potato rot	viii 25 °C	−				−
arginine dihydrolase	xi A 25 °C	−				−
tobacco	v	+				+
2-keto gluconate	iii A 25 °C	−				+
lipase (margarine)	viii A 25 °C	−				
nitrate reduction	viii A 25 °C	−	−	+	±	−
acid from sucrose	viii S 25 °C	+				
Action on peptone	x A 27 °C					
Production of NH_3		+	+		±	+
H_2S		−	−		−	−
Acid production from**	i S 27 °C					
Glucose		+	+	+	+	+
Mannose		+			+	
Sucrose		+	+	+	+	+
Galactose		+	+	−	+	
Glycerol		+			±	+
Mannitol		+			±	
Sorbitol		+				
Lactose		−			−	+
Maltose		−			−	
Starch		−				−
Salicin		−			−	−
Aesculin		−				
Action on tryptophane indole production	i A 27 °C	−	−	−		−
Starch hydrolysis	i S 27 °C	−	−	+	−	−
Litmus milk	S 27 °C					
coagulation		−	+	+	+	
peptonization		+	+	+	−	+
alkaline reaction		+	+	+	+	+
reduction of litmus		−	+	+		

Test	Method*	Results	Sackett, 1916	Stapp, 1937	Klement Lehoczky, 1960	Cirulli Ercolani, 1969
Aesculin hydrolysis	ix A 27°C	+				+
Presence of tyrosinase	xii A 25°C	+				+
Gelatine liquefaction	viii A 25°C	+	+	+	+	+
Pectolytic enzymes	ii A 27°C	−				−
Temperature (in NB)						
Minimum below		3°C	7°C	0°C		
Optimum about		25–26°C	27–28°C	28–30°C		
Maximum		Between 35.0° and 37.5°C	Below 37.5°C	Between 35.5° and 36.5°C		
Thermal death point	x		Between 49° and 50°C	Between 48° and 50°C		
of isolate II, between		50° and 51°C				
of isolate III, between		49° and 50°C				

* The roman figures refer to the methods mentioned under 3.3.2. They are not necessarily the same as those used by the other authors mentioned in this Table. The media were either autoclaved, usually for 15 min at 121°C (A), or steamed on three successive working days (S). The temperatures are the incubation temperatures.

** Brom thymol blue was used as an indicator. No gas was formed from any of these carbon compounds after 28 days of incubation.

3.4. The virulence of the pathogen towards beans (Phaseolus vulgaris)

Material and methods. Dark Red Kidney beans were grown in sterilized soil in 20 cm pots, two plants were grown in each pot. When the second and third trifoliate leaves were expanding, the plants were sprayed by means of a hand atomizer with suspensions in sterile, distilled water of the isolates II and III, and with a water suspension of a virulent isolate of *Pseudomonas phaseolicola*. The suspensions were prepared from one-day-old cultures grown on TGA at 27°C. The concentration of the inocula was about 4×10^7 cells/ml. Control plants were sprayed with sterile distilled water. Twenty-four hours before and 24 hr after inoculation the plants were kept in a moist chamber (see 4.7), in which the temperature varied between 18° and 24°C. Subsequently the plants were returned to the glasshouse, where the temperature varied between 12° and 30°C. The final observations were made 24 days after inoculation. Reisolations were made according to standard methods. The leaves were disinfected for 1 min in 0.1 % mercuric chloride to which a spreader had been added.

Results. Numerous typical halo blight lesions developed on the plants that had been sprayed with the *Ps. phaseolicola* suspension. The first water-soaked spots

were observed within a week after inoculation. After 24 days they were up to 3 mm in diameter and were surrounded by large halos. Parts of some of the infected leaves had become necrotic. On most of the twelve plants that had been sprayed with isolate II no symptoms developed at all, but on two of them a few brown spots, about 1 mm in diameter and surrounded by a small halo, developed. Similar results were obtained with isolate III, but on three trifoliate leaves of one out of the twelve plants that had been sprayed with this isolate, several small dark-brown spots with a distinct yellow halo developed. The spots were observed on leaves that had been at a very early stage of development when the plants were sprayed with the bacterial suspension. Twenty-four days after inoculation the spots were not more than 1 mm and the halos about 2 to 3 mm in diameter. Reisolations from the spots on the leaves that had been sprayed with *Ps. phaseolicola* were positive, but from those leaves that had been sprayed with *Ps. pisi* the reisolations were negative. No symptoms developed on the control plants.

3.5. Discussion

The characteristics of isolates II and III are well in agreement with those described by Dowson (1957) for the genus *Pseudomonas*. This genus can be divided into four groups by means of a determinative scheme, which was worked out by Lelliott et al. (1966). According to the results of the LOPAT and subsidiary tests the pathogen should be placed into group Ia of the scheme; the representative species of this group is *Ps. syringae*. However, because the pathogen does not produce typical 'brown spot' symptoms on beans it is classified as *Ps. pisi* Sackett as distinct from *Ps. phaseolicola* (Burkholder) Dowson and *Ps. syringae* Van Hall (Guthrie et al., 1965; Hoitink et al., 1968). There is a good correspondence between the characteristics described by Sackett (1916) and those of isolates II and III, and also with those described by other authors (Table 3). The main points of difference between Sackett's findings and those of the author are the following. 1) The cells of Sackett's isolates were somewhat smaller. 2) Sackett found only a single polar flagellum, whereas isolate III had 1 to 5 polar flagella. The latter is in agreement with i.a. Stapp's (1937) description. Isolate II had probably lost its flagella because it had been cultured for a considerable time before the flagella staining was done. 3) Sackett found that milk was coagulated. This is in agreement with the results of most authors, but Ludwig's (1926) and Skoric's (1927) isolates, like the author's, did not coagulate milk. 4) Sackett found that the litmus in litmus milk was reduced. 5) He also found that the pathogen did not grow in Uschinsky's medium, but isolates II and III did and so did those of Stapp (1937). 6) Sackett found an optimum temperature of 27–28 °C, but the difference with the optimum temperature of isolates II and III is probably not significant, because in the present study very little difference in growth was found between 25° and 30 °C. 7) The thermal death point of Sackett's isolates

was between 49° and 50°. This is in agreement with that of isolate III, but not with that of isolate II (Table 3).

There is full agreement between the results of the authors mentioned in Table 3 with regard to gelatine liquefaction, but LELLIOTT et al. (1966) found no liquefaction in six out of seven cultures tested. The differences between the results of the nitrate reduction tests may be at least partially ascribed to the nitrate concentration in the test solution. Isolates II and III produced negative results when LELLIOTT et al.'s (1966) solution (0.1% KNO_3) was used, but weakly positive results in the test solution (1.0% KNO_3) recommended by STAPP (1958).

The identity of isolates II and III was confirmed at the Commonwealth Mycological Institute in 1964. Isolate Ia was kept too long under liquid paraffin and could therefore not be included in the above study of the different characteristics. Not long after it had been isolated, however, it was identified as *Ps. pisi* at the Plant Pathology Laboratory, Harpenden, England on the evidence available in 1955.

4. EFFECT OF TEMPERATURE, MOISTURE AND MECHANICAL INJURY

Since the first severe outbreak of bacterial blight in 1951 it has always been obvious that weather conditions, particularly frost, have a great influence on the disease. Investigations on the effects of temperature, moisture as well as mechanical injury are recorded in this chapter; the reports are preceded by a review of the relevant literature.

4.1. LITERATURE REVIEW

a) *Temperature.* In the first article on blight, published by SACKETT (1916), the effect of frost was already mentioned. SACKETT inoculated plants by scarifying the stems and spreading loopfuls of culture over the prepared spot. The inoculated area became watery and olive-green in colour within six days after the inoculation, but during the first fortnight the symptoms did not spread, except for a small watery zone beyond the scarified area. After a few days with hard frost however, the disease had spread to the stipules and some distance along the stem. After a few more heavy frosts SACKETT observed that 'all of the plants in which the disease had made progress succumbed to the frost and wilted while every check... stood up bright and fresh and showed no effects of cold whatever'. SACKETT concluded from these observations: 'One of the most interesting points brought out in the experiment was the apparent lowering of frost resistance' (of diseased plants).

In field experiments in the early summer of 1915 SACKETT observed that the disease was most active for a week in the middle of June, but did not make much progress in the week thereafter; it had disappeared by July. He did not correlate the sudden activity of the disease with frost, but mentioned that he observed frost injury in the middle of June.

WARK (1954) found that exposure of the plants to frost was followed by a rapid increase in the number of bacteria in the tissue and that this was later followed by the development of large amounts of obviously diseased tissue in the plant. Symptoms even developed on plants that had been kept isolated and had shown no visible symptoms before frosting.

Information on the effect of temperature on the incubation period is scarce. An indication that high temperatures favour the development of symptoms was found by KLEMENT and LEHOCZKY (1960), who observed that the symptoms appeared a day or two earlier on inoculated plants kept at 25°–30°C, than on those kept at 2–10°C.

b) *Moisture.* LUDWIG (1926) found that infection took place only occasionally if there were no wounds and that transferring the plants to moist chambers did not increase the likelihood of infection. RIKER (1929) on the other hand observed that plants kept in a moist chamber for a day prior to inoculation and then

replaced in the moist chamber developed many more lesions than did those that were placed in the moist chamber only after having been inoculated.

c) *Injury*. SACKETT's (1916) laboratory experiments and field observations indicated that *Ps. pisi* enters the plants through stomata or wounds. In a cultivar trial he observed that all the cultivars became infected in a section that had been harrowed, whereas several of the same cultivars in the unharrowed part showed marked resistance. SACKETT pointed to the danger of sandstorms that bruise the vines and literally inject the tissue with contaminated soil particles. Because of results obtained in glasshouse trials, LUDWIC (1926) and SKORIC (1927) are also of the opinion that wounds encourage infection.

4.2. EXPERIMENTAL TECHNIQUE

The glasshouse experiments described in this chapter were done with pea plants grown in sterilized soil in 20 cm pots; two or three plants were grown in each pot. In most cases the cultivars Greenfeast and Morse's Progress were used. Greenfeast is a cultivar which is later than Morse's Progress and consequently there was a difference in the stage of development; at the time when the plants were treated Greenfeast had not yet flowered whereas Morse's Progress was in flower or already bearing pods. Before planting, the seeds were disinfected for 2 min in 0.1 % mercuric chloride. The temperature and relative humidity in the glasshouse, which was shaded with Saran cloth (52 % shade), could not be kept constant. However, the temperature was always well above freezing point and though the rh also varied considerably, it never reached the saturation point; in fact the rh was usually low. The same also holds for the laboratory, where the plants sometimes were inoculated.

Most of the experiments were done with isolate III. Bacterial suspensions in sterile, distilled water, prepared from one-day-old cultures grown on TGA at 27°C, were used for inoculation. The concentration of the suspensions was about 4×10^7 cells/ml, but ten times this concentration was used to inoculate the stems in the experiments described under 4.5.2. Those described under 4.3, 4.4.1, 4.5.1 and 4.7.2 were done before the preparation of the suspensions had been standardized; in these cases the actual concentration is unknown, but probably higher than 4×10^7 cells/ml.

Reisolations were made according to standard methods. Pods and stems were disinfected for 2 or 3 min and leaves for 1½ min in 0.1 % mercuric chloride to which a spreader had been added.

4.3. OPTIMUM TEMPERATURE FOR THE DEVELOPMENT OF SYMPTOMS

Following the temperature studies mentioned in the previous chapter, a study was made of the effect of temperature on the expression of symptoms on infected pea pods.

Material and methods. Pods of the green pea cultivar Little Marvel were surface sterilized for 1 min in 0.1 % mercuric chloride and subsequently washed in running tap water. They were inoculated as described under 3.1. Petri dishes with inoculated pods were put in a series of incubators kept at different temperatures (see under Results). Three experiments were done with isolates I, II and III respectively. The control pods were treated with sterile water. In each experiment three or four dishes with inoculated pods as well as one dish with control pods were put in each incubator, except in the first experiment where no controls were included. The final records were taken after six days.

Results. The temperature range in the first experiment was 0°, 6°, 9°, 13°, 17°, 21°, 26°, 30° and 35°C. No symptoms developed at 0°C. At 6°C two of the 16 inoculations resulted in slightly sunken spots. From 9° to 35°C sunken lesions developed on all pods; in the range 17° to 30°C they were brown and/or water-soaked. At 35°C a number of the pods had rotted and the development of symptoms was therefore obscured.

The temperature range in the second and third experiment was 4°–5°C, 10°, 15°, 20°, 25°, 30° and 35°C. In Table 4 the diameters of the lesions which developed at different temperatures after inoculation with isolates II and III are presented.

The lesions on the pods in the range 10° to 35°C were water-soaked and sunken; those caused by isolate II were distinctly browner than those caused by isolate III. Slightly sunken lesions developed on a few pods at the lowest temperature. No symptoms at all developed on the control pods. Some of the pods kept at 25°C and most of those kept at higher temperatures turned yellowish.

The experiments show that the minimum temperature for the development of symptoms is about 5°C. The maximum temperature determined with isolates I and II is above 35°C, whereas with isolate III it is between 30° and 35°C. No distinct optimum temperature was found in the first experiment, the development of symptoms being equal in the range 21° to 30°C. In the second experiment the optimum determined with isolate II was about 30°C and that with isolate III in Experiment 3 between 25° and 30°C.

TABLE 4. Average diameters (in mm) of pod lesions that developed at different temperatures six days after inoculation with isolates II or III.

Isolate	Temperature in °C						
	4–5	10	15	20	25	30	35
II	0.4	2.4	2.4	4.1	3.8	6.0	4.5
III	0.0	2.6	3.6	3.8	5.3	5.2	0.0

4.4. Effect of frost on infection

The following four experiments were conducted to investigate the effect of frost on infection. Two were done with pods alone; in the other two whole plants were exposed to frost.

4.4.1. *Experiments with frosted pods*

Material and methods. Pea pods with their peduncles were hung on 250 ml erlemeyer flasks filled with water. Only the peduncles were actually in the water; the pods themselves hung on the outside. There were 96 pods, which were divided into four groups, A, B, C and D, of 24 pods each. They were treated as follows. A. The pods were sprayed with a water suspension of isolate II and placed for one night (4 p.m. to 7 a.m.) in a cold room. B. Ditto A, but the pods were kept overnight in a glasshouse. C and D kept as A and B respectively, but sprayed with sterile water. During the night the temperature in the cold room dropped below freezing point and a layer of ice formed on the water in the erlemeyer flasks. After the treatments the flasks with the pods were kept in the

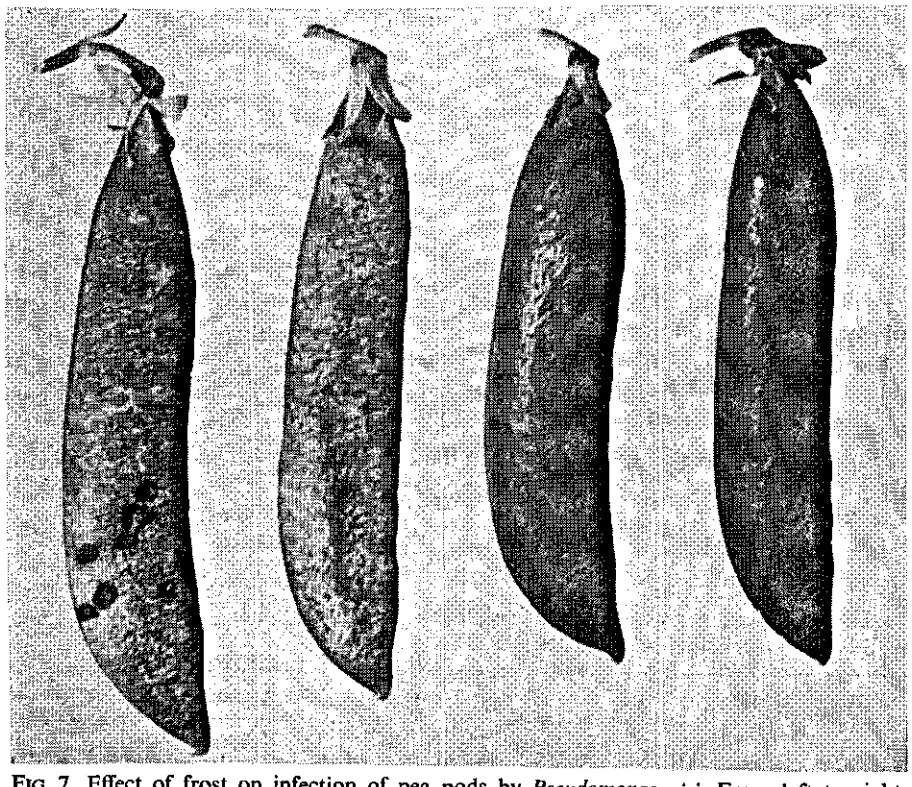

Fig. 7. Effect of frost on infection of pea pods by *Pseudomonas pisi*. From left to right: 1. frosted and inoculated, 2. frosted but not inoculated, 3. non-frosted, inoculated, 4. non-frosted, non-inoculated.

glasshouse. The final observations were made after five days. Two experiments were done.

Results. When the pods that had been exposed to frost were taken out of the cold room in the early morning they were water-soaked and dark-green, and many of them had small cracks in the epidermis. During the day most of them lost their water-soaked appearance and developed a white-green mottle, the same as that seen on pea pods in the fields after a frost. Fifteen of the 24 non-inoculated pods in the first experiment and eight out of 24 in the second showed signs of frost injury, but no blight symptoms were observed. On the pods that had been both exposed to frost and sprayed with a suspension of the pathogen sunken, water-soaked, dark-green to purplish lesions developed (Fig. 7). After five days they varied in size from a few millimeters in diameter to large coalesced infected areas. In the first experiment 16 out of 24 pods showed such symptoms. They were examined under the stereo-microscope for the presence of cracks, but no cracks were found on any of the lesions on seven of the pods. In the second experiment blight symptoms were found on 13 of the 24 pods in this group, some of the lesions showed cracks, others did not. Besides disease symptoms, signs of frost injury were also found on many of these pods, but eight pods in the first experiment and ten in the second showed neither signs of frost injury nor of disease symptoms.

No signs of disease or injury were found in Experiment 1 on pods that had been inoculated with *Ps. pisi*, but not frosted. In the second experiment, however, there was a small lesion about 1 mm in diameter on each of two of the 24 pods. None of the pods in group D, i.e. the control, showed signs of disease or frost injury. Reisolations from blight lesions were positive.

4.4.2. *Effect of the degree of frost and of the time of exposure*

Material and methods. Two experiments were conducted; the first with the cultivar Morse's Progress and the second with cv. Morse's Progress and cv. Greenfeast. The treatments were as follows.

A. The plants were kept for 6 hr in a dark, cold room, where the temperature was held at $-1\frac{1}{2}$°C (Exp. 1) or -2°C (Exp. 2) and the rh was about 80%.

B. The plants were kept for 3 hr in a dark room, in which the temperature was well above freezing point (18 ± 2°C in Exp. 1 and 14 ± 2°C in Exp. 2) and the rh varied between 50 and 85%. Subsequently they were transferred to the cold room and kept there for another 3 hr.

C. The plants were kept in the dark room for 6 hr.

D and E. As A and B respectively, but the plants were frosted in a dark, cold room where the temperature varied between $-2°$ and $-4°$ (average about -3°C) and the rh was about 70%.

After the 6 hr period, all the plants of treatments A–E were transferred to a laboratory, where they were sprayed with a water suspension of isolate III.

Treatments F–J were the same as A–E respectively, but the plants were sprayed with sterile, distilled water instead of a bacterial suspension. Treat-

ments D, E, I and J were not included in Experiment 2. After the treatments the plants were returned to the glasshouse and a fortnight later the final observations were made.

Results. Immediately after the plants had been taken out of the cold room, they were examined for water congestion. In Experiment 1 stems, leaves and pods of all the plants exposed to frost were to a certain extent water-soaked, but the leaves of the plants exposed to $-1\frac{1}{2}$°C much less so than those of the plants exposed to -3°C. Most of the stems of the plants in Experiment 2, that had been exposed to -2°C for 6 hr, were water-soaked and most of the Morse's Progress pods as well, but only a few stems and no pods were water-soaked after 3 hr exposure. In both experiments plants that had not been exposed to frost did not show signs of water congestion.

A fortnight later the plants were examined both for permanent frost injury and for blight symptoms. The observations on frost injury in Experiment 1 are summarized in Table 5.

The veins of the leaves of slightly injured plants were white and the pods showed a white-green mottle, but the plants did not show any other signs of frost injury. Apart from the above symptoms, the following signs of frost injury were observed on the moderately and severely injured plants. The leaflets and stipules were scorched, they became white to light-brown from the edges and were curled backwards. White spots appeared on the leaves. Some of the stems also turned white. The lower leaves were always the most severely injured. Some plants were so badly injured that only the tops were still green. Similar observations were made in Experiment 2, but the amount of injury was less than in the first experiment. The stipules and leaflets of a number of Greenfeast plants that had developed after exposure to frost, were malformed.

Table 5 shows that the degree of injury increased when the time of exposure was increased and the temperature lowered; the effect of the temperature was more marked than that of the time of exposure.

TABLE 5. Amount of frost injury on green pea plants, cv. Morse's Progress, after exposure to $-1\frac{1}{2}$°C and -3°C for 3 and 6 hr.

Treatment	Duration of frosting	Number of plants					
		Total	Not injured	Slightly injured	Moderately injured	Severely injured	Dead
A	6 hr at $-1\frac{1}{2}$°C	35	2	12	11	9	1
F	ditto	32	3	4	12	13	–
B	3 hr at $-1\frac{1}{2}$°C	35	7	19	6	3	–
G	ditto	33	6	24	3	–	–
C	0 hr	36	36	–	–	–	–
H	ditto	21	21	–	–	–	–
D	6 hr at -3°C	34	–	–	–	7	27
I	ditto	31	–	–	–	1	30
E	3 hr at -3°C	36	2	8	8	8	10
J	ditto	32	1	1	4	9	17

TABLE 6. Number of diseased internodes, leaflets and other organs of pea plants, cvs. Morse's Progress and Greenfeast, that had been exposed for 0, 3 or 6 hr to $-1\frac{1}{2}°$, $-2°$, or -3 °C before inoculation. The numbers are based on 30 living plants and given to the nearest whole number.

Experiment and Treatment	Cultivar	Duration of frosting	Internodes	Leaflets	Petioles	Stipules	Peduncles	Receptacles	Pods
Exp. 1 A	Morse's Progress	6 hr at $-1\frac{1}{2}$ °C	153	157	78	154	12	2	23
B		3 hr at $-1\frac{1}{2}$ °C	109	66	37	112	4	3	16
C		0 hr	1	7	0	6	0	4	3
E		3 hr at -3 °C	114	60	30	81	8	5	20
Exp. 2 A	Greenfeast	6 hr at -2 °C	97	71	14	74			
B		3 hr at -2 °C	25	63	17	47			
C		0 hr	0	30	2	23			
A	Morse's Progress	6 hr at -2 °C	51	47	8	58	9	8	17
B		3 hr at -2 °C	13	22	3	19	1	4	12
C		0 hr	1	8	0	5	0	14	1

In Table 6 the amount of blight in treatments A–C, as well as E in Experiment 1, is presented. The lesions that developed on the different organs were water-soaked, dark-green to brown-purplish. More or less round spots, up to 4 mm in diameter, developed on the pods. Some of the pods had water-soaked sutures. Spots on the leaves were also more or less round, but only 1–2 mm in diameter. In many cases the spots coalesced. The lesions on the internodes, petioles and peduncles varied from small streaks to lesions that covered a considerable part of the diseased organ. Reisolations were positive.

Table 6 shows that the plants became considerably more susceptible to blight as a result of exposure to frost. This applied to all the organs with the exception of the receptacles. Prolonging the duration of frosting from 3 to 6 hr caused a considerable increase in the amount of infection. The results of Experiment 1 show that lowering the temperature from $-1\frac{1}{2}°$ to -3°C (for 3 hr) had much less effect than prolonging the time of exposure from 3 to 6 hr (at $-1\frac{1}{2}$°C). It could be argued that the effect, which lowering the temperature might have had on the infection of most of the organs, was obscured by the damage caused by the severe frost, but it is improbable that this could have been the case with the internodes because these are more frost resistant. The results of treatment D are not included in Table 6 because most of the plants in this treatment were killed. The surviving plants were severely injured by frost and were heavily infected. No blight symptoms developed in treatments F–J.

4.5. Effect of frost on the spread of disease symptoms on the plant

In the Literature Review it is mentioned that Sackett (1916) observed that the symptoms spread to the stipules and along the stem after the plants had been exposed to frost. The following is a record of experiments conducted to study this phenomenon on plants naturally frosted in the field as well as on those artificially frosted in a cold room.

4.5.1. *Observations in field experiments*

Material and methods. During the winters of 1955, '56, '57 and '58 stems of pea plants which were sown at different times, were inoculated as described under 3.1. In the 1957 Experiment – the results are described in detail – two cultivars, Greenfeast and Morse's Progress, were used. The planting dates were 15/4 (Morse's Progress only), 27/4, 16/5 and 13/6/1957. Inoculations were made on 21/5, 10/6, 19/7 and 21/8/1957 with isolate Ia. This isolate was also used in 1955 and '56, but in 1958 the plants were inoculated with isolate II. Observations were made on the development and spread of the symptoms and the lengths of the diseased stem portions were regularly measured. These observations were correlated with temperature records taken by means of a thermograph placed within the trial plots in a Stevenson screen about 30 cm above the soil. In 1958 reisolations were made from the highest internode of the visibly

infected stem portion and the second symptomless internode above it. The experiment was done with the cultivars Morse's Progress and Greenfeast, planted on 18 April and inoculated on 17 June. Some Morse's Progress plants, planted on 23 April and inoculated on 10 July were also included. The reisolations from the plants inoculated in June and July were made in the beginning of July and August respectively.

Results. As a result of the inoculations done in May 1957 of the first Morse's Progress planting, typical lesions developed on the stems. The lesions were measured frequently; the average lengths of ten lesions measured at different times are presented in Table 7.

TABLE 7. Spread of stem lesions on Morse's Progress plants inoculated on 21 May 1957.

	Dates of observations									
	27/5	29/5	1/6	3/6	5/6	10/6	13/6	17/6	20/6	4/7
Average length of lesions (10 plants) in mm	24	24	24	24	24	92	101	103	106	226

There were two cold spells between the time of inoculation and the date on which the last measurements were made. The first was from 3 to 6 June and the second from 20 to 24 June. The minimum temperature during the first period was −4°C and during the second −5°C. If the incubation time is taken into account, it is obvious that there is a relationship between the spread of the disease along the stem and the occurrence of frost. When observed on 6 June portions of the stems on both sides of the original stem lesions had become olive-green and on 7 June they were purplish-brown. On some plants it was observed that while the water-soaked appearance of non-inoculated stems returned to normal, the inoculated stems remained water-soaked and later became olive-green to brown. Fan-like symptoms described under 2.1.1 developed on the stipules attached to the diseased stem portions. After the second cold spell the disease spread to the tops of the stems, eventually killing them.

The spread of blight lesions along the stems of the plants inoculated in June is shown in Table 8.

TABLE 8. Spread of blight lesions on the stems of pea plants of two plantings of Morse's Progress and Greenfeast, inoculated on 10 June 1957.

Cultivar	Time of planting	Average lengths of stem lesions in mm			
		13/6	17/6	20/6	4/7
Morse's Progress	15 April (10 plants)	21	22	22	181
	27 April (10 plants)	18	20	20	124
Greenfeast	27 April (10 plants)	17	17	17	79

During the cold spell from 20 to 24 June the temperature dropped to −5 °C. The effect on the spread of the disease can be seen in Table 8. On 4 July the infections had spread to the tops of the stems both in the Morse's Progress plants of the 15 April planting and in those of the 27 April planting; thus in this respect there was no difference between the plantings. In Greenfeast stems the disease did not spread as far as in those of Morse's Progress.

In Table 9 the spread of the blight lesions along the stems of the plants inoculated in July is shown.

During the period of observation the temperature dropped below zero on 5 August (−2 °C) and 13 August (−1 °C). Again the effect of frost is reflected in the figures of Table 9. The stem lesions on Greenfeast spread somewhat during the period between 29 July and 2 August, i.e. before the temperature dropped below freezing point. This may be explained by the low minimum temperature (0.5 °C) on July 30. It is possible that the actual temperature of the Greenfeast stems dropped below zero. In the previous period of observation (Table 8), the stem lesions on Greenfeast did not spread as far as did those on Morse's Progress. No such difference is seen in the figures of Table 9.

Though as a result of the inoculations made on 21 August in the 16/5 and 13/6 plantings typical lesions developed on the stems, they almost did not spread during the whole period of observation, i.e. from 27 August to 16 September. Individual lesions spread along a distance of up to 7 mm. The lowest temperature during the period from 21 August to 16 September was 5 °C.

Similar results as in 1957 were obtained in 1955. Fig. 8 illustrates the spread of the disease on a plant inoculated on 23 May. The stem lesion that developed after inoculation was not more than one internode long; only one stipule showed a fan-like lesion. The stem lesion did not spread beyond the inoculated internode for more than two weeks. On 9 June a minimum temperature of −3 °C was recorded; this was the first time since inoculation that the temperature dropped below freezing point. A few days later the disease was seen to have spread along more than five internodes and several of the stipules attached to the diseased stems showed typical fan-shaped symptoms. The 1956 Experiment was conducted with the cultivars Greenfeast and Little Marvel. The months of June and July were practically free from frost and for more than five weeks the blight lesions did not spread along the stem. During a few nights the temperature dropped to −1 °C, but this had little effect as only a few of the lesions increased at all in length.

TABLE 9. Spread of blight lesions on the stems of pea plants of two plantings of Morse's Progress and Greenfeast, inoculated on 19 July 1957.

Cultivar	Time of planting	Average lengths of stem lesions in mm						
		26/7	29/7	2/8	8/8	13/8	19/8	22/8
Morse's Progress	27/4 (4 plants)	20	20	20	44	53	83	83
	16/5 (6 plants)	17	17	17	19	25	44	44
Greenfeast	27/4 (7 plants)	21	21	37	43	55	76	87
	16/5 (9 plants)	20	20	31	40	49	58	61

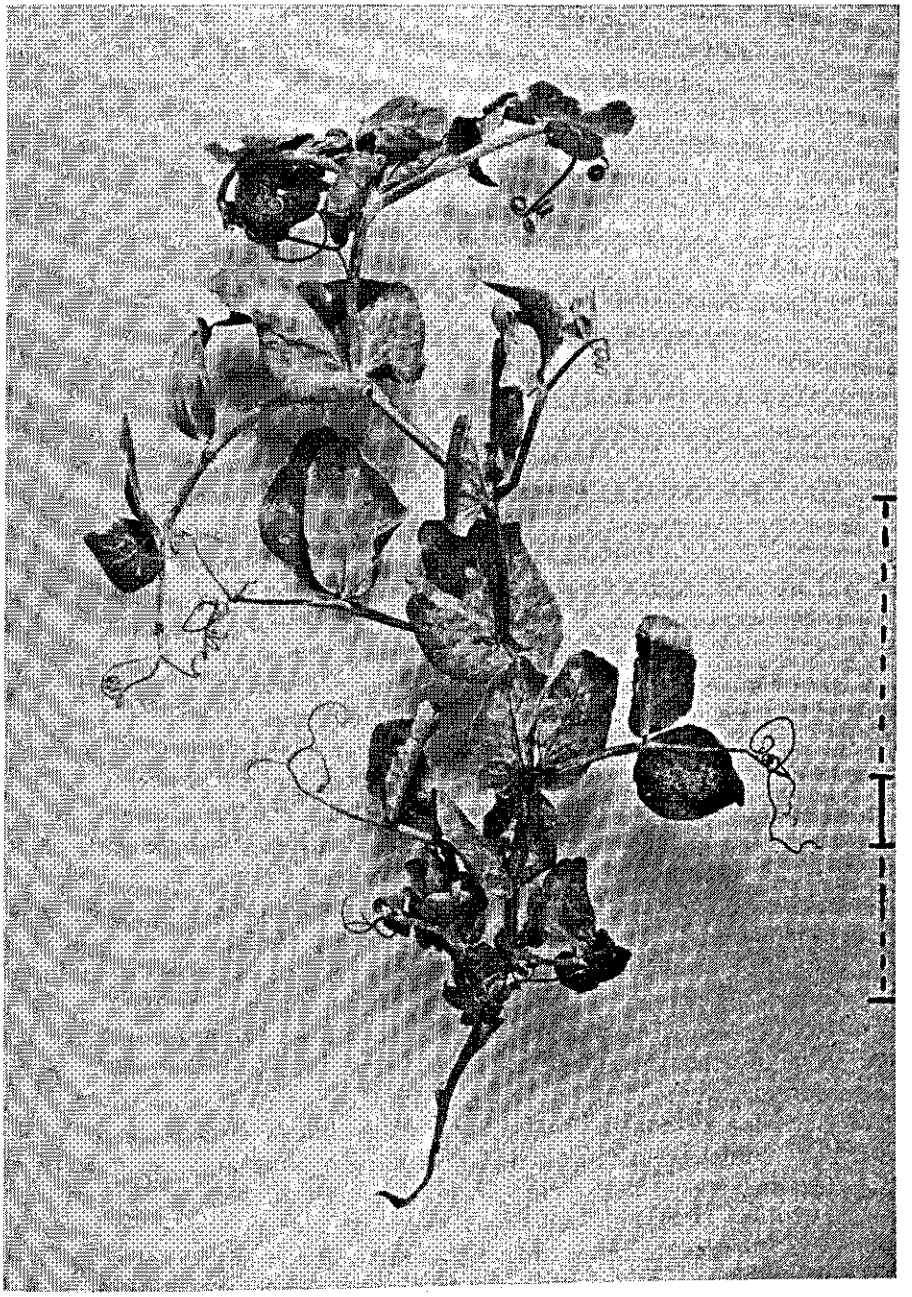

FIG. 8. Spread of bacterial blight symptoms on a pea stem.
⊢⊣ Indicates the length of the diseased stem portion before exposure to frost.

⊢----⊢⊣---------⊣ Ditto some days after exposure to frost. No signs of frost injury.

In the experiments described above the inoculated plants were never found to be more susceptible to frost than were the control plants in the same row. Though the disease spread along the inoculated stems, the tops were not more severely injured by frost than were those of the control plants. In many instances no permanent frost injury was visible. After repeated exposures to frost, it was often only the inoculated vine of a pea plant that was killed; the others remained healthy, often without signs of frost injury. It then looked as if the inoculated vine was more susceptible to frost, but in fact it had been killed by the pathogen.

The plants in the 1958 Experiment were inoculated according to the same method as in the previous years, but in June and July the temperatures often dropped below freezing point and the stem lesions that developed were several internodes long. The pathogen was easily reisolated from the visibly infected internodes of the Morse's Progress plants, but not from the internodes without symptoms. The results with Greenfeast plants were the same, but the reisolation from one out of eight internodes without symptoms was positive.

4.5.2. *Spread of blight lesions on artificially frosted plants*

Material and methods. Two experiments were done, the first with Greenfeast plants and the second with Morse's Progress. The treatments were as follows.

A. A water suspension of isolate III was injected under the epidermis of one internode of each plant. Eight days later the inoculated plants were put into a cold room and kept there for 5 hr at $-1\frac{1}{2}$°C (Experiment 1) or for 6 hr at -2°C (Experiment 2). The rh in the room was about 85%.

B. Ditto, but the plants were not exposed to frost.

C and D. As A and B respectively, but the plants were injected with sterile water instead of a bacterial suspension. Immediately after the plants of treatments A and C had been taken out of the cold room and returned to the glasshouse the stems were examined for water congestion. In Experiment 1 the pots were classified as follows: (a) no, (b) slight and (c) distinct water congestion of the plants. It was impossible to examine each individual plant within a short time, because of the luxurious growth of Greenfeast. About a fortnight after the plants of treatments A and C had been exposed to frost the final observations were made.

Reisolations were made from the second or third internode above the inoculated internode of nine frosted plants and of the same number of unfrosted plants.

Results. On the internodes injected with the bacterial suspension watersoaked brown to purplish lesions developed. Their lengths were measured immediately before the plants of treatments A and C were exposed to frost and again a fortnight later. In Experiment 1 the length of the lesions initially varied from 15 to 56 mm. On 21 out of 27 plants that had been inoculated but not exposed to frost the lesions did not spread. On six of them they did but not

more than 6 mm.

After the frosted, inoculated plants had been taken out of the cold room most of the stems were water-soaked to a greater or lesser extent. On 17 out of 50 plants the lesions did not spread more than 5 mm along the stem during the period of observation. Fifteen of these plants had not shown signs of water congestion after exposure to frost and two were from pots that had been marked 'slight water congestion'. On 24 of the remainder of the plants the lesions spread considerably along the stem and reached a length of up to 205 mm. The average lengths of these lesions just before frosting and a fortnight later were 25 and 107 mm respectively. The stems of most of these plants had been water-soaked after frosting. With the exception of one pot with two plants, all the pots had been marked 'distinct' or 'slight water congestion'. Many of the plants showed signs of permanent frost injury within a few days after frosting. There was no distinct correlation between the severity of frost injury and the distance that the lesions spread on the stem. For example in Experiment 2 – the results were very similar to those of Experiment 1 – the stem of a plant that had been water-soaked after freezing was not permanently injured, but the length of the lesion increased from 18 to 130 mm. On the stipules of this plant typical fan-shaped symptoms developed. Both in Experiment 1 and 2 more of the inoculated stems died after frosting than of the stems injected with water. In Experiment 1 the numbers were 9 out of 50 and 0 out of 50 respectively and in the second experiment 17 out of 41 and 2 out of 39. The control plants (26 in Experiment 1 and 19 in Experiment 2) that had not been exposed to frost neither showed signs of frost injury nor of blight.

The second or third internode above the inoculated internode on the frosted plants from which the reisolations were made showed blight symptoms and the reisolations were all positive in Experiment 2; one was negative in Experiment 1. Reisolations from these internodes on unfrosted plants, which did not show symptoms, were negative, except for one symptomless internode in Experiment 2 from which the pathogen could be isolated.

4.6. Effect of relatively high and low growth temperatures on the susceptibility of pea plants

In chapter 4 field experiments are described, which show that peas planted in April are more susceptible to blight than those planted later. One of the differences between early and late planted peas is that when young, those planted early grow at higher temperatures. Experiments to study the effect of growth temperatures on the susceptibility of pea plants are described below.

Material and methods. Two experiments were done, both with the cultivars Greenfeast and Morse's Progress. The following treatments were given.

A. The plants were grown in a glasshouse under 52% shade at temperatures that varied from 15° to 31 °C, while the rh varied between 25 and 75%. After

the plants had been inoculated in the laboratory they were returned to the glasshouse. The inoculation was done as follows. Sterile, distilled water was injected under the epidermis of a stem internode by means of a hypodermic syringe. Immediately thereafter the prick wound was sealed with sterile petroleum jelly and the internode sprayed with a water suspension of isolate III, to which a spreader had been added (see chapter 8). One internode was treated per plant.

B. The plants were grown outdoors under Saran cloth (52% shade). In Experiment 1 the temperatures varied between −2° and 27°C, but it seldom exceeded 21°C and the rh varied from 16 to 96%. In Experiment 2 the maximum and minimum temperatures outdoors varied between 27° and 2°C and the rh between 16 and 98%. The plants were inoculated in the laboratory, as described under A, and subsequently placed in the glasshouse.

C and D. Treated as A and B respectively, but the internodes were sprayed with sterile, distilled water to which a spreader (Triton B1956, 1 drop in 90 ml water) had been added.

The final observations were made a fortnight after inoculation. Not only were the internodes examined for disease symptoms, but the stomata frequency on the treated internodes of the control plants was also determined. For this purpose the household adhesive 'Samsonite' was used. The adhesive was applied thinly on the surface of the internode. After a few minutes when the adhesive had become hard it was taken off with a pair of tweezers and the number of stomata per square millimeter was determined by means of a haemocytometer. The stomata frequency was determined on 25 plants of each cultivar (12 to 20 plants in Experiment 2) grown in the glasshouse and on the same number grown outside.

Results. The plants grown in the glasshouse developed more rapidly than those grown outside. At the time of inoculation the Morse's Progress plants grown in the glasshouse were bearing pods in both experiments, whereas those grown outside had not yet started to flower (Experiment 1) or were in full flower, but were not yet bearing pods (Experiment 2). The Greenfeast plants were treated before they had started to flower. In Experiment 1 the plants grown in the glasshouse were up to 58 cm at the time of inoculation and those grown outdoors were 30 cm tall. In Experiment 2 little difference in height was found between Greenfeast plants grown in the glasshouse and those grown outside.

Water-soaked, dark-green to brown or purplish lesions developed on a number of internodes that had been sprayed with the bacterial suspension. The first symptoms were seen within a week after inoculation. The results after a fortnight are presented in Table 10. The stomata frequencies are also included in this table.

The internodes of the plants grown in the glasshouse had more stomata per square millimeter than those of the plants grown outdoors. This was positively correlated with the percentage of infection as is shown in Figure 9. Reisolations were positive.

TABLE 10. Percentages diseased internodes on Greenfeast and Morse's Progress plants, grown in a glasshouse or outside and sprayed with sterile water (controls) or with a suspension of *Ps. pisi*, as well as the stomata frequencies on the control internodes.

Cultivar	Experiment and Treatment	Glasshouse			Outside		
		diseased internodes number	%	stomata frequency	diseased internodes number	%	stomata frequency
Greenfeast	1, control	0/22	0	53.2 ± 1.1	0/25	0	31.0 ± 1.4
	1, *Ps. pisi*	10/16	63		6/20	30	
Morse's Progress	1, control	0/15	0	62.2 ± 1.9	0/19	0	29.3 ± 1.1
	1, *Ps. pisi*	5/16	31		3/19	16	
Greenfeast	2, control	0/20	0	43.1 ± 2.3	0/17	0	38.1 ± 2.5
	2, *Ps. pisi*	8/22	36		9/24	38	
Morse's Progress	2, control	0/12	0	62.6 ± 6.4	0/19	0	38.2 ± 1.2
	2, *Ps. pisi*	9/19	47		5/23	22	

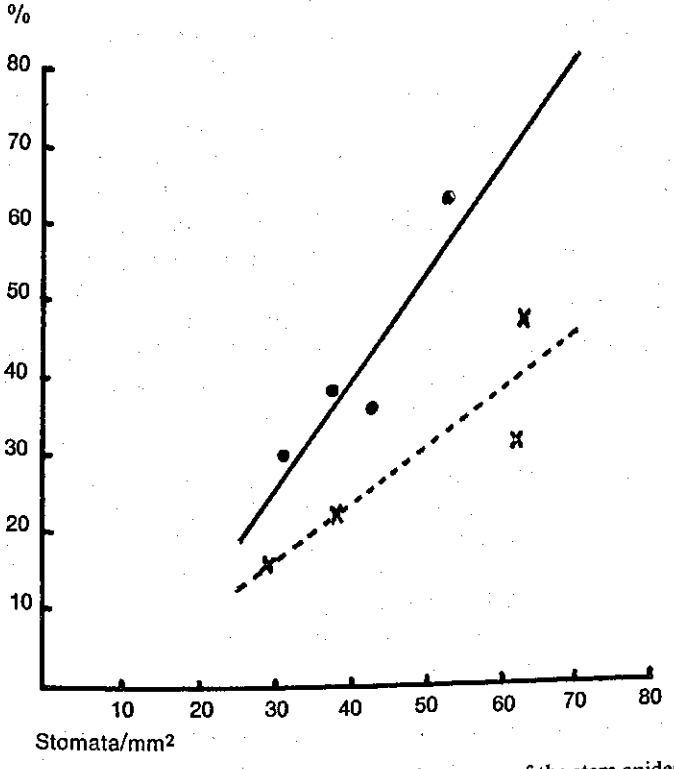

FIG. 9. Relationship between the stomata frequency of the stem epidermis and the percentage infected internodes of pea plants, cvs. Greenfeast (●——●) and Morse's Progress (×- - -×), sprayed with a suspension of *Pseudomonas pisi*.

4.7. Effect of moisture on infection

Frost in the early winter mornings in the Transvaal is often accompanied by rime and dew. The effect of artificial 'dew', in fact small droplets on the leaves, was studied in a series of experiments. Rain, particularly when driven by strong winds, may have an entirely different effect, because in this case the water may be forced into the leaves. Therefore, the effect of artificial rain was studied as well.

4.7.1. *Experiments with artificial 'dew'*

Material and methods. Three experiments were conducted, two with the cultivar Greenfeast, the third with Morse's Progress. The following treatments were given.

A. The plants were kept for 2 × 24 hr in a moist chamber.

B. The plants were kept for 24 hr in a moist chamber and subsequently for 24 hr in a dry chamber.

C. The plants were kept for 24 hr in a dry chamber and subsequently for 24 hr in a moist chamber.

D. The plants were kept for 2 × 24 hr in a dry chamber.

At the end of the first 24 hr all the plants of treatments A to D were sprayed with a water suspension of isolate III. Treatments E to H were the same as A to D respectively, except that they were sprayed with sterile, distilled water. At the end of the 48 hr all the plants were returned to the glasshouse. The dry chamber was made of a steel frame covered with cheese-cloth. The moist chamber was built in the same way but in this chamber a humidifier was placed, which was controlled by an electronic regulator similar to that described by Bohnen and Humm (1961). It was set in such a way that the plants inside the chamber were continuously covered with small water droplets. The temperature in the moist chamber varied from 16° to 23°C and in the dry chamber from 18° to 26°C. The rh in the dry chamber varied from 60 to 90%. The number of plants per treatment was between 9 and 12 in Experiment 1 and between 4 and 9 in Experiments 2 and 3.

Results. Within a week after inoculation the first symptoms were observed. In Table 11 the amount of blight in treatments A to D a fortnight after inoculation is presented. On leaflets and stipules more or less round, water-soaked green to brown spots developed, about 1–2 mm in diameter, some of them with a lighter centre and/or a yellow halo. Water-soaked green to purplish-brown lesions developed on some of the petioles and stem internodes. Lesions on the Morse's Progress pods were sunken, water-soaked and purplish. Reisolations were positive. No symptoms developed in treatments E to H.

In each of the four treatments only a few pods, internodes and petioles showed symptoms. Many more leaflets and stipules were infected and their numbers are therefore more suitable to evaluate the differences between the treatments. Leaflets and stipules of plants kept for 48 hr in a moist chamber were

TABLE 11. Number* of diseased internodes, leaflets, etc. that developed on pea plants, cvs. Greenfeast and Morse's Progress, after 0, 24 and 48 hr in a moist chamber.

Moist chamber		Internodes			Leaflets			Petioles			Stipules			Pods
Before inoculation	After inoculation	Exp. 1	2	3	Exp. 1	2	3	Exp. 1	2	3	Exp. 1	2	3	Exp. 3
24 hr	24 hr	0	1	0	113	138	25	7	4	0	69	51	28	4
24 hr	0 hr	1	0	0	82	138	45	6	1	2	57	49	12	3
0 hr	24 hr	1	0	0	53	54	35	3	0	5	24	20	38	3
0 hr	0 hr	0	0	0	63	42	7	3	0	0	28	22	0	2

* The numbers are based on 10 plants, rounded off to whole figures.

in all three experiments more infected than those of the plants that had not been in the moist chamber at all. Generally speaking more leaflets and stipules were infected if the plants were kept in the moist chamber before they were inoculated than thereafter, but the results of treatment D show that keeping the plants in a moist chamber was not essential for infection.

4.7.2. *Effect of artificial rain*

Material and methods. The experiment was done with 12 two-months old plants, cv. Perfection, which were divided into four groups and on two successive days were treated as follows.

A. Plants were kept for 3 min under a shower with the object of imitating rain and subsequently sprayed with a water suspension of isolate II.

B. Plants were sprayed with a water suspension of isolate II.

C. As A, but the plants were not inoculated.

D. Control, no treatment.

Results. Yellow-brown to rust-brown angular spots with dark-brown edges and mostly 1–3 mm in diameter, developed on 35 leaflets and 37 stipules of the plants that had been inoculated after having been put under the shower. On some of these stipules large irregular infected areas up to 7 × 30 mm were observed. Only one stipule of the inoculated plants, that had not been put under the shower, became infected, the spots being ½ to 1 mm in diameter. No symptoms developed on the plants in treatments C and D, but two stipules and two leaflets in treatment C were slightly damaged by the water.

4.8. THE EFFECT OF MECHANICAL INJURY ON INFECTION

The effect of wounds was studied in two glasshouse experiments; observations were also made in a field experiment.

4.8.1. *Glasshouse experiments on the effect of mechanical injury on infection*

Material and methods. Two experiments were conducted, both of them

TABLE 12. Number of internodes that showed symptoms out of a total number of treated internodes of Greenfeast and Morse's Progress plants; the treatments consisted of scarifying the internodes and painting them with a bacterial suspension or sterile water.

Treatment	Greenfeast				Morse's Progress			
	Exp. 1		Exp. 2		Exp. 1		Exp. 2	
	number	%	number	%	number	%	number	%
Ps. pisi suspension	0/8	0	0/9	0	0/6	0	0/12	0
Ditto + wounding	8/8	100	10/10	100	5/9	56	8/8	100
Sterile water	0/12	0	0/11	0	0/9	0	0/8	0
Ditto + wounding	0/9	0	0/8	0	0/4	0	0/5	0

with Greenfeast and Morse's Progress plants. The following treatments were given.

A. One internode of each plant was slightly scarified on one side and the wound was painted with a water suspension of isolate III.

B. The internode was painted with the bacterial suspension but not scarified.

C and D. As A and B respectively, but the internodes were painted with sterile, distilled water.

A fortnight after the inoculations the final observations were made.

Results. Water-soaked, purplish brown lesions developed on the inoculated wounds. Most of those on the Greenfeast plants were about the same size as the wounds, but on Morse's Progress plants the lesions were smaller. At the time when the final observations were made the wounds that had not been inoculated had completely healed. Reisolations were positive. The numbers of diseased internodes in the different treatments are presented in Table 12.

4.8.2. *Field observations*

The field observations were made in a trial which had been laid out to study what effect topping of pea plants had on yield and quality of dry seed. When the numbers of diseased plants were counted in this trial, a distinction was made between the infection of the topped stems and the laterals which had developed after topping. The experiment was planted on 16 May 1955. The individual plots in this field trial consisted of 4 rows of 6 m long. The plots were not sprayed with a suspension of *Ps. pisi*, so the infection was natural.

No blight was found in the control plots. A total of four diseased plants were found in the four replicates, where the plants had been topped. On three of these plants the disease started on the topped vines and on one it started on an untopped vine.

4.9. Discussion

The results of the experiments described under 4.4 show that pea plants that have been exposed to frost are very susceptible to *Ps. pisi*, while unfrosted plants are only slightly susceptible. Similar results were obtained in experiments with pods alone. In an experiment in which the degree of frost and the duration of exposure were varied, prolonging the exposure from 3 to 6 hr (at $-1\frac{1}{2}$°C) rendered the plants much more susceptible to blight, than did lowering the temperature from $-1\frac{1}{2}$° to -3°C (for 3 hr). No correlation was found between susceptibility and the amount of frost injury. The amount of frost injury increased much more by lowering the temperature than by increasing the time of exposure. Also, in the experiments with pods alone, severe frost injury was not a prerequisite for infection; many blight lesions developed in places where no frost cracks could be found on the pods.

The effect of frost is apparently twofold: first, as stated above, plants that have been exposed to frost are more susceptible to infection, and second, the bacteria spread more rapidly in tissue that has been frozen. In experimental fields of peas it was observed that stem lesions did not spread far along the stem as long as the temperature remained above freezing point. After a frost, however, the disease spread for a considerable distance along the stems and into the stipules, on which the typical fan-like symptoms developed. These results were confirmed in experiments with artificially frosted plants. The explanation of this phenomenon is probably that a network of temporarily enlarged intercellular spaces and ruptures, filled with water and other cell components, forms in the stems and leaves (Levitt, 1941). In this network the bacteria can easily move. A similar phenomenon was observed on tobacco leaves infected with *Pseudomonas tabaci*. In this case the water-soaking was not caused by frost, but by heavy rains. Under dry conditions the pathogen only caused small spots, but after heavy rains the bacteria spread and multiplied so rapidly in the water-filled intercellular spaces, that large parts of the leaves became necrotic (Clayton, 1936).

The observations on the spread of the disease after frost were made on peas planted at different times. Different planting times and cultivars were included in the experiments because these factors had a great effect on the occurrence of the disease in the field experiments described in chapter 5. In 1957 the disease spread over a somewhat longer distance on the stems of earlier planted peas, but in 1955 no such difference was observed. The differences in spread of the disease on the stems of Morse's Progress and Greenfeast plants were not consistent either.

The observations on the effect of frost on the spread of the disease are in agreement with those of Sackett (1916), who concluded that diseased plants were more sensitive to frost. This was also found in the experiments with artificially frosted plants described under 4.5.2, but never in field experiments. What did happen in the field was that after repeated exposure to frost the

disease spread over the whole length of the infected stems, which were eventually killed.

It appears that the pathogen does not easily travel in the vascular system otherwise it would have been detected more often in the internodes above the visibly infected portion of the stem. Bacterial blight, as it occurs in South Africa, is apparently not a vascular or systemic disease.

The experiments to determine the optimum temperature for the development of symptoms on the pods were performed in incubators. A disadvantage of this relatively simple apparatus was that the pods, removed from the plants and kept in the dark, eventually became yellowish at the higher temperatures. This shortcoming may at least partly explain the differences in results obtained in the three experiments described under 4.3. Nevertheless the conclusion that the optimum temperature for the development of symptoms is about 25°–30°C seems justified. If the effect of frost and the relatively high temperature, necessary for the rapid development of symptoms, are taken into account it becomes obvious why the climate in those areas of South Africa, where outbreaks of blight may be severe, is so conducive to the disease. Here low night temperatures alternate in winter with high day temperatures. For example, one day in July the air temperature, registered at about 30 cm above the ground in a Stevenson screen in an experimental field at the Horticultural Research Institute, rose from −3°C to 20°C; the rise from −3°C to 10°C took place in one hour. Thus frost renders the plants susceptible to infection and also promotes the spread of the disease in the plant, whereas relatively high temperatures during the day favour the growth of the pathogen.

The temperature also has another effect on the plants. Those grown at relatively high temperatures (see 4.6) are more susceptible to blight than those grown at lower temperatures. The difference in susceptibility could be correlated with the stomata frequency (Fig. 9).

In the Transvaal frost in the early winter mornings is often associated with rime and dew. Artificial 'dew', i.e. small droplets on the leaves, encourages infection but not to a great extent. It appears that artificial 'dew' for 24 hr before inoculation is more favourable for infection than artificial 'dew' after inoculation. This is in agreement with the findings of RIKER (1929). In most countries, where the disease has been observed, it occurs mainly during rainy weather. This was also the case at the Horticultural Research Institute in the winter of 1957 with its exceptionally high rainfall. Rain not only promotes the spread of the disease in the field, but it also increases the likelihood of infection taking place as was shown in the experiment with artificial rain; it appeared that heavy rain had a greater effect on infection than dew.

Ps. pisi is not a typical wound parasite. SACKETT (1916), SKORIC (1927), STAPP (1937) and others found that wounds are not a prerequisite for successful inoculations. Neither can the importance of frost be attributed to the fact that it causes wounds, because infection can also take place in frosted tissue in which no wounds occur. Wounds do, however, increase the likelihood of infection, as was shown in glasshouse trials. SACKETT's (1916) experiments indicate that

mechanical injury encourages the disease in the field, but the results of the experiment described under 4.8.2 do not support this finding, at least not under the conditions under which this experiment was conducted. No indication was found that the rifts in the epidermis and cortex, which arise as a result of frost or vigorous growth (see 2.3) are points of infection in the field.

Finally, the question arises which of the three factors, frost, rain and dew or mechanical injury is the most important in South Africa. As outbreaks of blight are severe only where and when severe frost occurs, it appears that frost is the most important predisposing factor. Mechanical injury appears to be of minor importance. The effect of the rain in the winter of 1957 has already been mentioned. It must, however, be taken into account that the disease was severe only in a field trial, which had been sprayed with a suspension of *Ps. pisi* shortly before a heavy shower. In the other pea trials the disease symptoms (Fig. 5) were found only sporadically.

5. CULTIVAR, PLANTING TIME AND IRRIGATION TRIALS

Differences in varietal susceptibility of peas to *Ps. pisi* have been reported elsewhere in the literature. Differences in resistance between peas planted at different times have been found as well. In this chapter field trials are described which were done to study these differences under local conditions. In chapter 4 the effect of frost as a predisposing factor to blight was discussed. Because there is often a relationship between the moisture content of the soil and the amount of frost damage, the effect of soil moisture on frost injury and blight was also investigated.

The following information on cultivars, time of planting and effect of soil moisture is known from the literature.

5.1. Literature review

(a) *Cultivars.* In Colorado Sackett (1916) found that in field trials there were differences in resistance between cultivars of field peas. In laboratory experiments he found varietal differences as well: the green pea cultivar Wellington for instance showed remarkable resistance even after exposure to frost. Differences in resistance between cultivars were also reported by Jennison (1921) in Montana and by Ludwig (1926) in South Carolina, but these authors did not mention the names of the cultivars. Brown and Evans (1932) in Arizona maintained that Extra Early and Lightning Excelsior are more resistant than Telephone; they also mentioned differences in resistance between cultivars of forage peas. Harter et al. (1945) stated that they do not expect bacterial blight to disappear before resistant cultivars have been developed. This implies that no satisfactory resistant cultivars were known in the U.S.A. in 1945. Neither were any available in Canada (Machacek and Brown, 1948). Stapp (1937) found differences in resistance between German cultivars, Heines Folgererbse and Heines Viktoriaerbse being more resistant than others.

In Australia Greenfeast was first described as a less susceptible cultivar (Anonymous, 1948), but later Wark (1950), having tested more than 300 cultivars, reported that not one showed any appreciable resistance at all. In tests carried out in New Zealand a number of cultivars showed some resistance e.g. Greencrop, Merit Wisconsin, Marathon, Giant Stride, Delwiche Commando, Perfection, English Wonder and Duplex; among the susceptible were Onward, Greenfeast, Little Marvel and Kelvedon Wonder (Brien et al., 1955). Results of trials published in 1949, however, showed that the least susceptible cultivar, Greencrop, was 53 % infected (Anonymous, 1949).

(b) *Times of planting.* In Tasmania bacterial blight was observed only in early planted peas. Wade (1951) therefore recommended planting late in spring. Similar advice was given by Sackett (1916) in Colorado, but this author does

not state his reasons. CROSBY and CHUPP (1934) recommended that peas be planted early in spring in the state of New York, because conditions became more favourable to the disease later in the season.

(c) *Soil moisture content.* WARK (1954) planted contaminated pea seed in soil kept at different levels of soil moisture content, namely at 40, 60 and 80% of the water holding capacity, and found that the disease was more prevalent at the higher levels, symptoms developing only after exposure to high atmospheric humidity. He assumed that the humidity in the intercellular spaces was higher in peas grown in soil at the higher moisture levels and that this might have encouraged the multiplication of the bacteria.

5.2. CULTIVAR TRIALS

Two field experiments were done, in which varietal differences only were studied. One was done in 1957 under rainy conditions, the second in 1958 in a normal, dry winter with occasional frost.

Material and methods.

Cultivars: Black-eyed Susan, English Wonder, Greenfeast, Merit, Onward, Perfection and Little Marvel (in 1958 only).

Dates of planting: 18 April 1957 and 18 April 1958.

Size of plots: 6.6 × 3 m.

Spacing: 60 × 5 cm.

Irrigation: In furrows when necessary.

Fertilization: Superphosphate, 500 kg/ha.

Design: Randomized block trial with 4 replicates.

Inoculations: The experimental fields were sprayed respectively with a suspension of isolate Ia on 20 May, 1957 and twice with isolate II in July 1958.

TABLE 13. Average percentages and numbers of diseased plants per plot in the cultivar trials 1957 and 1958.

Cultivar	Diseased plants		
	1957		1958
	percentage	transformed	number
Black-eyed Susan	27.9	31.6	32.3
English Wonder	10.0	18.2	97.0
Greenfeast	19.5	25.9	238.3
Merit	40.7	39.6	172.0
Onward	14.2	22.0	152.0
Perfection	53.5	47.0	148.0
Little Marvel			242.0
S.S.D. (P = 0.05)		6.6	66.6
C.V. %		14.2	29.0

Results. During the period 20–25 May 1957, i.e. immediately after the inoculation on 20 May, a total of 10 mm of rain fell. Under these conditions rust-brown spots (Fig. 5) developed within a week on the leaves. The average percentages of diseased plants per plot, as observed on each of the cultivars on 1 June, are presented in Table 13. In 1958 symptoms as described under 2.1.1 were observed. The diseased plants were counted on 28 August and the average numbers per plot are presented for each cultivar in Table 13. After a cold spell in June 1958 it was observed that Little Marvel was much more susceptible to frost injury than any of the other cultivars.

Table 13 shows that English Wonder, Onward and Greenfeast were the most resistant cultivars in 1957 and English Wonder and Black-eyed Susan in 1958.

5.3. Cultivar/planting time trials

Trials with different cultivars, which were planted at different times, were done in the winters of 1953, '54 and '55. In these winters bacterial blight was common and it was not necessary to spray the trials with a suspension of the pathogen.

Material and methods.

Cultivars: Two or more of the following cultivars were included (see Tables 14, 15 and 17): Greenfeast, Morse's Progress, Kelvedon Wonder and Little Marvel.

Times of plantings: Plantings were made from March to July (see Tables 14, 15 and 17).

Size of plots: In 1953: 14.4 × 0.6 m. In 1954: 6.6 × 2.25 m. In 1955: 6.6 × 3 m.

Spacing: 60 × 5 cm (in 1953 and '55) and 45 × 5 cm (in 1954).

Fertilization: Superphosphate, 450 kg/ha (in 1953) and 500 kg/ha (in 1954 and '55).

Irrigation: In furrows when necessary.

Design: Randomized block trial with split plots for cultivars; 4 replicates.

Table 14. Average numbers of diseased plants, in the cultivar/planting time trial 1953, counted in the beginning of August.

Cultivar	Time of planting				
	20/4	11/5	2/6	22/6	14/7
Greenfeast	19.3	0	0	0	0
Morse's Progress	60.0	1.5	0	0	0
Kelvedon Wonder	106.0	1.5	0	0	0
Little Marvel	120.5	5.0	0	0	0
S.S.D. (P = 0.05)	27.0				
C.V. %	22.0				

Results. In Tables 14 to 18 the average numbers of diseased plants in each treatment are presented. The statistical analyses were made of the data of only one planting time of each trial. In all three experiments the symptoms were like those described under 2.1.1.

Experiment 1953. The diseased plants were counted in the first week of August and the results are presented in Table 14.

The difference in infection between the first and the later plantings was very marked. While the susceptible cultivars in the first planting were severely infected, those in the second were almost free from blight and even the susceptible cultivars in the last three plantings were healthy. In this trial Greenfeast was much more resistant than Little Marvel, Kelvedon Wonder and Morse's Progress.

Experiment 1954. The diseased plants were counted on 17 June, 15 and 26 July and 12 August; the results are presented in Table 15. Observations on frost injury were made shortly after the first frost on 23 May; it was found that Morse's Progress and Kelvedon Wonder were badly injured.

TABLE 15. Average numbers of diseased plants in cultivar/planting time trial 1954.

Cultivar	Planted on	Number of diseased plants on:			
		17/6	15/7	26/7	12/8
Greenfeast	15/3	2.3	13.0	–	–
	5/4	0.5	5.8	9.3	16.0
	26/4	0	0.5	0.8	1.5
	17/5	0	0.3	0.3	0.3
	7/6	–	0	0	0
	28/6	–	–	0	0
Morse's Progress	15/3	6.0	54.0	–	–
	5/4	1.3	9.5	13.8	26.8
	26/4	0	0.3	0.3	1.5
	17/5	0	0	0	0.5
	7/6	–	0	0	0
	28/6	–	–	0	0
Kelvedon Wonder	15/3	15.3	42.8	–	–
	5/4	0.8	4.0	4.0	6.3
	26/4	0	0.3	1.3	3.5
	17/5	0	0	0.3	5.3
	7/6	–	0	0	0
	28/6	–	–	0	0

TABLE 16. Analysis of data taken on July 15 in the March 15 and April 5 plantings of the cultivar/planting time trial 1954.

Cultivar	Average	Date of planting	Average
Greenfeast	9.4	15/3	36.6
Morse's Progress	31.8	5/4	6.4
Kelvedon Wonder	23.4		
S.S.D (P = 0.05)	9.5	S.S.D. (P = 0.05)	17.6
C.V. 40.4%			

In Table 16 the statistical analysis is given of the numbers of diseased plants in the 15/3 and 5/4 plantings as observed on July 15.

Table 15 shows that Greenfeast was again the most resistant cultivar and that the disease was less severe or non-existent in the later plantings. This is in agreement with the results of 1953. On July 15 the differences between the cultivars were most pronounced, but they were much smaller in the 5/4 than in the 15/3 planting. The interaction cultivar × planting date was statistically significant. After July 15 no further observations could be made in the 15/3 planting because the crop was already maturing.

Experiment 1955. The diseased plants were counted on 27 June, 13 and 30 July and 29 August. The results are presented in Table 17.

The statistical analysis of the numbers of diseased plants as observed on August 29 is given in Table 18.

Greenfeast was significantly more resistant to blight than Little Marvel. The differences in resistance between the planting times were statistically not significant, probably because of the high coefficient of variation. The differences between the planting times were small for Greenfeast, but big for Little Marvel; the interaction cultivars × dates of planting was significant.

On June 9 the temperature dropped to −3 °C and a few days later differences in the extent of frost injury to the plants in the different treatments were observed. The first planting was about a foot high and had already started to flower, but the second was only a few inches high at the time. Except for the flowers, neither the first nor the second planting of Greenfeast was injured at all. The tops of the first Little Marvel planting were badly injured, but those in the second showed only a slight yellowing.

TABLE 17. Average numbers of diseased plants in cultivar/planting time trial 1955.

Cultivar	Planting date	Number of diseased plants on:			
		27/6	13/7	30/7	29/8
Greenfeast	20/4	0.5	1.5	4.0	10.3
	13/5	0.0	0.3	2.3	3.8
Little Marvel	20/4	0.8	14.3	61.0	107.3
	13/5	0.3	2.8	12.5	40.5

TABLE 18. Analysis of data taken on August 29 in the cultivar/planting time trial 1955.

Cultivar	Average	Date of planting	Average
Greenfeast	7.0	20/4	58.8
Little Marvel	73.9	13/5	22.1
S.S.D. (P = 0.05)	25.7		N.S.
C.V. 52%			

5.4. IRRIGATION TRIAL

Material and methods.

Cultivar: Morse's Progress.

Date of planting: 16/4/1958.

Fertilization: Superphosphate, 500 kg/ha.

Plot size: 7.1 × 2.2 m.

Spacing: 45 × 5 cm.

Number of plots: 15. The plots were laid out along a concrete furrow; the distance between them was 90 cm.

Inoculation: The plots were sprayed with a suspension of *Ps. pisi* (isolate II) on 3/7, 22/7 and 1/8/'58.

Irrigation: The critical moisture contents of the soil were:

(a) Field capacity: 17.5%.

(b) Wilting point determined with sunflower (*Helianthus annuus*): 8%.

A plot was flood irrigated when the moisture content of the soil dropped below the specific irrigation level for that plot. The numbers of the plots and their specific irrigation levels are given in the diagram below.

Plot number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Irrigation level in % moisture	14	10	12	8	8	12	10	12	8	14	10	8	10	14	12

The moisture content of the soil in the different plots was determined twice a week by the gravimetric method. The soil samples were taken at a depth of 22.5 cm. The plots were, if necessary, always irrigated the day after sampling.

On April 11, i.e. 5 days before planting, the plots were irrigated with an amount of water equal to 25 mm of rain. The rainfall during April amounted to 75 mm. After that the rainfall was so low that it was of no importance.

Results. At the end of May differences in the colour of the plants in the different treatments were already noticeable; the plants growing in the soil at the two highest irrigation levels were bright green, whereas those at the lowest levels were dull grayish-green. After a cold spell from 15 to 18 June, when the temperatures registered in a Stevenson-screen, at 30 cm above the ground, dropped to −5°C, distinct differences in frost injury were observed. The severity of injury decreased progressively from the lowest to the highest irrigation level. The most conspicuous differences were found between 8% and 10% soil moisture on the one hand and 12% and 14% soil moisture on the other, the plants in the first mentioned plots being more severely injured by frost than those in the plots with high irrigation levels. In July the temperature seldom dropped below freezing point, the plants started growing again and those that were severely injured produced new laterals.

The first disease symptoms were observed towards the middle of July. The numbers of diseased plants, counted on August 25, are given in Table 19.

TABLE 19. Average numbers of diseased plants in irrigation trial 1958.

Irrigation level	Average number of diseased plants
8%	266.0
10%	214.8
12%	253.5
14%	256.7
S.S.D. (P = 0.05)	35.5
C.V. %	9.0

The differences in number of infected plants, between the treatments were not great: they were not statistically significant for P = 0.01. There were indications that the plants in the plots with an irrigation level of 10% were less susceptible than those in plots with lower or higher levels.

5.5. DISCUSSION

The effect of the time of planting on the occurrence of bacterial blight was the most remarkable phenomenon in these field trials. The disease was always more severe in early planted peas and the chances of infection were very small if the peas were planted after 15 May. These findings were confirmed by observations in field trials planted at the Horticultural Research Institute for breeding purposes: if the peas were planted after the middle of May bacterial blight was seldom observed. The question arises, what is the nature of the resistance of late planted peas. Because the disease does not reach its peak before August and the incubation time is relatively short, the differences in resistance cannot be explained by assuming that the later plantings simply escape the disease. The results of the experiments described in the previous chapter show that the differences in resistance between the different plantings cannot be ascribed either to differences in the rate of spread of the disease in the plants after infection has taken place. In fact, the individual diseased plants in the later plantings were usually as badly attacked as those in the early plantings. Thus, if the resistance to infection has been overcome, the time of planting will have no effect on the development of the disease.

The difference between early and later plantings must therefore be attributed to a difference in resistance to infection. Results of the experiments described under 4.6 show that pea plants grown at higher temperatures are more susceptible to blight than plants grown at lower temperatures. This difference in susceptibility is correlated with the stomata frequency (Fig. 9), but it is unlikely that the number of stomata would fully explain the effect of the time of planting on the occurrence of the disease. However, the differences in susceptibility to blight between early and late plantings can possibly be correlated with differences in sensitivity to frost. These differences can be explained as follows. Resistance to frost as well as the ability to become winterhardy de-

creases as the plant develops (LEVITT, 1941). As young plants are more resistant to frost, the injury is, in the beginning of the winter, less severe in the late than in the early plantings, whereas the ability of young plants to become winter-hardy enables them to maintain their resistance right through the season. Big fluctuations in temperature on sunny days from relatively high temperatures to near or below freezing point are optimal for the hardening of annual winter crops (TUMANOV, 1931). Such conditions occur frequently at the Horticultural Research Institute during June, July and August, but not in April and early May, because the minimum temperatures are then too high. The early plantings are, in the beginning of their growing period, therefore not subjected to the right conditions to become winterhardy and later they have partly lost their ability to do so. When the temperatures drop below freezing point in winter these plants are sensitive to frost and susceptible to infection by *Ps. pisi*.

In the Literature Review it was mentioned that in other countries peas can also be planted at such times as to minimize their chances of becoming infected with blight. Thus by planting at the right time, weather conditions favourable to blight e.g. rain (CROSBY and CHUPP, 1934) and frost (WADE, 1951), can be avoided. The effect of the time of planting in these countries is apparently not the same as in the Transvaal, where the crop apparently becomes hardened against predisposing conditions.

Besides the effect of planting time on the occurrence of blight, differences in susceptibility between cultivars were also observed. The results of the experiments, which are described under 4.5 and were done with the cultivars Morse's Progress, Little Marvel and Greenfeast, show that these differences cannot be ascribed to resistance to spread. In later years more such experiments were done in the field which are not described here and in which all the cultivars mentioned under 5.2 and 5.3 were included. In these experiments the disease spread after frost somewhat more rapidly in the very susceptible cultivars Morse's Progress, Kelvedon Wonder and Little Marvel than in most of the other cultivars, but the differences were not of any practical importance. It seems, therefore, that the resistance of certain cultivars is mainly aimed against infection, the differences in susceptibility being small once infection has taken place. As the resistance which SACKETT (1916) observed in his cultivar trials was lost by injuring the plants, his cultivars showed what was probably the same type of resistance.

The results of the trials conducted in 1953, 1954 and 1955 indicate that frost sensitive cultivars appear to be more susceptible to blight than are the frost resistant cultivars. The resistance of these cultivars has, therefore, two features in common with the resistance of late plantings: both are directed against infection and both are correlated with frost resistance. As big differences in susceptibility were also observed within the group of frost resistant cultivars, other factors besides frost possibly play a rôle as well.

The relative susceptibility is not always the same from year to year. Onward for instance was more resistant than Perfection in 1957, but in 1958 they were about equally susceptible. This inconsistency may be the result of differences in

the weather: the winter of 1957 was rainy; that of 1958 dry. But if the relative susceptibility of Greenfeast in 1958 is compared with that in the 1953, '54 and '55 trials it is obvious that there are annual differences which cannot be explained by differences in the weather. The information on the resistance of cultivars obtained in the literature is also somewhat contradictory; this makes it difficult to distinguish between susceptible and resistant cultivars. Of all the research workers, it was only SACKETT (1916) who found in his experiments a cultivar, viz. Wellington, which seemed immune. The experiments were unfortunately not repeated and the name Wellington is moreover a synonym for several different cultivars. There is at present no immune cultivar and the results of WARK's (1950) investigations in Australia indicate that it is unlikely that such a cultivar will soon be found.

In the 1958 irrigation trial it was found that pea plants irrigated frequently were less sensitive to frost than were those suffering from lack of water. This phenomenon, which is well known to farmers, can possibly be ascribed to a difference in thermal conductivity between dry and wet soil. The amount of irrigation water had no appreciable effect on the occurrence of the disease. These results appear to be different from those which were obtained by WARK (1954) and which are described under 5.1. WARK, however, investigated the appearance of symptoms on plants which were infected from the seed; in the irrigation trial described above the occurrence of the disease was studied on plants which had been sprayed with a suspension of the pathogen when they were fullgrown.

6. SOURCES OF INFECTION AND SPREAD IN THE FIELD

The disease symptoms on seed, described in chapter 2 suggest strongly that bacterial blight is a seed-borne disease: in fact several research workers have shown that pea seed can be infected with *Ps. pisi* and that the disease can be transmitted in this way. However, in cases when it is uncertain that the disease had its origin in the seed, the question arises whether the causal organism can survive in the soil or in other host plants. Another important question is how the disease spreads in a pea field after it has made its appearance on one or a few plants. In this chapter some experiments conducted with the object of finding answers to the above questions are described; a review of the relevant literature is given first.

6.1. LITERATURE REVIEW

(a) *Transmission of bacterial blight in the seed, in contaminated pea straw and in the soil.* JENNISON (1921), who was the first to report pod infection in the U.S.A., found that seeds of infected pods are often contaminated. He thought that dissemination was largely due to contaminated seed. His findings were confirmed by other research workers e.g. STAPP (1937) in Germany and WADE (1951) in Tasmania. SKORIC (1927) showed that the pathogen could remain viable in contaminated seed for at least ten months. He found that most of the plants grown from contaminated seed showed symptoms on the lower stipules, but that a few showed them on all the organs. He supposed that the plumule becomes infected through contact with the infected seed coat and that the number of organs which become infected depends on the speed at which the seedlings develop. Similar observations were made by WARK (1949, 1954) and by WALLACE and WALLACE (1951), but LUDWIG (1926) did not observe symptoms on plants grown from infected seed.

BROWN and EVANS (1932, 1937) found that seed of several cultivars offered for sale in Arizona was contaminated with *Ps. pisi*, but seed from the arid areas in the western U.S.A. was apparently free from the disease (DELWICHE et al., 1939; CONNERS and SAVILE, 1950). JONES and LINFORD (1925), however, could not find any correlation between the occurrence of blight and the source of seed.

Diseased plants left in the field are considered to be sources of infection in Australia, but of much less importance than infected seed (ANONYMOUS, 1939). According to another Australian report (ANONYMOUS, 1956), available evidence indicates that the disease may be soil-borne, but in the U.S.A. SKORIC (1927) did not succeed in isolating the pathogen from soil on which diseased plants had been grown for several years. In New Zealand it is believed that the pathogen does not survive in the soil once the host tissue has decomposed (BRIEN et al., 1955).

(b) *Host plants of* Ps. pisi. A fair amount of information is available about the susceptibility of other plants, besides peas, but much of it was obtained in glasshouse experiments (i.a. SKORIC, 1927). Under natural field conditions, apart from green peas (*Pisum sativum*), also field peas (*P. sativum* var. *arvense*), vetch (*Vicia* sp.) and purple vetch (*Vicia atropurpurea*) were found to be susceptible (see SACKETT, 1916; THANASSOULOPOULOS, 1965 and ARK, 1944 respectively). In field trials WARK (1950) found that the following species of *Pisum* and *Lathyrus* were susceptible as well: *P. abyssinicum, P. elatius, P. humile, P. jomardi, P. aphaca, L. hirsutus, L. ochrus, L. pubescens, L. sativus* and *L. tingitanus.*

(c) *Origin and spread of bacterial blight in the field.* SKORIC (1927) distinguished between primarily and secondarily infected plants. The first were infected directly from diseased seed and the second from infected plants. The secondarily infected plants were usually found in the same row as the primarily infected plants or in the row next to it. The number of primarily infected plants was usually small. SKORIC sometimes found what appeared to be secondarily infected plants well away from those primarily infected. As they were usually found in the lower parts of the fields he believed that the disease had been spread by means of contaminated drainage water.

The same origin and manner of spread of the disease in the field was observed by WALLACE and WALLACE (1951). They found that the intensity of the disease could increase within six weeks from a few isolated leaf spots to blackening, decay and collapse of all the plants in a field.

Besides being splashed by rain and carried in running water, the disease can, according to the literature, be spread in sand during dust storms, by contact between healthy and diseased plants, by insects, birds and by man (SACKETT, 1916; BROWN and EVANS, 1937; ANONYMOUS, 1939; WALLACE and WALLACE, 1951; BRIEN et al., 1955). In Hungary KLEMENT and LEHOCZKY (1960) observed infection on leaves damaged by *Sitona* spp., mainly *S. lineatus.* They believe that these insects play a part in spreading the disease.

Factors known to prevent the spread of the disease are dry weather (WALKER and HARE, 1943) and high temperature, but STAPP (1937) in Germany found that the disease did spread in spite of such conditions.

6.2. SEED TRANSMISSION OF BACTERIAL BLIGHT IN SOUTH AFRICA

6.2.1. *Conditions favourable for pod infection*

Two factors are important for seed transmission of blight: (a) the stage of development of the crop when it becomes infected and (b) the occurrence of frost. Frost is, firstly, a direct cause of flower and pod injury and is therefore a limiting factor in the production of seed. Secondly, it is a predisposing factor for infection and spread of the disease in the plant.

If the plant is infected when it is still young, the main stem is usually killed and can therefore not produce pods. Subsequent infection of the side shoots may be a result of direct infection or of internal spread of the disease from the

infected to the healthy stems. In both cases frost is a predisposing factor. If the laterals remain healthy they may flower and produce pods and seeds. If the crop is exposed to frost in the flowering and early pod stage, the flowers as well as the pods may be killed. Even if the pods have already grown to their full length, the undeveloped seed inside will be killed by the frost, making transmission of the disease by the seed impossible. In a crop which has been injured by frost at this stage and become infected by blight, diseased plants can be found bearing pods with typical symptoms of frost injury and blight. The seeds in these pods are usually undeveloped and dead. If the disease remains restricted to the lower parts of the plants, buds may develop in the upper parts and, if no more frost occurs, they may produce seed.

From the above it follows that healthy-looking pods with normal seeds may sometimes be found on diseased vines. In chapter 4 it was shown that bacterial blight is apparently not a systemic disease. The chances of seed from apparently healthy pods being infected with *Ps. pisi* are therefore very small. This hypothesis is supported by the results of an experiment described under 6.2.2.

If, however, a crop showing signs of disease is exposed to moderate frost when it is bearing pods with swollen seeds, then both pods and seeds may become infected. A description of symptoms on diseased pods was given in chapter 2. Under 6.2.3 an attempt to isolate the pathogen from seeds, borne in pods with these symptoms, is described. An experiment on the survival of *Ps. pisi* in pea seed is described under 6.2.4.

6.2.2. *Isolation from seeds borne in apparently healthy pods on diseased plants*

Material and methods. In July 1958 plants of the cultivar Morse's Progress were inoculated with isolate II by means of a hypodermic syringe. The pathogen was injected into the base of the stem (see 3.1), but after frost in July and August it spread to the higher internodes. In the second half of August apparently healthy pods were picked from stems in which the disease had spread over two internodes or more. The pods were picked when they were still green, but the seeds were already fully swollen. Some of the peduncles of the pods showed disease symptoms. The pods were washed, dipped in 96% alcohol to drive off the air from the surface, and surface sterilized for 3 min in 0.1% mercuric chloride. The seeds were aseptically removed and plated out on NA. Fifteen Morse's Progress pods and one from the cultivar Greenfeast were investigated in this manner. Symptomless pods from the diseased stems of a naturally infected pea plant, cv. Nugget, were also included in these investigations.

Results. *Ps. pisi* could not be isolated from any of the seeds, though the pathogen was detected in three diseased peduncles.

6.2.3. *Isolation from seeds from an infected pod*

Material and methods. In the winter of 1958 a pod with typical symptoms (natural infection) on the proximal part of the ventral suture was found in a field of Morse's Progress. The five seeds attached to the visibly infected part of

the suture had not developed normally, but had remained small and soft. In the distal part of the pod, which did not show any signs of disease, two seeds had developed normally. The pod was disinfected externally and the seeds were removed aseptically and plated out on NA. The funiculus of each seed was plated out separately. Colonies growing from these seeds were transferred to NA-slants and tested for pathogenicity according to the method described under 3.1.(a).

Results. *Ps. pisi* was isolated from the two fully developed seeds and the five undeveloped seeds, as well as from the funiculi.

6.2.4. *Survival of* Ps. pisi *in dry pea seeds and pods*

Material and methods. Pods of the cultivar Morse's Progress were inoculated with *Ps. pisi* in the field on August 30, 1962. They were pricked in the ventral suture with a hypodermic syringe containing a suspension of isolate III. When the seed was mature the pods were harvested and kept in a paper bag in the laboratory. The following winter at the end of July, i.e. eleven months later, reisolations were made from the pod walls and the seeds. (a) Reisolations from pods: Pieces of pod showing lesions were crushed and suspended in tubes with sterile water, which were incubated for two days at 15°C. Streak plates were made from these suspensions on TGA and colonies were transferred from these plates to slants of the same medium. (b) Reisolations from seeds: Seeds with blight symptoms were dipped for a moment in 96% alcohol and soaked for 3 min in 0.1% mercuric chloride. They were then washed in sterile water, put in tubes with sterile water and incubated at 15°C. After two days the seed coat was removed and cut into small pieces, which were suspended in sterile water. The suspensions were allowed to stand for a few hours and were subsequently streaked out on TGA. The isolates were tested for pathogenicity according to the method described under 3.1.(a).

Results. *Ps. pisi* was reisolated both from the pod walls and the seeds.

6.3. Origin and spread of bacterial blight in an experimental field

In a field trial both healthy seed and seed from artificially inoculated pods were planted in different plots and a survey was made of the occurrence of the disease.

Material and methods.

Cultivar: Morse's Progress.
Date of planting: 28/4/1959.
Spacing: 60 × 5 cm.
Fertilization: Superphosphate, 500 kg/ha.

Design: See Figure 10. The field was divided in two sections by a path.

Irrigation: The flow of irrigation water is indicated in Figure 10 by arrows; it did not flow from one section to the other. Dates of irrigation: May 8 and June 23.

Weather: Rain fell on: May 3 (0.7 mm), May 12 (6.8 mm), May 13 (7.2 mm), June 8 (0.2 mm) and June 9 (0.6 mm). Frost occurred on: May 23 (−2°C), May 24 (−½°C), May 25 (−1½°C), May 28 (−2½°C), June 10 (−3½°C), June 15 (−2½°C) and June 21 (−2½°C). The figures in brackets are the minimum temperatures measured 45 cm above the soil in a Stevenson screen.

Procedure: The trial was laid out in a field where infected pea plants had grown the previous year. Healthy mother seed, harvested in 1957, was planted in the experimental field, except for a plot in the middle. After the mother seed had first been treated with an organic mercury compound and then planted, infected pea seed, not previously treated, was planted in the centre plot. This seed had been artificially infected the previous year with isolate II according to the method described under 6.2.4 and had shown distinct symptoms of blight. The experimental field was not tilled after planting. All suspicious looking plants were subsequently marked and after the plants had been touched, the hands were washed in a disinfectant to prevent the blight bacteria from being carried to other plants in the field.

Results and discussion. The plants were carefully inspected for the first time between May 21 and May 27, but none of them showed typical blight symptoms, though many were marked as suspicious. On May 29 the first plant with typical symptoms was found during a superficial inspection of the centre plot. Between June 2 and 18 the whole field was carefully inspected for the second time. Plants that showed typical blight symptoms are indicated in Figure 10. All the diseased plants found in the centre plot, i.e. those grown from infected seed, had already shown suspicious spots at the first inspection, but none grown from healthy seed had been marked as suspicious during the first inspection.

The distribution of the diseased plants in the experimental field can be interpreted in three possible ways:

(a) One possibility is that all diseased plants, i.e. in the centre plot as well as in the remainder of the field, were infected from the seed. This is, however, very unlikely, because the seed planted in the section outside the centre plot had been harvested from a crop where no blight had been observed and because the seedlings did not show any signs of disease at the first inspection.

(b) Another possibility is that only the plants in the centre plot contracted the disease from the seed. This is probable, because the seed was heavily infected and the seedlings showed suspicious symptoms at the first inspection. Exposure to frost between the two inspections may have caused the symptoms to become more distinct and typical. It could be argued that the bacteria may have spread from the centre plot to the rest of the field, that they infected the healthy plants only after these had been exposed to frost and that consequently the disease

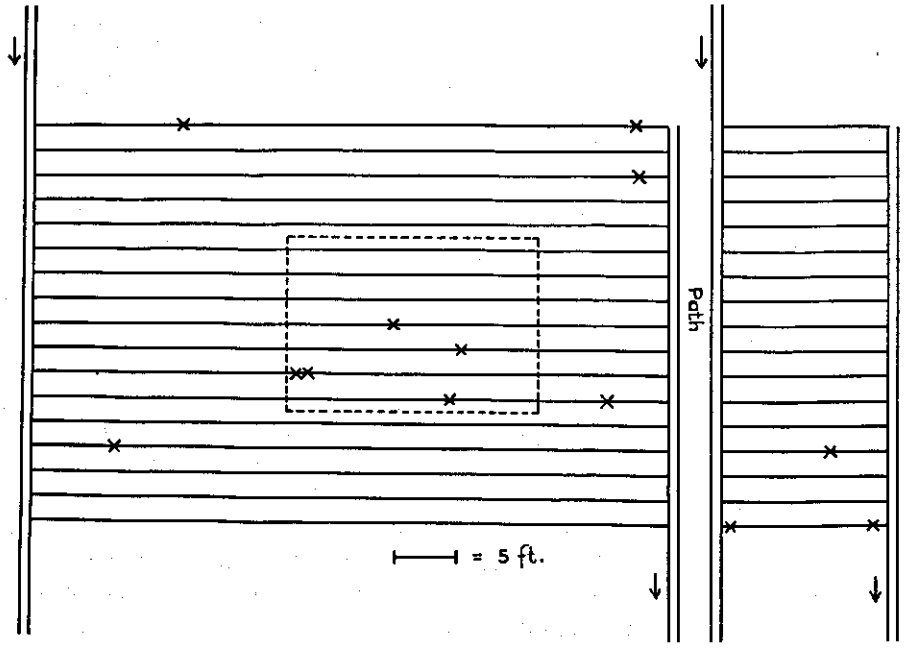

FIG. 10. Occurrence of bacterial blight in a field trial (ch. 6.3.). x = diseased plants.

made its appearance in the centre plot and in the remainder of the field at about the same time. Possible means of spread were: in irrigation water, by insects, by labourers working in the field and by rain on May 12 and 13. It is unlikely that the pathogen spread in irrigation water from the centre plot to the remainder of the field: most diseased plants outside the centre plot were found on places situated higher than the centre plot or in the section that was separated by a path. No indications were found that the disease could have been transmitted by insects and transmission by the workers had been avoided as much as possible. This then leaves the possibility that the disease was spread by the rain. A pea disease which is transmitted by seed and spreads by means of rain and wind is ascochyta-blight: as is to be expected in such a case most secondarily infected plants were found near plants infected from the seed (DEKKER, 1957). A similar distribution pattern was not found in the experiment under discussion and it is doubtful whether rain played a part in the spread of the pathogen.

(c) This leads to the possibility that the disease originated at least partly in the soil, which had been contaminated by diseased plants ploughed in the previous year.

6.4. Discussion

Research work in other countries has shown and experiments described in this chapter have confirmed that bacterial blight is a seed-borne disease. Spots on the lower stipules of pea seedlings grown from naturally infected seed were observed by Skoric (1927). These symptoms are very similar to those observed in the experiments with artificially infected seed, described in 3.1 (c). As naturally infected seed is difficult to obtain in South Africa no experiments were done with such seed, except that the pathogen was isolated from it (6.2.3). That seed infection is relatively rare in South Africa was, earlier in this chapter, attributed to the weather. Only if moderate frost occurs when the seeds are swelling in the pods and provided there is a source of infection nearby, do the pods and seeds become infected. Pods can probably also become infected under rainy conditions, but the chances of timely rain in the seed producing areas are so remote that this manner of infection can be ignored in most years.

Skoric (1927) found that the pathogen can remain viable in the seed for at least ten months. This was confirmed and it was also shown that the pathogen could survive the summer in infected pod walls. The disease can therefore be transmitted in and on the seed and probably in dry pea straw from one season to the next. In practice, the straw is mostly ploughed into the soil, where it decomposes rapidly if the soil is moist. The question arises whether the disease can be soil-borne. It appears from literature information, as well as from the experiments described under 6.3, that contaminated soil may be a source of infection.

Besides infected seed, pea straw and soil, host plants other than peas might be a source of infection. In other countries, besides green peas, only field peas, purple vetch (Ark, 1944), vetch (Thanassoulopoulos, 1965), and the species, investigated by Wark (1950) and mentioned under 6.1, were found to become naturally infected. In South Africa natural infection has been found only on green peas. Infected seed and contaminated soil are probably the main sources of infection.

Skoric (1927) found a clear pattern in the origin and spread of bacterial blight. Seedlings were infected from contaminated seed and from these foci the disease spread to plants growing nearby, which subsequently served as sources of infection to other plants in the vicinity. Similar observations were made in other countries, where the disease is associated with rain (Brien et al., 1955; Wallace and Wallace, 1951). The origin and spread of bacterial blight at the Horticultural Research Institute is much more difficult to explain. Not only in the 1959 field trial, but also in other experiments, the first diseased plants were found randomized over the field and no obvious connection was found between new and already existing infections. Sackett (1916), who made his observations under conditions very similar to those in the South African seed producing areas, was of the opinion that the disease spreads by means of sand, blown by the wind, which literally injects the tissue with germ-laden soil particles.

7. CONTROL

7.1. Time of planting and resistance of cultivars

The results of the experiments described in chapter 5 show that bacterial blight was hardly ever observed in pea crops planted after the middle of May. Thus it follows that the time of planting is of great importance for the control of the disease. The results of the cultivar trials described in the same chapter were not very consistent, but an indication was found that frost sensitive cultivars are more susceptible to blight than those which are frost resistant.

7.2. Spraying trials

The object of the spraying trials was firstly to find an effective bactericide for the control of blight and secondly to obtain information on the best times of application.

7.2.1. *Preliminary experiments*

In preliminary experiments the following bactericides were tested: Bordeaux mixture (8 kg/500 l of water), cuprous oxide (1¼ kg Perenox/500 l of water) and streptomycin (220 i.u./ml). The first two were applied once a week and the third once a fortnight. Bordeaux mixture gave the best results, cuprous oxide was also effective but slightly phytotoxic. Streptomycin was ineffective: this could be expected since the sensitivity test conducted by Morgan and Goodman (1955) had shown that *Ps. pisi* was not sensitive to streptomycin in the laboratory. In experiments done by Katznelson and Sutton (1951) only one of the two strains of *Ps. pisi* was sensitive.

7.2.2. *Spraying trial 1955*

Material and methods.

Cultivar: Morse's Progress.
Time of planting: 21 April 1955.
Size of plots: 6.6 × 3 m.
Spacing: 60 × 5 cm.
Irrigation: In furrows when necessary.
Fertilization: Superphosphate, 500 kg/ha.
Bactericide: Bordeaux mixture, 8 kg/500 l of water.
Applications: See Table 20.

Results. The average numbers of diseased plants (natural infection), counted on 4 August 1955 are presented in Table 20. Two sprayings with Bordeaux mixture apparently decreased the number of diseased plants to about a quarter

TABLE 20. Effect of differently timed applications of Bordeaux mixture on the occurrence of bacterial blight.

Treatment	Average number of diseased plants
1. Control	53.3
2. Sprayed 1 ×, on 16/5	51.5
3. Sprayed 2 ×, on 16/5 and 30/5	11.3
4. Sprayed 3 ×, on 16/5, 30/5 and 14/6	12.0
5. Sprayed 4 ×, on 16/5, 30/5, 14/6 and 27/6	14.8
6. Sprayed 5 ×, on 16/5, 30/5, 14/6, 27/6 and 26/7	10.5
S.S.D. (P = 0.05)	9.7
C.V. %	25.2

of that in the control plots. It is remarkable that the 3rd, 4th and 5th spraying had no apparent effect on the numbers becoming diseased. As the number of infected plants gradually increased in the control plots during the course of the winter and as the disease did not reach its peak before August, the ineffectiveness of the sprays after May 30 cannot be explained by assuming that the time favourable for infection was restricted to a short period after this date. It is probable that the new growth, which developed in the second half of June and later, was highly resistant to the disease and therefore did not need a protecting bactericidal cover. This resistance is probably of the same nature as that of late plantings. A similar result was found in an experiment conducted in 1957.

7.3. SEED INSPECTION AND PLANT QUARANTINE

As bacterial blight is a seed-borne disease the importance of healthy seed is stressed wherever the disease occurs. If possible, seed should therefore be imported from places where the disease does not occur. Reports from the U.S.A. and Canada suggest that this control measure would be successful (DELWICHE et al., 1939; CONNERS and SAVILE, 1950). In 1954 the Working Party on Seed-Borne Diseases convened by the European Plant Protection Organization in co-operation with the International Seed Testing Association decided that countries would be justified in demanding that pea seed be accompanied by a phytosanitary certificate stating that the seed crop had been inspected in the field, or had been grown in a place where the disease was unknown (ANONYMOUS, 1954). Several countries, including South Africa, require a certificate stating that imported pea seed is free from *Ps. pisi.*

To prevent the spread of blight within South Africa a Proclamation was issued by the government in 1953 prohibiting the transport of pea seed and plants from any part of South Africa to the pea producing areas near Upington, but this precautionary measure did not have the desired effect. A similar attempt in Tanganyika to restrict the disease to a certain area was also unsuccessful

(WALLACE and WALLACE, 1952).

In South Africa the Division of Seed Control of the Department of Agricultural Technical Services, responsible for the inspection and certification of seed, does allow a certain degree of blight infection in a seed crop, provided the pods do not show any signs of the disease; seeds from plants with only infected leaves and stems must first be treated with an approved seed dressing.

7.4. SEED DRESSING

Experiments described in chapter 6 show that the pathogen is not killed inside seed soaked for 3 min in 0.1% mercuric chloride. Similar results were obtained by WARK (1949) with naturally infected seed, but his experiments with solutions containing mercury bichloride were promising if the seed was soaked for more than 20 min. The most effective solution was 1:500 mercury bichloride + 1:20000 gentian violet in 70% ethyl alcohol, acidified with 3% acetic acid. Soaking the seed in this solution for four hours was very effective and did not harm the germination.

7.5. CROP ROTATION

SACKETT (1916) advised crop rotation as a control measure. When, however, WALKER and HARE (1943) plotted rotation against disease index the correlation, evident with ascochyta-blight, did not hold.

7.6. DISCUSSION

Planting at the right time is the most effective way of preventing bacterial blight. At the Horticultural Research Institute the disease was never important in peas planted after the middle of May. Peas should, however, not be planted at the Horticultural Research Institute much later than June, because late crops are likely to become heavily infected with powdery mildew (*Erysiphe pisi*) at the beginning of summer and with ascochyta-blight if there are early summer rains. The same holds true for the areas under irrigation in the Transvaal and at Oudtshoorn, where the production of pea seed is concentrated. Experience will show what the right planting time is in each of these areas, but it will probably not be before May and after June. Early cultivars can be planted in the Transvaal Middle Veld as early as March and harvested before the first frost occurs, but if peas are planted still earlier the young plants usually become infected by soil fungi. In other countries it was also found that the planting time is important in preventing blight (see chapter 5).

In severe blight years it was sometimes observed that peas in higher situated fields were only slightly infected, whereas those in the lower parts were severely

attacked. These low lying so-called frost pockets should therefore be avoided; similar advice is also given by WADE (1951).

It is important to use government certified seed. The use of infected seed is considered the most important means of spreading the pathogen over long distances as was illustrated by its spread from Groblersdal and Lydenburg to Vaalhartz in 1951–'52. Dressing the seed is recommended in South Africa as well as in other countries (SKORIC, 1927; STAPP, 1937), but its value is very limited if only the surface of the seed is sterilized. The method developed by WARK (1950) is not practical for big quantities and is only applied in the case of very valuable seed, e.g. breeding material.

Results of the experiments described in chapter 5 show that there are differences in susceptibility between cultivars: preference should be given to those resistant to frost and blight. As, however, bacterial blight is not an important disease in South Africa, the choice of the cultivars will be mainly determined by the demand on the local and world market, particularly in the case of seed production.

SACKETT (1916) recommended crop rotation and careful cultivation of the crop to prevent injury, and BROWN and EVANS (1937) the use of wind breaks, but the value of these control measures under South African conditions is uncertain.

Control by means of copper sprays is possible, but not of practical value because, if the above mentioned control measures are practised, the disease is seldom of any practical importance in South Africa.

8. ANALYSIS OF THE EFFECT OF FROST ON INFECTION

In this chapter experiments are described which were done to investigate the basic causes of the effect of frost on infection. The word infection is used here in its wider sense: it refers not only to the penetration by the bacteria through the stomata, but also to the establishment of the pathogen in the intercellular spaces.

In the preceding chapters it has been mentioned several times that pea plants which have been exposed to frost become water-soaked. Thus the question arises whether water congestion alone offers an explanation for the increased susceptibility of frosted plants, which was observed in the experiments described under 4.4. To answer this question the effect of water-soaking of non-frosted plants on infection was studied (8.3). Pea plants have a waxy surface and it is important for successful inoculation that contact be made between the bacterial suspension sprayed on the plants and the fluid in the intercellular spaces (JOHNSON, 1947); therefore the effect of adding a spreader to the inoculum was also investigated. In all these experiments the inoculum was sprayed on the plant by means of an atomizer and the main object was to study the penetration of the pathogen into the plant. Another question is whether frost has any effect on the multiplication of the bacteria after they have entered the plant. Experiments to study this problem are described under 8.4; in these experiments the pathogen was introduced into the plant by means of injection. Studies on the effect of water congestion of non-frosted plants on the multiplication of the pathogen introduced by means of vacuum-infiltration were included as well. Finally the growth of *Ps. pisi* in intercellular fluid obtained from frosted and non-frosted plants was investigated (8.5). The object was to determine whether nutrients, which might have a favourable effect on the pathogen's growth or enzyme production, are exuded from the cell into the intercellular spaces after the plants have been exposed to frost.

The description of the experiments is preceded by a review of the relevant literature.

8.1. LITERATURE REVIEW

Several diseases have been reported to be associated with frost, many of them being fungus diseases of trees (GÄUMANN, 1945; PANAGOPOULOS and CROSSE, 1964b; YARWOOD, 1959). The relationship between canker of willow trees, caused by *Nectria galligena* and the occurrence of frost was studied by MOOI (1948). He found that the cankers originated on the bark around the scars of thin lateral branches or on the bark of the stubs of lateral branches. The bark at these places is especially sensitive to frost and is killed if subjected to freezing weather below a certain critical temperature. *Nectria galligena*

penetrates the dead tissue and from there infects the adjacent living bark. From a study of the relevant literature he concluded that cankers of many other trees originate in the same way. Infection of frosted pea plants by the fungi *Botrytis cinerea, Sclerotinia sclerotiorum, Fusarium avenaceum* and *Fusarium solani* was investigated by KERLING (1952). She observed that these fungi penetrated tissue, that had become water-soaked as a result of exposure to frost, though they could not or hardly infect vigorous pea plants under normal conditions. KERLING refers in this respect to the work of JOHNSON (1947), who found that water congestion of plant tissue facilitates the entrance of fungi and she adds that apart from this, the interruption of the normal gas exchange by the presence of water in the intercellular spaces may lead to an increase in the cells' susceptibility to infection.

The multiplication of *Ps. pisi* in frosted pea plants was studied by WARK (1954). He found that two days after a frost the number of bacteria in plants, which had been grown from infected seed, had increased enormously. He suggested that the sudden multiplication is due to the favourable micro-environment, supposedly created by an increase in the air humidity of the intercellular spaces following the frost and by the nutrients set free by the rupture of frosted cells.

Thus – to sum up – five possible causes of the effect of frost have been mentioned in the above: (a) selective killing of plant tissue, (b) a decrease in the resistance as a result of the interruption of gas exchange in water-soaked tissue, (c) water congestion as a factor that favours the penetration of the pathogen, (d) the availability of more nutrients and (e) the higher air humidity in intercellular spaces.

8.2. EXPERIMENTAL TECHNIQUE

All the experiments described in this chapter were done with the cultivars Morse's Progress and Greenfeast. Unless otherwise stated, they were grown under the same conditions and treated when they were in the same stage of development as described under 4.2. The experiments were all done with suspensions of one-day-old cultures of isolate III grown on TGA at 27 °C. The concentrations of the suspensions are mentioned under Material and methods. Reisolations were made according to standard methods. Stems and leaflets were disinfected for 1½ min in 0.1 % mercuric chloride to which a spreader had been added.

8.3. EFFECT OF FLUID IN THE INTERCELLULAR SPACES ON INFECTION

Pea plants that have been exposed to frost have a dark-green, watery appearance, which is caused by the water congestion of the plant tissue. In a series of experiments it was investigated whether fluid in the intercellular spaces of pea

stems, that had not been exposed to frost, rendered them more susceptible to infection by *Ps. pisi*. Fluid was introduced into the intercellular spaces either by injecting the stems with sterile, distilled water or by bruising the leaves. After these treatments the plants were sprayed with a suspension of the pathogen, but because pea plants have a waxy surface and are therefore difficult to wet, the effect of adding a spreader to the suspension was studied as well.

8.3.1. *Effect of injecting pea stems with sterile, distilled water*

Material and methods. Four experiments were done, two with the cultivar Morse's Progress (Exp. 1 and 2) and two with Greenfeast (Exp. 3 and 4). The treatments were as follows.

A. Sterile, distilled water was injected under the epidermis of a stem internode by means of a hypodermic syringe. Immediately thereafter the prick wound was sealed with sterile petroleum jelly and the internode was sprayed with a suspension of *Ps. pisi* in sterile, distilled water. The concentration of the suspension was about 4×10^7 cells/ml. In Experiments 1 and 3 one drop of Triton B1956 was added to each 90 ml of suspension, but no spreader was added to the inoculum in Experiments 2 and 4. One internode was treated per plant.

B. The same treatment as A, but the internode was pricked only with the needle of the syringe; no water was injected.

C and D. The same treatments as A and B respectively, but the internodes were sprayed with sterile, distilled water, with or without the spreader. A fortnight after the plants had been treated the final observations were made.

Results. The first symptoms started to develop within a week after inoculation. After a fortnight the lesions on the treated internodes were water-soaked, green to brown or purplish. They varied in size from small streaks to areas covering one side of the internode over almost its entire length. The numbers and percentages of diseased internodes are presented in Table 21. The reisolations were positive. No symptoms developed on the plants sprayed with sterile water (treatments C and D). The numbers of internodes in these treatments were about the same as those of the internodes that had been sprayed with a bacterial suspension.

TABLE 21. Percentages and numbers of diseased internodes out of the total numbers of internodes that had (or had not) been injected with distilled water and subsequently sprayed with a water suspension of *Ps. pisi* with or without a spreader.

Experiment	Bacterial suspension	Internodes injected with sterile water	Diseased internodes on			
			Morse's Progress		Greenfeast	
			Number	%	Number	%
2 and 4	without spreader	injected	3/14	21	3/26	12
	ditto	not injected	0/16	0	0/19	0
1 and 3	with spreader	injected	12/16	75	5/17	29
	ditto	not injected	0/19	0	0/20	0

From the results presented in Table 21 it follows that the internodes that had not been injected with water before they were sprayed with a suspension of *Ps. pisi* were not infected by the pathogen. If the results of Experiments 1 and 3 on the one hand and those of Experiments 2 and 4 on the other, are compared, it can be concluded that there is a distinct indication that adding a spreader improves the chances of infection. The experiments were, however, not done under identical conditions, therefore a separate experiment (8.3.3) was done to investigate the effect of a spreader.

8.3.2. *Effect of bruising*

In the experiments described above, water congestion was induced by injecting water into the internodes. Water congestion can also be induced in non-frosted plants by bruising them. Experiments on the effect of bruising on infection are described below.

Material and methods. Two experiments were done, both with the cultivar Greenfeast. The treatments were as follows.

A. A number of leaflets were pressed for 5 sec at 0.726 kg/cm^2; the apparatus pictured in Fig. 11 was used for this purpose. Immediately after a leaflet had been pressed the under surface was painted with a suspension of *Ps. pisi*, to which a spreader (one drop of Triton B1956 in 90 ml suspension) had been added. The concentration of the suspension was about 4×10^7 cells/ml.

B. Ditto, but the leaflets were painted about 24 hr (Exp. 1) or 2 hr (Exp. 2) after they had been bruised.

C. Painted as in A, but the leaves were not bruised.

D, E and F. As treatment A, B and C respectively, but the leaves were painted with sterile distilled water to which a spreader had been added (Triton B1956, 1 drop in 90 ml of water).

The pressed leaves were carefully inspected for wounds, which might have resulted from the treatment; those that were wounded were discarded. For the painting a soft camel hair brush was used, which was sterilized in boiling water for 10 min before each experiment.

Results. The main veins of the leaflets as well as narrow bands on both sides of these veins, became water-soaked after having been bruised, but in Experiment 1 the water-soaking disappeared within an hour and in Experiment 2 most of the water congestion had disappeared after two hours. The results as observed after a fortnight are presented in Table 22. The figures show that as a result of bruising, the leaflets became much more susceptible to bacterial blight, but after 2 hr the effect was reduced to about a half and after 24 hr it had disappeared. The main veins of the diseased leaflets had turned brown and small water-soaked, brown to biscuitbrown spots or similarly coloured bands had developed on both sides of these veins. In Experiment 2 the diseased portions of the main veins and the leaf blades were considerably smaller on those leaflets, that had been inoculated 2 hr after bruising, than on those inoculated

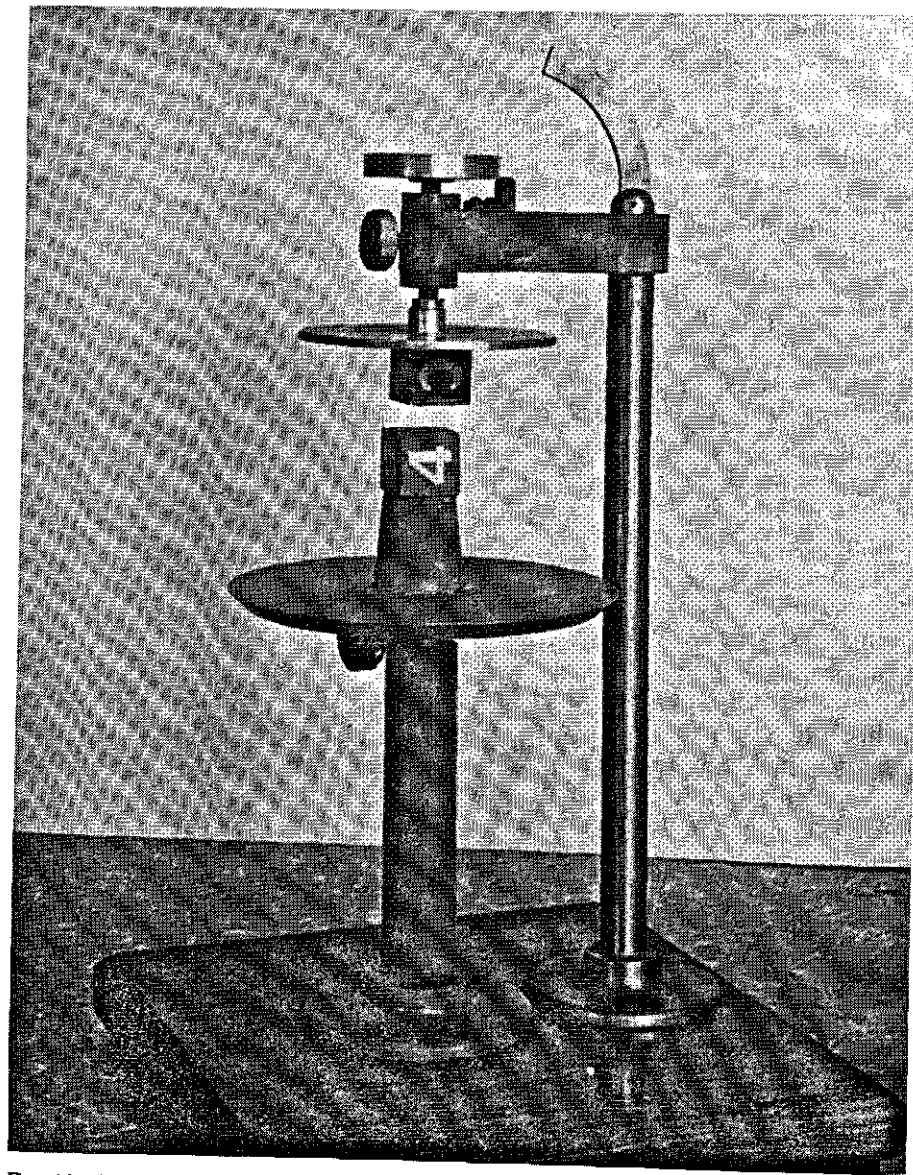

FIG. 11. Apparatus used for bruising pea leaflets (ch. 8.3.2). A weight is placed on the upper disc. The leaflet is carefully clamped between blocks C and 4 by raising the lower disc. Block 4 rests on a rubber stopper. The leaflet is bruised by loosening the upper screw.

immediately after this treatment. Thus the experiments show that the inoculations were only successful as long as the tissue was water-soaked. Reisolations were positive. No symptoms developed on the leaflets that had been painted with water.

TABLE 22. Percentages and numbers of diseased leaflets out of the total numbers of leaflets which had (or had not) been bruised and were painted with a water suspension of *Ps. pisi* 0, 2 or 24 hr later.

Bruising	Inoculated hr after bruising	Diseased leaflets			
		Experiment 1		Experiment 2	
		number	%	number	%
Bruised	0 hr	26/30	87	23/23	100
	2 hr			13/27	48
	24 hr	0/47	0		
Not bruised		0/29	0	0/26	0

8.3.3. *Effect of adding a spreader to the inoculum*

Material and methods. Four experiments were done, two with the cultivar Morse's Progress and two with Greenfeast. The treatments were as follows.

A. Sterile, distilled water was injected under the epidermis of a stem internode by means of a hypodermic syringe. Immediately thereafter the prick wound was sealed with sterile petroleum jelly and the internode was sprayed with a suspension of *Ps. pisi* in sterile, distilled water. One internode was treated per plant. The concentration of the suspension was about 4×10^7 cells/ml.

B. Ditto, but a spreader was added to the bacterial suspension (Triton B1956, 1 drop/90 ml suspension).

C and D. Treated as A and B respectively, but the internode was sprayed with sterile, distilled water instead of a bacterial suspension.

Results. The first symptoms developed within a week after inoculation. After a fortnight the results were as follows. No symptoms had developed on the internodes sprayed with water. The numbers of infected internodes in the other two treatments are presented in Table 23. The lesions on the infected internodes were water-soaked, green to brown or purplish. Reisolations were positive.

In Experiments 1 and 4 the percentage of diseased internodes increased considerably if a spreader was added to the inoculum. This is in agreement with what was found under 8.3.1. In Experiment 2 the percentage of infected inter-

TABLE 23. Effect of adding a spreader to the inoculum. Percentage and number of diseased internodes out of the total number of internodes that had been sprayed with a water suspension of *Ps. pisi* with or without a spreader

Bacterial suspension	Diseased internodes on							
	Morse's Progress				Greenfeast			
	Exp. 1		Exp. 2		Exp. 3		Exp. 4	
	number	%	number	%	number	%	number	%
With spreader	13/25	52	14/18	78	7/40	18	15/25	60
Without spreader	9/30	30	11/16	69	10/45	22	10/27	37

nodes was also somewhat higher if a spreader was added to the inoculum, but this was not the case in Experiment 3.

8.4. The multiplication of Ps. pisi in pea plants

In the experiments described under 4.4 it was shown that frost is a predisposing factor for infection. This effect of frost on infection can at least partly be explained from the fact that frost causes the tissue to become water-soaked, a condition which facilitates the entrance of the pathogen (8.3). Experiments on the effect of frost on the multiplication of the pathogen once it is in the plant are described below. The multiplication of the pathogen in non-frosted, water-soaked plant tissue was studied as well.

8.4.1. *Effect of frost on the multiplication of* Ps. pisi *in stems of pea plants*

Material and methods. Two experiments were done, the first with four-weeks-old Morse's Progress plants and the second with five-week-old plants of the cultivar Greenfeast. One internode of each plant was injected with a suspension of *Ps. pisi* in sterile, distilled water; injections were made into both axils of each stibule. The concentration of the bacterial suspension was 7.7×10^6 cells/ml. Immediately after the inoculation a number of the plants were put in a dark room, where the temperature was about 0 °C ($-\frac{1}{2}$° to $+1\frac{1}{2}$ °C), and the remainder of the plants were put in a room where the temperature was about -2 °C ($-1\frac{1}{2}$° to -3 °C). The plants were kept in these rooms for $5\frac{1}{2}$ hr. Subsequently they were transferred to the laboratory and the following morning, i.e. after about 18 hr, the relative numbers of bacteria in the inoculated internodes of the two treatments were assessed as follows. Ten (Exp. 1) or eight (Exp. 2) injected internodes of each treatment, each cut to a length of 15 mm, were homogenized for 5 min in 13 ml of sterile, distilled water in a Bühler homogenizer. The homogenates were allowed to stand for $1\frac{1}{4}$ hr with intermittent shaking. Dilution series of the suspensions were prepared and 1 ml of each 1/10 dilution was mixed with 10 ml of molten Bacto Nutrient Agar (Difco) +5% sucrose (pH 6.3) kept in a water bath at 48 °C. The medium was poured into Petri dishes and the numbers of white, domed, mucoid colonies, presumably *Ps. pisi*, were determined; the final countings being made after five days of incubation at 27 °C. Three days after inoculation the relative numbers of bacteria were again assessed according to the same method.

During the time that the plants were kept in the laboratory the temperature varied from 14° to 23 °C in the case of Experiment 1 and from 21° to 28 °C in the case of Experiment 2.

Results. The internodes of the plants that had been exposed to -2 °C were water-soaked when they were taken out of the cold room. The following day these internodes had regained their normal appearance, but the tops of the plants were slightly damaged. The plants that had been exposed to 0 °C did not show any external signs of the effect of the treatment. In Experiment 2, the

TABLE 24. Effect of the exposure of pea plants, cvs. Morse's Progress and Greenfeast, to 0° and −2°C for 5½ hr on the multiplication of *Ps. pisi* in the stems.

Cultivar	Time after inoculation	Relative numbers (in multiples of 10^5 cells) of bacteria in stems exposed to	
		0°C	−2°C
Morse's Progress	1 day	0.25	0.26
	3 days	740	120
Greenfeast	1 day	1.2	0.29
	3 days	810	2300

injected internodes in both treatments showed a beginning of water-soaking three days after inoculation, probably the first blight symptoms. The relative numbers of *Ps. pisi*, one and three days after inoculation, are presented in Table 24.

The differences between the 0° and the −2°C series were relatively small. In the internodes of cv. Morse's Progress the relative numbers of *Ps. pisi* were about equal after one day and somewhat lower in the −2°C series after three days. In the Greenfeast internodes the number was a little lower in the —2°C series after one day, but somewhat higher after three days. Thus in both experiments the effect of frost on the multiplication of *Ps. pisi* was relatively slight.

8.4.2. *Effect of the presence of fluid in the intercellular spaces on the multiplication of* Ps. pisi *and saprophytic bacteria*

Pea plants which have been exposed to frost in the fields early on a winter morning often have a watery appearance, but the water congestion usually disappears later in the morning. If, however, the day temperatures are low and if it freezes again during the night, the plants may remain water-soaked for at least two days and nights. The results of the experiments described under 8.3 indicate that prolonged water congestion could increase the chances of infection. In the following experiments the effect of prolonged water-soaking on the multiplication of *Ps. pisi* bacteria once they have entered the plant is investigated.

Material and methods. Three experiments were done, one with the cultivar Greenfeast and two with Morse's Progress. The plants were grown in a glasshouse and when they were about five weeks old (two weeks old in Exp. 3) they were taken out of the soil and washed in running tap water. Subsequently the leaves were infiltrated under a vacuum of 0.75 kg/cm² with a suspension of *Ps. pisi* in sterile, distilled water. The concentrations of the suspension in Experiment 1 and 2 were 8.0×10^6 and 1.3×10^6 cells/ml respectively. In Experiment 3 the plants were infiltrated with sterile, distilled water. The vacuum was not sufficiently low to infiltrate all the leaves. Then the plants were grown for two days in a laboratory in 250 ml erlemeyer flasks filled with water. Half the number of plants were enclosed in translucent plastic bags in order to

TABLE 25. Relative numbers of *Ps. pisi* and other bacteria (in multiples of 10^2) in pea leaflets, that had been water-soaked for a few hours or for two days, two hours and two days after inoculation.

Cultivar	Concentration of *Ps. pisi* in the inoculum (cells/ml)	Time after infiltration	*Ps. pisi*		Other bacteria	
			Leaflets water-soaked		Leaflets water-soaked	
			for a few hr	for two days	for a few hr	for two days
Greenfeast	8.0×10^6	2 hr	25	62	1.9	1.1
		2 days	160	150	3.0	52
Morse's Progress	1.3×10^6	2 hr	0.7	0.8	1.3	1.7
		2 days	52	14	2.0	130
Morse's Progress	0	2 hr	0	0	16	16
		2 days	0	0	11	1200

check the transpiration, the other half were not enclosed. Two hours as well as two days after the infiltration the relative numbers of bacteria in the leaflets of each treatment were assessed as follows. A circular disc, 16 mm in diameter, was cut out of each of ten leaflets, that had been water-soaked immediately after the infiltration. After the ten discs had been washed in sterile, distilled water, they were homogenized and dilution plates were made as described under 8.4.1. Both the white, domed, mucoid colonies, presumably *Ps. pisi*, and the other colonies, presumably saprophytic bacteria, were counted. The final countings were made after five days' incubation at 27°C.

The temperature in the laboratory during the time of the experiments varied from 16° to 26°C.

Results. The water congestion of the leaflets not enclosed in plastic bags disappeared within a few hours after infiltration, whereas it lasted for at least two days in the leaflets enclosed in plastic bags. The relative numbers of *Ps. pisi* and saprophytic bacteria and actinomycetes two hours as well as two days after inoculation are presented in Table 25.

The results of these experiments hint that *Ps. pisi* multiplied more rapidly in the normal intercellular spaces than in those that were filled with water. Saprophytic bacteria on the other hand did not multiply in the normal intercellular spaces, but rapid multiplication occurred in those that were filled with water.

8.5. INTERCELLULAR FLUID AS A GROWTH MEDIUM FOR PS. PISI

In the experiments described under 8.4.2 the intercellular spaces were infiltrated with distilled water. If, however, water congestion is caused by frost the fluid originates from the cells and might therefore be much richer in nutrients or might contain compounds that stimulate the production of enzymes.

Therefore the growth of *Ps. pisi* and the production of enzymes by the pathogen in intercellular fluid from frosted plants was compared with its growth in intercellular fluid from plants infiltrated with distilled water.

8.5.1. *Multiplication of* Ps. pisi *in intercellular fluids from frosted and non-frosted plants*

The multiplication of *Ps. pisi* was investigated in intercellular fluid obtained (a) from plants that had been infiltrated with distilled water, (b) from water-soaked plants which had been frosted, as well as (c) from water-soaked plants which, after frosting, had been allowed to return to normal in the glasshouse and which were subsequently infiltrated with distilled water. The intercellular fluid was obtained according to a method, which was based on the one described by KLEMENT (1965). The growth in the three above mentioned media was compared with that in diluted nutrient broth. In two of the media the effect of the initial concentration of the pathogen in the intercellular fluid was studied as well.

Material and methods. Fluid from the intercellular spaces of pea plants, cvs. Morse's Progress and Greenfeast, which were grown in a glasshouse, was obtained as follows.

A. Intercellular fluid from infiltrated plants. After the roots and the base had been cut off the plants were infiltrated with distilled water under a vacuum of 0.75 kg/cm^2. Subsequently they were carefully dried with blotting paper and centrifuged for 10 min at 3600 rpm (i.e. about 1200 $\times$ *g*).

B. Intercellular fluid from frosted plants. The plants were exposed for 5 to 6 hr to -2°C, except where otherwise stated. At the end of this treatment the upper three-quarters of the plants were cut off and centrifuged as described under A.

C. Intercellular fluid from plants first frosted and later infiltrated with distilled water. The plants were exposed for 6 hr to temperatures between -1° and -2°C. Then they were returned to the glasshouse and those vines that were water-soaked were marked. The following morning when the plants had lost their water-soaked appearance, those that did not show signs of permanent frost injury were treated as described under A.

The fluid was collected in capped medicine bottles and kept in a solid frozen state. The experiments were conducted with full-grown plants, but pods and flowers were cut off before the treatments.

The intercellular fluid was sterilized by means of a Sartorius filter syringe (filter number SM11307). To each 4 ml of sterile, intercellular fluid 1 ml of a *Ps. pisi* suspension in sterile, distilled water was added. Tubes, each with 4 ml of a 0.1% solution of Bacto Nutrient Broth (Difco), were inoculated in the same way. The tubes with intercellular fluid or Nutrient Broth were incubated in a water bath at 15°C. The concentration of *Ps. pisi* in the inoculum and in the tubes at different times was determined according to the method described under 8.4.1.

The following five series of experiments were carried out:

Series 1: A comparison was made between the growth of *Ps. pisi* in fluid from frosted and from infiltrated pea plants, cv. Morse's Progress.

Series 2: Ditto, but the pathogen was grown in fluids from the cultivar Greenfeast.

Series 3: As series 2, but Greenfeast plants from a different planting were used and the initial concentration of *Ps. pisi* was varied.

Series 4: The growth of *Ps. pisi* in intercellular fluid from infiltrated, frosted plants (Treatment C) was investigated and the initial concentration of the bacteria was varied. The plants in this series were from a different planting than those used in series 2 and 3.

Series 5: The growth of *Ps. pisi* was studied in Nutrient Broth diluted to such a concentration that the growth rate was a little slower than in the intercellular fluids.

Results. The Morse's Progress plants, that had been exposed to frost (Treatment B), were distinctly water-soaked after the treatment and because the temperature dropped for a while to $-3\frac{1}{2}$°C permanent damage would probably have been observed if the plants had been returned to normal growing condi-

TABLE 26. Multiplication of *Ps. pisi* in 0.08% Nutrient Broth and in the intercellular fluid of pea plants obtained after (a) infiltration with distilled water, (b) exposure to frost or (c) after infiltration with distilled water about 18 hr after the plants had been frosted. In series 3 and 4 the initial concentration of the bacteria was varied.

Series of experiments and treatments	Cultivar	Intercellular fluid from plants	Concentration of *Ps. pisi* (cells/ml) after n days of incubation			
			0	1	2	3
1. a	Morse's	infiltrated	6.0×10^1	2.0×10^2	2.6×10^4	2.2×10^6
b	Progress	frosted	6.0×10^1	3.4×10^2	2.1×10^5	1.9×10^7
2. a	Green-	infiltrated	2.8×10^1	$< 1 \times 10^1$*	4.4×10^2	7.0×10^4
b	feast	frosted	2.8×10^1	3.0×10^1	1.6×10^3	5.6×10^5
3. a	Green-	infiltrated	6.6×10^2	2.5×10^4	7.6×10^6	
b	feast	frosted	6.6×10^2	1.4×10^5	1.3×10^7	
a		infiltrated	1.5×10^1	4.7×10^2	1.0×10^5	
b		frosted	1.5×10^1	3.4×10^3	1.2×10^6	
a		infiltrated	1.1×10^1	4.1×10^2	1.3×10^5	
b		frosted	1.1×10^1	1.1×10^3	5.6×10^5	
a		infiltrated	0.3×10^1	4.9×10^2	1.4×10^5	
b		frosted	0.3×10^1	1.1×10^3	4.5×10^5	
4. c	Green-	frosted and	8.4×10^2	3.4×10^4	3.9×10^6	1.8×10^7
c	feast	infiltrated	3.6×10^2	1.5×10^2	2.7×10^4	
c			1.6×10^1	$< 1 \times 10^1$	1.8×10^2	
5.	Nutrient Broth		1.6×10^1	2.2×10^2	1.2×10^4	1.8×10^5

* The number, calculated by extrapolation of the line in Figure 12, is about 3.

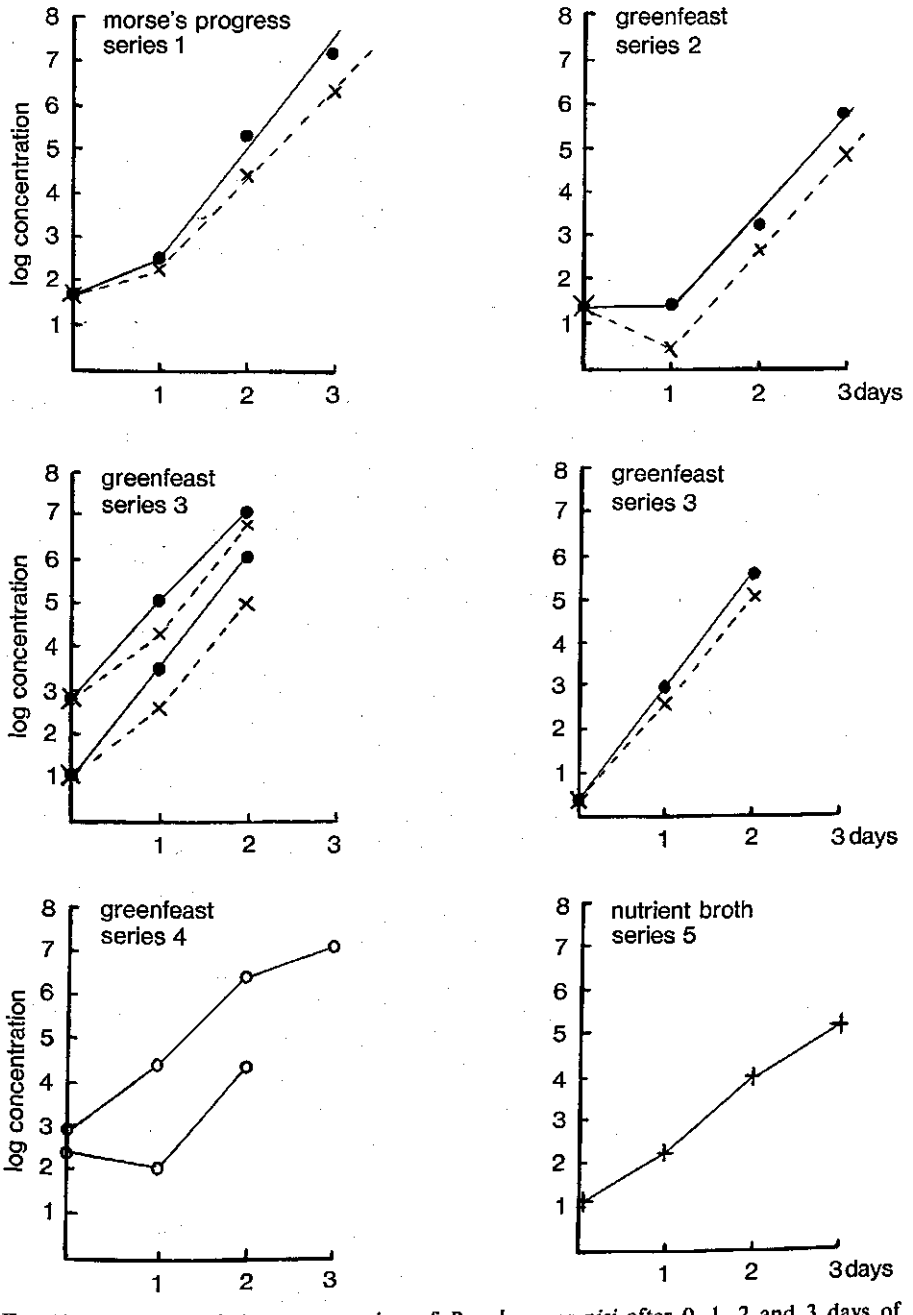

FIG. 12. Logarithm of the concentration of *Pseudomonas pisi* after 0, 1, 2 and 3 days of incubation at 15°C in Nutrient Broth (+ —— +) or in intercellular fluid from pea plants, cvs. Greenfeast and Morse's Progress, obtained either after infiltration with distilled water (×- - -×) or after exposure to frost (●——●) or after infiltration with distilled water 18 hr after the plants had been frosted (○——○). See Table 26.

tions. Most of the frosted Greenfeast plants of this treatment were also water-soaked, but in this case the temperature did not drop below −2°C and all plants would probably have recovered had they been returned to the glass-house.

The intercellular fluid both from Morse's Progress and Greenfeast plants collected after exposure to frost was yellow-green, but turned dark-yellow during storage. A sediment was formed in the liquid. The fluid collected after vacuum infiltration (Treatment A) was almost colourless and had little sediment. Intercellular fluid from Greenfeast plants treated as described under C was very similar to fluid from Greenfeast plants that had been frosted only (Treatment B).

The initial concentrations of the bacteria in the intercellular fluid and in the Nutrient Broth solution, and the concentration after one, two and three days of incubation are presented in Table 26. If no colonies grew on the first dilution plate the concentration is indicated as $< 1 \times 10^1$.

From the results presented in Table 26 and the graphs in Figure 12 the following conclusions were drawn. During the first day of incubation a lag in the multiplication of *Ps. pisi* or even a decrease in the number of bacteria was observed in fluid from plants infiltrated with sterile, distilled water. Considerable differences were found in this respect between series 1, 2 and 3, though in series 2 and 3 fluids from plants of the same cultivar were used. Hardly any lag in growth was found in diluted Nutrient Broth (series 5). If in series 3 the initial concentration was very low, i.e. 3 cells/ml, the growth of the bacteria showed hardly any lag. A more pronounced lag was found in the other three experiments of this series, the degree of lag being about the same. In fluid from frosted plants the lag was less pronounced than in fluid from infiltrated plants; in series 3 there was no lag at all in the fluids from frosted plants. After the lag phase there was little difference between the growth rates in fluids from frosted and from infiltrated plants in all the experiments.

In series 4 the growth in fluid from infiltrated plants which had been frosted the previous day was studied. In these experiments the growth rate during the second day of incubation was independent of the initial concentration and of the same order as in series 1, 2 and 3. During the first day, however, there was a decrease in the number of bacteria if the initial concentration was low, but not if it was relatively high. The slow growth during the third day suggests that the maximum concentration which the medium could support was between 10^7 and 10^8 cells/ml.

8.5.2. *Production of enzymes in intercellular fluids from frosted and non-frosted plants*

Since medium composition is known to affect the synthesis of numerous microbial exo-enzymes (DAVIES, 1963), it was investigated whether intercellular fluid from frosted pea plants was more suitable for the production of proteolytic, pectolytic and cellulolytic enzymes than intercellular fluid from plants infiltrated with distilled water.

Material and methods. Intercellular fluid from frosted and non-frosted (but infiltrated) Greenfeast and Morse's Progress plants was collected and sterilized as described under 8.5.1 (A and B). To each 4 ml of sterile, intercellular fluid 1 ml of a suspension of *Ps. pisi* was added and the tubes were incubated at 27°C in a shaking apparatus for two days. The concentration of the inoculum was about 4×10^7 cells/ml. Two days after inoculation the tubes were centrifuged at 10 000 rpm and the supernatant fluid was kept in a solid frozen state. In the series of tests that were done to detect the presence of proteolytic enzymes a peptone-yeast extract medium (0.5% Bacto Peptone and 0.3% Bacto Yeast Extract in distilled water) was included as well; 4 ml of the medium was inoculated with 1 ml of inoculum. The pH of the media before inoculation was 6 and after incubation 7 or 7½; it was measured with indicator paper (Riedel-de Haën, both 1–11 and 5–9). The presence of pectolytic and cellulolytic enzymes was detected both in intercellular fluid and in KNÖSEL's (1970) culture medium, containing citrus pectin and the sodium salt of carboxy methylcellulose (Na-CMC). Non-inoculated media and intercellular fluids were used as controls.

The presence of proteolytic enzymes was determined according to a slightly modified method of LAWRENCE and SANDERSON (1969). Merthiolate 0.008% was added to the calcium caseinate agar, which was adjusted to a pH of 6.5. The hole in the agar was 5 mm in diameter. The slides with the caseinate agar were incubated for 18 hr; the tests were done in four replicates.

The presence of pectolytic and cellulolytic enzymes was determined according to the method used by KNÖSEL (1970), but in the test mixtures 0.4% sodium polypectate and 0.4% Na-CMC were used respectively and the viscosity was measured in an Ostwald viscosity meter (E-mil B BS/U) at 30°C. The pH values of the test mixtures were adjusted as follows: pH 6.0 for the detection of polygalacturonase (PG), pH 8.5 for pectinlyase (PL) and pH 6.0 for cellulase (Cx).

Results

A. Proteolytic enzymes. In Table 27 the average diameters of the precipitation zones as produced by the different fluids that were tested, are presented.

TABLE 27. Average diameters in mm of precipitation zones produced in caseinate agar by a peptone-yeast extract solution or by intercellular fluids from frosted or non-frosted Morse's Progress and Greenfeast plants, either not inoculated or inoculated with *Ps. pisi*.

Medium	Precipitation zones	
	not inoculated	inoculated with *Ps. pisi*
Intercellular fluid from		
Morse's Progress, unfrosted	13	12
Morse's Progress, frosted	18	17
Greenfeast, unfrosted	11	10
Greenfeast, frosted	10	11
Peptone-Yeast extract	nil	9

Caseinolytic proteases were apparently present in sterile, intercellular fluid. The concentration of these enzymes was higher in fluid from frosted than in fluid from non-frosted Morse's Progress plants, but such a difference was not found in the case of fluid from Greenfeast plants. The concentration was not or hardly affected by the growth of *Ps. pisi* in the fluid. *Ps. pisi* did produce proteolytic enzymes in peptone-yeast extract.

B. Pectolytic and cellulolytic enzymes. The Ostwald viscosity meter could not be used for the detection of PL in inoculated or non-inoculated intercellular fluid from frosted Morse's Progress and Greenfeast plants, because as soon as the intercellular fluid was mixed with the test fluid the mixture solidified. The same happened when non-inoculated intercellular fluid from frosted Morse's Progress plants was mixed with the test fluid for the detection of PG. Because these mixtures remained solid for the duration of the experiment, i.e. for 20 hr, it was assumed that no pectolytic enzymes were present. In most of the tests conducted with the viscosity meter some change of viscosity was found during the course of the experiment, e.g. the test mixtures for the detection of Cx lost in all cases, including the controls, some of their viscosity, but no indication was found of any effect of *Ps. pisi*. It was concluded that *Ps. pisi* does not produce one or more of the above pectolytic and cellulolytic enzymes either in the artificial medium or in the intercellular fluids.

8.6. Discussion

From the results of the experiments described in chapter 4 and from observations in field experiments it was concluded that pea plants become more susceptible to bacterial blight when they are exposed to frost. One of the effects of frost is that water diffuses from the plant cells into the intercellular spaces and as a result the tissue becomes water-soaked on thawing. This is a normal reversible process, but if the temperature is too low or the rate of thawing too rapid the cells may die or rupture and the whole cell content may diffuse into the intercellular spaces (Levitt, 1941, 1956).

In a series of experiments water congestion was induced either by injecting distilled water into stem internodes of pea plants (8.3.1) or by bruising the leaflets (8.3.2). Internodes and leaflets that had been treated in this way became much more susceptible to blight, even though they had not been exposed to frost. Both the water congestion and the increased susceptibility to blight were, to a great extent, reduced within two hours and disappeared within 24 hours. The results are in agreement with those of Johnson (1947), who concluded from elaborate studies on the subject that water congestion predisposes plants to bacterial infection. When Goodman et al. (1967) deal with the concept of bacterial infection, they distinguish between the penetration and the establishment of the pathogen in the plant. Because in the experiments described under 8.3.1 the state of water congestion induced by injecting distilled water soon disappeared, it was concluded that water congestion mainly promotes the

first phase of infection, i.e. the penetration. Adding a spreader to the inoculum resulted in a higher percentage of diseased internodes in the experiments described under 8.3.1. The same was found in three of the four experiments described under 8.3.3. It is a generally accepted theory that surface tension of water inhibits the transfer of liquid water through small natural openings (GOODMAN et al., 1967). Thus the effect of the spreader is probably that it facilitates the penetration of the bacterial suspension into the stomatal openings and that in this way contact is made between the inoculum and the water in the intercellular spaces. In this continuous water film the bacteria are sucked into the plant by capillary forces or they enter the plants by means of their own motility (JOHNSON, 1947).

The effect of frost on the establishment of the pathogen was studied in the experiments described under 8.4 and 8.5. It was found that the growth rate of *Ps. pisi* in pea plants, that had been exposed to frost, did not differ to a great extent from the growth rate in non-frosted plants. Neither did continuous water-soaking of plant tissue have a favourable effect on the multiplication of *Ps. pisi*. Nor was the production of caseinolytic proteases, pectolytic and cellulolytic enzymes stimulated by compounds that had diffused from the cells into the intercellular spaces as a result of frost. Finally, during the period following the first day of incubation, there was little difference between the growth rate of *Ps. pisi* in intercellular fluid from frosted plants and the rate in fluid from plants infiltrated with sterile water. The results of the last mentioned experiments (8.5.1) showed that there were sufficient nutrients in the intercellular fluid of non-frosted plants. This agrees with the findings of KLEMENT (1965), who injected tobacco leaves with water and observed that *Ps. tabaci* grew well in the fluid obtained by centrifuging the injected leaves.

The graphs of Figure 12, however, show that during the first day of incubation (at 15°C) there was a difference between the growth rate of *Ps. pisi* in intercellular fluid from frosted plants and the growth rate in fluid from water-infiltrated, non-frosted plants. Always when *Ps. pisi* was grown in the intercellular fluid from infiltrated, non-frosted plants, but also in some cases when fluid from frosted plants was used, a lag phase was observed even though the fluids were inoculated with bacteria from an actively growing culture. Without exception the lag was more distinct in the intercellular fluid from infiltrated plants than in fluid from frosted plants of the same planting. The fact that in some instances there was not only a lag but even a decrease in the number of bacteria suggests the possible presence of bactericidal compounds in the intercellular spaces. A comparison of the results of the experiments in series 2 and 3 (Table 26; Fig. 12) indicates that great differences may exist between fluids obtained from different plantings of the same cultivar. A detailed study of the growth of *Ps. pisi* in living pea plants during the first day after inoculation was not made. KLEMENT et al. (1964), however, found in some of their experiments that in tobacco plants the numbers of a saprophytic and an incompatible, pathogenic bacterium dropped sharply during the first day of incubation before they increased again (see the non-idealized growth curves in their article). Similar

observations were made by SAALTINK (1963), who studied the development of *Fusarium oxysporum* in resistant and susceptible varieties of *Lupinus luteus* and found that after the roots of susceptible cultivars had been injected with a spore suspension the amount of fungus decreased during the first two days of incubation before rising sharply during the following two days.

If a culture of *Ps. pisi* is transferred to intercellular fluid of pea plants one would expect that some of the bacteria of the population would adapt themselves more rapidly to the new medium than others. Thus the greater the initial number of bacteria the more likely it is that some of them adapt themselves and start multiplying. This would explain the effect of the initial concentration on the degree of lag in series 4 (Table 26, Fig. 12). This effect, however, was not found in the experiments of series 3.

From the above review of the results of the experiments described in this chapter it follows that the effect of frost on infection is primarily that by inducing water congestion of the tissue it facilitates the invasion of the plants by the pathogen, and in the second place, compounds, exuding into the intercellular spaces after the cells have been exposed to frost, favour the establishment of the bacteria.

The results of the experiments described under 8.4.1 are not in agreement with those of WARK (1954), who found that two days after frost the number of bacteria in plants exposed to frost had increased enormously. This was followed by the appearance of typical blight symptoms which covered large areas of stem and leaf tissue and which did not appear on non-frosted plants. Exposure of the plants to high humidity had the same effect as frost on the appearance of symptoms. For his investigations WARK used plants grown from seed known to be contaminated with *Ps. pisi*. It is possible that in these experiments the pathogen was present in the vascular system where it multiplied slowly or not at all, but that after it had moved from the vessels to the intercellular spaces in tissue which was water-soaked either after exposure to frost or to high humidity (JOHNSON, 1947) rapid multiplication occurred.

The results of the experiments described under 8.4.2 show that saprophytic bacteria start multiplying rapidly if the intercellular spaces are filled with water and that their numbers remain constant under normal conditions. The latter is a well-known phenomenon (KLEMENT et al., 1964), but it is not yet fully known why pathogenic bacteria multiply in the host plant and saprophytes do not. The factor which prevents the saprophytes from multiplying is apparently neutralized by water congestion. A reason could be that the bactericidal compounds present in the intercellular spaces of healthy plants are diluted to such an extent that the saprophytes can multiply. It is more likely, however, that saprophytic bacteria are unable to attach themselves to the multiplication sites (ERCOLANI, 1970) in non-flooded intercellular spaces, but that they can use the available nutrients when these are in solution.

The results of the enzymological investigations show that *Ps. pisi* produces caseinolytic proteases. This is in agreement with the result of the litmus milk test (3.3). These enzymes, however, were also found in sterile, intercellular

fluid and it is therefore doubtful whether they play any major part in the virulence of *Ps. pisi*. LANGE and KNÖSEL (1970) also question their importance as a virulence factor, but for different reasons. The strains of *Ps. pisi* used in these investigations produced none of the cellulolytic or pectolytic enzymes that were tested for. Thus the enzymological characteristics of these strains are very similar to those of the virulent B73 strain of *Ps. lachrymans* studied by KEEN et al. (1967). The question arises how can strains of *Ps. pisi*, or *Ps. lachrymans* for that matter, be virulent if they do not produce pectolytic or cellulolytic enzymes. The following is an attempt at an answer. On the curve, which depicts the multiplication of saprophytic and pathogenic bacteria in a plant (KLEMENT et al., 1964) three stages can be distinguished. They can possibly be characterized as follows: During the initial stage it is decisive whether or not the bacteria can attach themselves to the multiplication sites (ERCOLANI, 1970). This is probably dependent on the physico-chemical nature of the surfaces of the bacterium and plant cells. Seeing that the saprophytes cannot attach themselves to these loci, they do not multiply, though they can do so if the intercellular spaces are flooded with water. The pathogenic bacteria, however, do multiply in non-flooded, intercellular spaces and in this stage it is decisive whether they induce a hypersensitivity reaction or not. The HR of tobacco leaves induced by *Ps. pisi* was studied by GOODMAN and PLURAD (1971), who found that i.a. the plasmalemma and the tonoplast were profoundly deranged; particularly the latter appeared to be very sensitive to the presence of HR-inducing bacteria. Evidence from their and other authors' work, which is reviewed in their article, suggests that the denaturant is ammonia, produced in vivo by the bacteria. The pathogens which are congenial to the host plant, however, do not induce an HR, but continue to multiply and during this stage the disease symptoms start developing. Pectolytic and cellulolytic enzymes may play an important part at this stage (LANGE and KNÖSEL, 1970), but are apparently not of essential importance. Bacterial blight of peas is similar to many other diseases caused by *Pseudomonas* species in that the first visible symptom is water congestion of the infected tissue, which must be the result of a disturbance of the selective permeability of the membranes. It was mentioned above that if the host-pathogen relationship is non-congenial, ammonia, a relatively simple compound, can derange the membranes to such an extent that the cells completely collapse. It could therefore be that ammonia or an equally simple compound, produced in vivo by bacteria in a congenial host plant, also affects the membranes, but to a much lesser extent, resulting in a relatively slow exosmosis of the cell fluid. Evidence to support this theory was in fact found by LOVREKOVICH et al. (1969) in their work on the wildfire disease of tobacco caused by *Ps. tabaci*.

SUMMARY

In the beginning of the nineteen fifties bacterial blight caused much damage to pea crops in South Africa, particularly to those grown for seed production. A study has been made of the causal organism and the conditioning factors of the disease, special attention being paid to frost as a predisposing factor.

The symptoms of the disease vary according to weather conditions during and after infection. In dry weather with occasional frost they usually start on the stem near the soil and extend upwards to stipules and leaflets, where a characteristic fan-like pattern develops. In rainy weather they appear as scattered spots on the stems and leaves. The bacterium that causes the disease was identified as *Pseudomonas pisi* Sackett.

Although frost is not essential to infection, the susceptibility of pea plants increased considerably when they were frosted before inoculation. There was no correlation between the increase in susceptibility and the amount of permanent frost injury. In freezing experiments with pods alone, it was confirmed that frost is a predisposing factor for infection, but frost cracks in the tissue were not a prerequisite. A second effect of frost is that the disease spreads more rapidly in tissue that has been frosted. Stem lesions did not spread far on the stems of plants in experimental fields as long as the temperature remained above freezing point. After a frost, however, the disease spread for a considerable distance on the stems and to the stipules, on which the typical fan-like symptoms developed. These results were confirmed in experiments with artificially frosted plants. A possible explanation is that the pathogen spreads in the temporarily enlarged, water-filled intercellular spaces of the stems and leaves that have been exposed to frost.

Because frost is a predisposing factor and because the optimum temperature for the development of symptoms is relatively high (about 25°–30°C), the winter climate in the seed producing areas of South Africa where frost in the early winter morning is followed by high day temperatures, is very conducive to the disease.

The time of planting had a considerable effect on the occurrence of the disease; it was much more severe in crops planted in April than in those planted in the second half of May or later. The difference in susceptibility between crops planted at different times is probably caused by the fact that early planted peas are more sensitive to frost than those that are planted later. Furthermore it was found in glasshouse experiments that pea plants that were grown at high temperatures were more susceptible to blight than those grown at low temperatures. The differences in susceptibility were correlated with the stomatal frequency. These results may also partially explain the differences in susceptibility between early and late plantings. Varietal resistance investigated in cultivar trials was not consistent over the years. Pea plants suffering from lack of water were more sensitive to frost than plants that were regularly irrigated, but

not much difference in the occurrence of blight was found between peas grown at different irrigation levels.

Bacterial blight is a seed-borne disease and the pathogen remains viable in infected seed from one season to the next. Only if moderate frost occurs when the seeds are swelling in the pods and if there is a source of inoculum nearby, the pods and seeds may become infected in normal dry winters. Because the South African isolates of the pathogen do not easily move in the wood vessels, it is unlikely that seeds from apparently healthy pods will be infected, even if they are borne on diseased plants.

In South Africa no other natural host plant for *Ps. pisi* was found. In a pea field the spread of the disease was erratic, no obvious connection was found between new and already existing infections.

The following preventive measures are recommended: (a) plant at the right time of the year, (b) avoid frost pockets and (c) plant government certified seed.

Experiments were done to investigate how frost renders the plants more susceptible to infection. One of the effects of frost is that the plant tissue becomes water-soaked on thawing. Water-soaking, induced either by injecting the stems with sterile water or by bruising the leaflets, rendered pea plants considerably more susceptible to infection, probably because the pathogen penetrated the water-soaked tissue more easily. In experiments to study the effect of frost on the establishment of the pathogen it was found that the growth rate of *Ps. pisi* in pea plants, that had been exposed to frost, did not differ much from the growth rate in non-frosted plants. Neither did prolonged water-soaking of plant tissue have a favourable effect on the multiplication of *Ps. pisi*. Saprophytes did, however, multiply rapidly in water-soaked leaves while they remained stationary in normal plant tissue. During the first day of incubation the growth of *Ps. pisi* in intercellular fluid from non-frosted pea plants, which had been infiltrated with distilled water, showed a lag phase, even though the fluid was inoculated with actively dividing bacteria. In one case a decrease in the concentration of *Ps. pisi* was observed. In intercellular fluid from plants which had been frosted, the lag was less distinct than in fluid from infiltrated plants of the same planting, or it was entirely absent. After the lag phase little difference was observed between the growth rate of *Ps. pisi* in intercellular fluid from plants that had been frosted and that in intercellular fluid from infiltrated, non-frosted plants.

The results described above show that the effect of frost on infection is primarily, that by inducing water congestion of the tissue it facilitates the invasion of the plants by the pathogen; and in the second place compounds, exuding into the intercellular spaces from cells which have been exposed to frost, favour the establishment of the bacteria.

The *Ps. pisi* strain tested did not produce polygalacturonase, pectinlyase or the cellulolytic Cx enzyme in artificial media or in intercellular fluid. *Ps. pisi* did, however, produce caseinolytic enzymes, but it is doubtful whether they are important as a virulence factor, because they were also found in sterile intercellular fluid.

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SAMENVATTING

De bacterieziekte van de erwt deed in het begin van de vijftiger jaren veel schade in Zuid Afrika, vooral aan gewassen die verbouwd werden met het doel om zaad te winnen. Een studie werd gemaakt van de bacterie die de ziekte veroorzaakt en van de omstandigheden die de ziekte beïnvloeden, waarbij vooral aandacht werd geschonken aan de invloed van vorst als een predisponerende factor.

De symptomen van de ziekte zijn afhankelijk van de weersomstandigheden gedurende en na de infectie. Als het weer droog en helder is en er af en toe nachtvorst voorkomt, beginnen de symptomen gewoonlijk op de stengel dichtbij de grond en breiden naar boven uit naar de steunbladen en blaadjes, waarop een karakteristiek waaiervormig patroon ontstaat. Bij regenachtig weer daarentegen ontstaan verspreide vlekken op de stengels en bladen. De bacterie die de ziekte veroorzaakt, werd geïdentificeerd als *Pseudomonas pisi* Sackett.

Hoewel het niet een absolute voorwaarde voor infectie was, nam de vatbaarheid van erwten wel aanzienlijk toe, wanneer de planten voor de inoculatie aan vorst blootgesteld werden. Geen verband werd gevonden tussen de toename in vatbaarheid en de mate van permanente vorstschade. De invloed van vorst als een predisponerende factor voor infectie kon bevestigd worden in proeven met peulen, maar de aanwezigheid van door vorst veroorzaakte scheurtjes in het weefsel was geen voorwaarde voor het slagen van de infectie. De invloed van vorst kwam ook hierin uit, dat de ziekte zich sneller in weefsel, dat bevroren was geweest, uitbreidde. In veldproeven bleek n.l. dat stengelvlekken niet veel groter werden zolang de temperatuur boven het vriespunt bleef. Onder invloed van vorst echter breidde de ziekte zich over een aanzienlijke afstand uit zowel op de stengel als naar de steunbladen, waarop de typische waaiervormige symptomen ontstonden. De resultaten van de veldproeven konden bevestigd worden in proeven met kunstmatig bevroren planten. De verklaring van het verschijnsel is waarschijnlijk dat de bacteriën zich verspreiden in de tijdelijk vergrote, met water gevulde intercellulaire ruimten van planten die aan vorst blootgesteld zijn geweest.

Omdat vorst een predisponerende factor is en omdat de optimum temperatuur voor de ontwikkeling van de symptomen betrekkelijk hoog is (ongeveer 25° tot 30°C), is het winterklimaat in die gebieden van Zuid Afrika, waar erwten voor zaad verbouwd worden, en dat gekenmerkt wordt door vorst in de vroege morgenuren gevolgd door hoge temperatuur gedurende de dag, bijzonder gunstig voor de ziekte.

De zaaitijd had een grote invloed op het voorkomen van de ziekte, die n.l. veel ernstiger was in gewassen welke in april dan in die, welke in de tweede helft van mei of later gezaaid waren. Dit verschil in vatbaarheid tussen de zaaitijden wordt waarschijnlijk veroorzaakt doordat vroeg gezaaide erwten meer gevoelig voor vorst zijn dan laat gezaaide. Bovendien werd gevonden dat erwte-

planten, die bij hoge temperaturen in de kas gekweekt waren, meer vatbaar waren voor de bacterieziekte dan die welke bij lage temperaturen opgroeiden. Deze verschillen in vatbaarheid waren gecorreleerd met de stomata-frequentie. De resultaten van de kasproeven verklaren misschien ook gedeeltelijk het verschil in vatbaarheid tussen vroeg en laat gezaaide erwten. In veldproeven waargenomen resistentieverschillen tussen erwtecultivars waren van jaar tot jaar niet gelijkblijvend. Erwten die leden aan een tekort aan water, waren meer gevoelig voor vorst dan erwten die regelmatig bevloeid werden, maar de invloed van de frequentie van bevloeiing op het voorkomen van de bacterieziekte was gering.

Pseudomonas pisi infecteert ook het zaad en de bacterie kan daarin van het ene seizoen tot het andere in leven blijven. Peulen en zaden worden in normale droge winters echter alleen geïnfecteerd wanneer in het stadium dat de zaden zwellen er matige vorst is en er bovendien een bron van infectie dichtbij is. Omdat de Zuid-Afrikaanse isolaten van de pathogeen zich blijkbaar niet gemakkelijk in de houtvaten verplaatsen is het onwaarschijnlijk dat zaden in uiterlijk gezonde peulen van overigens zieke planten, geïnfecteerd zullen zijn.

In Zuid Afrika werden geen andere waardplanten voor *Ps. pisi* gevonden dan erwten. De ziekte breidde zich in het gewas op zo'n wijze uit dat er geen duidelijk verband te bespeuren viel tussen nieuwe infecties en reeds eerder aangetaste planten.

De volgende maatregelen ter voorkoming van de ziekte worden aanbevolen: (a) zaai op de daartoe geschikte tijd van het jaar, (b) vermijd laaggelegen percelen waar vorst vaker voorkomt en zwaarder is dan elders, en (c) zaai alleen zaad dat door de betreffende regeringsinstantie gecertificeerd is.

Proeven werden gedaan om te onderzoeken hoe erwteplanten door blootstelling aan vorst meer vatbaar worden voor infectie door *Ps. pisi*. De invloed van vorst op planten komt o.a. hierin tot uiting dat het weefsel na ontdooiing glazig wordt. Wanneer nu stengels van erwteplanten ingespoten werden met gedistilleerd water of de blaadjes gekneusd werden en het weefsel zodoende waterig werd, nam de vatbaarheid aanzienlijk toe, blijkbaar omdat de pathogeen gemakkelijker kon binnendringen. In proeven die gedaan werden om de vestiging van de pathogeen in het weefsel te bestuderen, werd gevonden dat de groeisnelheid van *Ps. pisi* in erwteplanten die aan vorst blootgesteld waren geweest niet veel verschilde van die in niet bevroren planten. Ook had een enkele dagen durende toestand van waterigheid van het weefsel geen gunstige invloed op de vermeerdering van *Ps. pisi*. Saprophyten daarentegen, die zich niet of nauwelijks vermeerderden in normaal bladweefsel, vermeerderden zich snel indien het blad met water geïnfiltreerd was.

De groei van *Ps. pisi* in intercellulaire vloeistof verkregen uit erwteplanten, die met gedistilleerd water geïnfiltreerd waren, vertoonde gedurende de eerste incubatiedag een 'lag'-phase, hoewel de vloeistof met actief delende bacteriën geënt was. In een geval werd een daling in de concentratie van *Ps. pisi* waargenomen. In intercellulaire vloeistof afkomstig van planten, die bevroren waren geweest, was deze vertraging in groei minder geprononceerd dan in vloeistof

van met water geïnfiltreerde planten, die terzelfdertijd gezaaid waren, of de 'lag'-phase was geheel afwezig. Na deze phase bestond er weinig verschil tussen de groeisnelheid van *Ps. pisi* in intercellulaire vloeistof van bevroren planten en die in intercellulaire vloeistof van met water geïnfiltreerde, niet bevroren planten.

Uit het bovenstaande volgt dat de invloed van vorst op infectie in de eerste plaats hieruit bestaat, dat de bacterie de plant gemakkelijker kan binnendringen, omdat het weefsel na blootstelling aan vorst, waterig wordt. In de tweede plaats zijn stoffen, die vanuit cellen, welke aan vorst blootgesteld zijn geweest, in de intercellulaire ruimten diffunderen, gunstig voor de vestiging van de bacterie.

De stam van *Ps. pisi* die onderzocht werd, produceerde geen polygalacturonase, pectinelyase of het cellulolytische Cx-enzym in kunstmatige media of in intercellulaire vloeistoffen. De stam produceerde wel caseïnolytische proteasen, maar omdat deze ook in steriele, intercellulaire vloeistof gevonden werden, is het te betwijfelen of ze voor de virulentie van belang zijn.

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