***Phytophthora porri* in leek: epidemiology and resistance**

Phytophthora porri in prei: epidemiologie en resistentie

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Bibliographic abstract

In winter, *Phytophthora porri* is an important pathogen of leek (*Allium porrum* L.) in the Netherlands. The fungus survives the crop-free period in summer by oospores in soil, and infects the leaves in autumn. Field studies indicated that dispersal by rain splash is crucial for initiation of an epidemic. Disease foci expanded at a rate of ca. 3 $cm.d^{-1}$ in the first month after artificial inoculation. At temperatures below 5°C the fungus is still able to grow in leaf tissue. Temperatures above 45°C are detrimental to all fungal structures, including oospores. Cultivars differed in partial resistance, both in naturally inoculated field tests and in zoospore-inoculated glasshouse tests. Within cultivars, a broad variation range for partial resistance was demonstrated by means of clone tests. High levels of partial resistance were observed in several landraces. Two landraces were crossed and backcrossed to cultivars.

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Stellingen

1. Prei-selekties van het ras 'Blauwgroene Winter' bevatten eenvoudig selekteerbare partiële resistentie tegen papiervlekkenziekte. Bepaalde typen zomerprei, o.a. selekties van het ras 'Bulgaarse Reuzen' en landrassen uit voormalig Joegoslavië en Egypte, bevatten een hogere mate van deze ziekteresistentie die ingekruist kan worden in winterprei.
   Dit proefschrift

2. Bladafval met papiervlekkenziekte is, op tuinbouwgrond, een biologische tijdbom.
   Dit proefschrift

3. De Engelse benaming van papiervlekkenziekte (white tip disease) is misleidend.
   Dit proefschrift

4. *Phytophthora porri* op *Brassica* spp. verdient op grond van moleculair-genetisch onderzoek een nieuwe soortsnaam.
   De Cock, A.W.A.M., A. Neuvel, G. Bahnweg, J.C.J.M. de Cock & H.H. Prell, 1992. Netherlands Journal of Plant Pathology 98: 85-105.

5. De hogere opbrengst van prei-hybriden kan worden toegeschreven aan het ontbreken van inteeltnakomelingen in de hybride-populatie.
   Schweisguth, B., 1970. Annales de l'Amélioration des Plantes 20: 215-231
   Kampe, F., 1980. Zeitschrift für Züchtungsforschung 10: 123-131

6. De sterke inteeltdepressie bij autotetraploïden kan mogelijk worden verklaard door interactie tussen drie of vier allelen van één gen, of door interactie tussen allelen van drie of vier gekoppelde genen in afstotingsfase. Bewezen is nog niets.
   Bingham, E.T., R.W. Groose, D.R. Woodfield & K.K. Kidwell, 1995. Crop Science 34: 823-829

7. Bij prei is het grootste deel van de genetische informatie waarschijnlijk verdeeld over 16 groepen sterk gekoppelde genen die ver van het centromeer af liggen.
   Gohil, R.N., 1984. Eucarpia 3rd Allium Symposium: 99-105
   Stack, S.M., 1994. American Journal of Botany 80, Supplement: 78-79

8. De neergang van het preigeelstreepvirus in de jaren tachtig is waarschijnlijk een gevolg van resistentie, en kan zeker niet worden toegeschreven aan de invoering van een gewasvrije periode in de jaarcyclus van de intensieve preiteelt.
   Brewster, J.L., 1994. Onions and other Vegetable Alliums. CAB International, Wallingford

9. Overgevoeligheidsresistentie voor necrotrofe pathogenen kan niet veroorzaakt worden door lokale necrose.
   Prusky D., 1988. In: W.M. Hess *et al.*, Experimental and Conceptual Plant Pathology, Volume 3, Gordon & Breach, New York: 496

10. De eerste bewegingswet van Newton is een bijzonder geval van de tweede.

11. Als de publicatie van het Communistisch Manifest (1848) en het ontstaan van het voetbalspel (ca. 1850) een gemeenschappelijke oorzaak zouden hebben, zou na de val van de Berlijnse Muur het voetballen overal moeten instorten zoals in Wageningen.
    Van den Bergh, J.H., 1963. Leven in Meervoud. Callenbach, Nijkerk

12. De gedachte dat een toename van het electronisch verkeer vanzelf leidt tot een afname van het auto- en vliegverkeer getuigt van een naïef optimisme.
    Toffler, A., 1980. The Third Wave. Bantam Books, New York

13. Als 'missie' en 'taakstelling' van een organisatie botsen, is de bewegingsrichting onvoorspelbaar.

14. Een stadsprovincie is geen stad en geen provincie, en verstoort de balans tussen stedelijke en andere landsbelangen.

15. Zolang de Europese Unie onvoltooid is, is de kans op een gewapend conflict binnen de Unie klein. Daarom is verbreding van de Europese samenwerking urgenter dan verdieping.

16. Spellinghervormers mogen geen financiële relatie hebben met een uitgever van woordenboeken; ze kunnen niet conservatief genoeg zijn.

Stellingen, behorend bij het proefschrift

'*Phytophthora porri* in leek: Epidemiology and resistance'

door W.D. Smilde

Wageningen, 22 maart 1996

'There is no doubt but many vegetables and animals have qualities that might be of great use, to the knowledge of which there is not required much force of penetration, or fatigue of study, but only frequent experiments, and close attention.'

Samuel Johnson. On Spring. Rambler 5, April 3, 1750

'We feel that the more one knows about a plant, the more interesting it becomes, and that work with it provides something beyond the purely utilitarian.'

H.A. Jones & L.K. Mann. Onions and their Allies, xv, 1963

Woord vooraf

Dit proefschrift is het resultaat van vijf jaar onderzoek. Het meeste werk werd verricht in een zeer klein en zeer hecht team, bestaande uit mijzelf en mijn assistent, Marcel van Nes. Alle voordelen van teamvorming zijn mij dankzij Marcel duidelijk geworden. Zijn zelfstandige, zeer betrouwbare manier van werken zijn mij goed bevallen. Bijzonder nuttig was de stugheid waarmee hij onuitvoerbare proefvoorstellen benaderde. Zijn stelling 'Prei kan een hoop hebben' kreeg in de loop der jaren door herhaling een steeds diepzinniger inhoud. Vanuit die stelling ontstond het idee om de prei voor het selekteren domweg in besmet water onder te dopen. Achteraf lijkt zoiets simpel. Toch doet men dat niet snel. Onvergetelijk waren verder het bokje en de roodmus. Marcel, bedankt!

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Ik hoop dat mijn proefschrift gelezen zal worden door onderzoekers die om wat voor reden dan ook geïnteresseerd zijn in prei, door preiveredelaars die uit zijn op commerciële successen, en door voorlichters die voor de moeilijke taak staan om met een minimum aan betrouwbare gegevens en technieken een doeltreffend teeltadvies te geven aan preitelers met percelen die besmet zijn met *Phytophthora porri*.

Diederik Smilde,
Wageningen,
24 November 1995.

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Chapter 1

General introduction

1.1 Leek

1.1.1 Origin and classification

Leek (*Allium porrum* L.) probably originates from *A. ampeloprasum* L., a diverse species which is found in the Mediterranean region and the Near East (Van der Meer & Hanelt, 1990). The centre of origin of *A. ampeloprasum* is probably located in the eastern Mediterranean region, where the species occurs on coastal cliffs, in ravines and garigues (Von Bothmer, 1974; Bonnet, 1976). One taxonomic opinion is that *A. ampeloprasum* comprises both the wild species and at least four vegetable groups: leek, kurrat, great-headed garlic and pearl onion (Hanelt, 1990). In this view the correct botanic name of leek is *A. ampeloprasum* L. var. *porrum* (L.) Gay. This view is followed by Jones & Mann (1953), Siemonsma & Piluek (1993), Pink (1994) and Havey (1995). Nevertheless, Stearn (1978) maintains that 'the cultivated leek *A. porrum* L. is a cultigen probably derived from *A. ampeloprasum* L. but distinct enough now (...) to be kept apart'. Stearn's view is supported in the list of stabilized plant names (ISTA, 1988) and hence in all national seed lists. *A. porrum* is also accepted in the latest monography of *Allium* sect. *Allium* by Mathew (in press). In the present work we use therefore *A. porrum* for cultivated leek.

A. ampeloprasum belongs to the *ampeloprasum*-complex within the section *Allium* of the genus *Allium* (Family Alliaceae). The *ampeloprasum*-complex comprises among others *A. bourgeaui* Rech fil., *A. commutatum* Guss. and *A. atroviolaceum* Boiss. The species within the *ampeloprasum* complex are probably interfertile (Von Bothmer, 1974). Leek and kurrat were crossed by Van der Meer & van Dam (1982), and leek and *A. ampeloprasum* var. *lussinense* by Wietsma & de Vries (1992). Recently, a vigorous, fertile hybrid of leek and *A. commutatum* was reported by Verbeek *et al.* (1995).

A. ampeloprasum represents a polyploid series (x = 8) with tetraploids (2n = 4x = 32), pentaploids (2n = 5x = 40) and hexaploids (2n = 6x = 48). Rare diploid variants occur in the endemics *A. ampeloprasum* subsp. *truncatum* (Kollman, 1972) and *A. ampeloprasum* var. *lussinense* (unpublished data). Tetraploids are most common. Leek is probably an autotetraploid, but the evidence for the occurrence of tetrasomic inheritance is weak, because only few genes have been studied (Berninger & Buret, 1967; Potz, 1987). Chiasmata are typically localized near the centromere, thus preventing the formation of tetravalents (Stack, 1993).

Most wild forms are biennial and form bulbs to survive the seasonal dry period of the Mediterranean climate. Often, numerous bulblets are produced around the parent bulb. The cultivated leek is a biennial (winter leek) or annual (summer leek) in which bulb formation and shoot splicing are almost wholly suppressed. Biennial leek usually requires vernalization before flowering. Bolting is promoted by short days during and long days after vernalization (Wiebe, 1994). There are also perennial leek types (Van der Meer & Hanelt, 1990).

1.1.2 Early history

Leek has been grown as a vegetable in the Middle East from Egypt to India since ancient times. The oldest records of leek stem from Egypt. Already before 2000 B.C. leeks were probably used to pay the wages of pyramid builders, together with onions and garlic (Hehn, 1911; Täckholm & Drar, 1954; Van Beekom, 1952). In the Bible 'leeks' are mentioned as an Egyptian vegetable (Numeri 11 : 5). In classical times, Theophrastus (ca. 327-ca. 287 B.C.) mentions different *Allium* crops, some of which must have resembled leek, which was apparently widely grown in Greece and the Near East. Galen (131-200 A.D.) mentions four *Allium* crops (Heyser, 1928), among which *porrum* and *ampeloprasum*, which names he used in roughly the same sense as Linnaeus would do later, viz. *porrum* designating the cultivated vegetable and *ampeloprasum* the wild species, called after the vineyards (vine = Gr. ampelos) in which they apparently grew well.

The crop was probably imported to Western Europe in Roman times. The patron saint of Wales, St. David (ca. 600 A.D.), is associated with leek, and leek is still used as the national emblem of Wales. The origin of this tradition seems untraceable (Davies, 1992). In the Middle Ages leek was mentioned in several European herbals and were probably grown in monastic gardens from Bulgaria to Ireland. Local landraces, adapted to local climates and market demands, were developed in many European countries. Although leek from Egypt and southern Europe cannot survive severe frost, several European landraces are winter hardy. The most winter hardy landraces are 'Winterreuzen' and 'Blauwgroene Winter' which have been important sources for modern Dutch cultivars.

1.1.3 Economic significance

Leek is an important vegetable in Europe. The total leek area in the EC was 27,000 ha in 1989 (Eurostat). Outside Europe the significance of leek is limited and data are scarce. The crop is important in Turkey (Astley, 1982). Currah & Proctor (1990) found that leek

Table 1.1 Leek production (area, weight and value) and export (weight) in the Netherlands, 1975-1995. Sources: CBS for areas, CBT for production and export '35-'70, PGF for production and export '75-'95.

Year	Production			Export
	Area 10^3 ha	Weight 10^6 kg	Value 10^6 Dfl	Weight 10^6 kg
1935		7	0.2	
1950	0.8	12	4	6
1960	1.2	24	21	6
1970	1.3	28	11	5
1975	1.3	48	22	7
1980	2.0	51	38	7
1985	2.5	58	68	12
1990	2.9	94	95	40
1991	3.6	99	117	46
1992	4.1	114	93	56
1993	3.9	106	105	53
1994	4.3			
1995	3.9			

is grown on a commercial scale in at least 16 countries in tropical and subtropical regions, among which Egypt, India and Indonesia. In the USA, Canada and Australia the crop is grown on a small scale (Maynard, 1988; Sward, 1991).

The Netherlands are the world's third producer of leek, in 1991 only surpassed by France (9,000 ha) and Belgium (4,800 ha), and followed by the United Kingdom (3,300 ha) and Germany (1,500 ha).

In the Netherlands, the leek area increased over 1975-1995 from 1,300 to about 4,000 ha. In the same period, the market production of leek increased from 22.10^6 to almost 100.10^6 kg per year, and the total sales increased from Dfl 60 to 120 million (US$ 40 to 80 million) per year (Table 1.1). Leek is widely grown in home gardens. Almost 50% of the Dutch leek production is exported, mainly to Germany, while only 5% of the consumption is imported. The Dutch seed industry has the largest share of the world market for leek seed.

The Dutch leek production is concentrated in the southern part of the country (Fig. 1.1). Before 1940, leek was a relatively unimportant crop on clay soils in the western part of the country, near the cities (Kemmers *et al.*, 1952). After 1945, the crop was mainly grown in the south of the country on sandy soils, which require less labour during

harvest. Leek is one of the few winter crops with high labour requirements and profit margins, and is therefore an indispensable source of income for many farmers, who tend to grow the crop in very narrow rotation schemes. The intensity of leek cropping may be estimated for each Dutch municipality from the ratio of the leek area and the area under field grown vegetables (CBS, 1994). For 10% of the leek area this ratio is above 0.5, suggesting that ca. 400 ha of the Dutch leeks are grown on the same field at least every second year. In many places continuous cropping is practised.

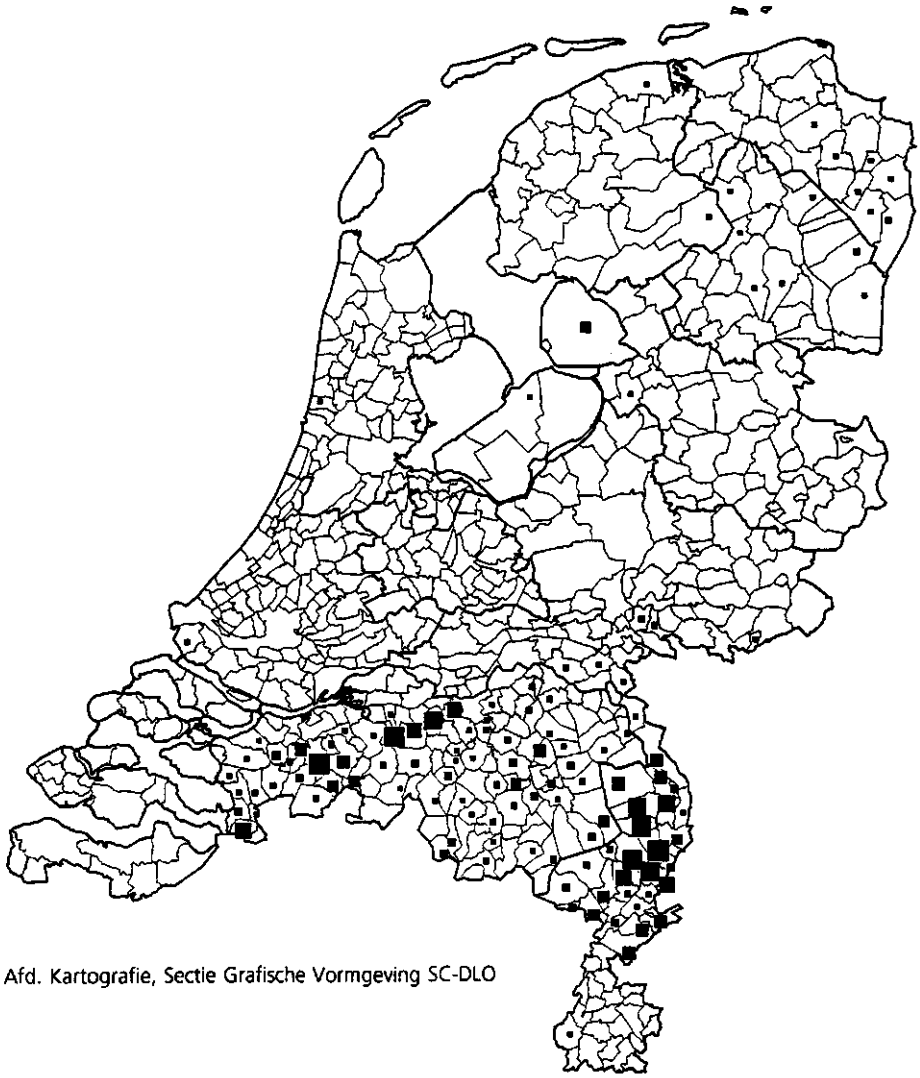

Figure 1.1 Geographic distribution of 4000 ha commercial leek in the Netherlands, 1994 (CBS). The areas of the black squares are proportional to the measured leek areas. The smallest squares correspond with 5-15 ha, the largest with 165-175 ha.

Table 1.2 Characteristics of leek cropping seasons for direct marketing. Recommended varieties, percentages of the total production, expected yields, and average prices are given for each crop type.

Cropping season	Variety[a]	Harvest[b] Month	Yield[c] % Weight	Yield 10^3 kg.ha^{-1}	Price[d] Dfl.kg^{-1} avg. (min - max)
Very Early Summer[e]	-	6	3	25	-
Early Summer	HR	7	5	35	1.05 (0.88-1.09)
Summer	HR	8	7	40	0.89 (0.57-1.10)
Early Autumn	BH	9-10	19	40	0.76 (0.57-1.05)
Late Autumn	BH	11-12	19	35	0.83 (0.65-1.02)
Winter	BW	1-2-3	30	28	1.18 (0.91-1.74)
Late Winter	BW	4-5	17	35	1.06 (0.78-1.38)

[a]recommended by CRG (1995). HR = Herfstreuzen, BH = Blauwgroene Herfst, BW = Blauwgroene Winter
[b]1 = January, 2 = February, etc.
[c]auctioned leek weight averaged over 1988-1992
[d]auctioned leek in 1988-1993, avg = middle-price averaged over weeks and years, min = lowest middle price averaged over weeks, max = highest middle price averaged over weeks (Balk-Spruit & Spigt, 1995)
[e]Van der Meer & Hanelt (1990)

1.1.4 Utilization and marketing

The blanched pseudo-stem of leek has a softly pungent taste which adds flavour to soups, salads, and Italian and oriental dishes. Consumers desire a long white shaft and dark green leaves, and dislike soil in the shafts. Bolting and (even slight) bulbing of plants are undesirable.

Leeks are harvested all year round (Table 1.2). Only in June there is a technical constraint for production, as at that time the winter leeks have bolted and the summer leeks are still too small. In the rest of the year the production is determined mainly by market forces.

The market requirements for leeks may vary greatly over seasons and among countries. Summer leeks are generally harvested very young. They are valued for their leaves, which should be tender. Autumn and winter leeks are valued for their shafts and the leaves are less important. In the UK the leaves are even cut off, but in other countries the leaves are important for market presentation of the crop, although they are not always eaten. On the Spanish market the leeks should be relatively thin, while the Japanese market requires very thick pseudo-stems. Swiss and Bulgarian landraces have long stems and a light leaf colour. Such types are preferred by the public in these countries.

In the Netherlands, most leeks are produced for the fresh market, and are marketed through auction houses. About 10% of the leeks are supplied directly to supermarkets, and another 10% is used for industrial purposes, mainly in dried and tinned soups. The auction prices vary from Dfl. 0.76 for early autumn to Dfl. 1.18 per kg in winter (Table 1.2). During prolonged periods of severe frost the prices may rise sharply due to a reduced supply.

Leeks are auctioned in three quality classes. The criteria for classification may vary somewhat, but as a rule the Class I leeks should be almost totally free of diseases and soil, and should be evenly trimmed (De Kraker & Bosch, 1993). Class I leeks get a 10-30% higher price than Class II, and double the price of Class III. Growers generally aim for the production of Class I, which often involves high investments in crop protection and labour. About 50% of the auctioned leeks are Class I (Spaans, 1992). Manual trimming of plants is particularly important for Class I when leaf spot diseases are present, and may require about 25% of the production costs (Van der Spank, 1995).

1.5 Cropping methods

Commercial leek is grown in various ways (De Kraker & Bosch, 1993). Usually, leek is sown at high density in seed beds by specialized companies, who sell the plants to the grower after ca. 12 weeks when they have 3-6 leaves. In the Netherlands, leeks are seldomly sown in the production field, but in the UK direct drilling is quite common. Direct sowing has several disadvantages: there will be more plants which express genetic deficiencies in the production field, less opportunity for weed control, and the plants must be 'earthened up' to produce long white shafts. Leek transplants are planted into 12-15 cm deep planting holes, often at 12 x 50 cm distance.

Leek is harvested 3-10 months after transplanting. The plants are sometimes stored at -1°C for 1-2 months after harvesting to obtain a better price. In the last decade, the labour requirements for leek harvesting were reduced through mechanization. Share-lifters (Du: klembandrooiers) are now used to cut the roots and lift the plants from the field. The cost of decrease in product quality by mechanical damage is small compared with the benefit of higher labour productivity. Washing of leeks is more or less automated, but manual trimming is still necessary.

Mechanization leads to further specialization, and an increase in scale of leek growing farms. For small farmers, the crop rotation schemes become very narrow. At the same time, it becomes attractive for larger farms to grow leeks. It may be expected that an increase in scale will take the form of new cropping regions competing with traditional cropping regions.

Trimming and washing of leeks may create environmental problems. Dutch leek farmers have to abide by laws and regulations for the disposal of leaf debris (Edel *et al.*, 1994; Van de Pol, 1995). The common practice of returning leaf debris to the land is

forbidden, although it is allowed to leave leaf debris behind when the leek is trimmed on the land. It is forbidden to make piles of leaf debris on or in the soil, as this will lead to nitrogen leakage to the subsoil. Therefore, the leaf debris should either be transported to special depots, or the leeks should be cleaned on the land.

The disposal of washing water is also regulated. Farmers have to pay for a licence to wash their leeks, related to the amount of pollution. In this way the authorities hope to minimize the pollution of surface waters.

1.1.6 Diseases, pests and disorders

Leeks may be affected by a number of disorders and parasitic organisms. The scope of the present study is limited to only one disease, *Phytophthora porri* or white tip disease, which is the most important disease of Dutch leek crops in the winter season. The names of a number of important pests, diseases and non-parasitic disorders are listed in Table 1.3. The list is based on reviews of onion pests and diseases, which frequently also treat some leek pests and diseases (Rabinowitch & Brewster, 1990; Snowdon, 1991; Brewster, 1994; Schwarz & Mohan, 1995; Howard *et al.*, 1995), listings of leek diseases (Bonnet, 1976; Crüger, 1983; Grill, 1985; Snoek & de Jonge, 1985; Forrat, 1988; Van der Meer & Hanelt, 1990; De Kraker & Bosch, 1993; Messiaen *et al.*, 1993), and a taxonomic check list (Boerema *et al.*, 1993). There are no reliable data on the relative importance of leek pests and diseases in terms of crop loss. For each disease in Table 1.3 the present author gives a rough, subjective estimation of its importance in the Netherlands, based on information collected over the years 1990-1995 from extension workers and researchers at experimental stations. Some extra information about various points of interest is given in the text below.

Onion thrips (*Thrips tabaci*) was probably the most important problem of leek in the Netherlands in 1990-1995. Severe epidemics developed during the summer seasons. The insects often multiplied in spite of frequent attempts at chemical control. Onion fly (*Delia antiqua*) and leek moth (*Acrolepiopsis assectella*) were also a hazard, but were controlled relatively easy with insecticides.

Leek rust (*Puccinia porri*) was second in importance. Severe epidemics of leek rust occurred every year. Intensive chemical control was not always effective when the disease came in early.

White tip disease (*Phytophthora porri*) was the most important disease during winter. Severe losses were reported from the south of the Netherlands where leek is grown intensively, but the disease was also found in other areas, on farms and sometimes in home gardens. Although the disease was widespread, it was not present in all leek fields, even within leek cropping areas.

Table 1.3 Diseases, pests and disorders of leek, and their importance in the Netherlands. The entries are grouped together with 1. fungal leaf pathogens, 2. fungal root pathogens, 3. bacteria, 4. viruses, 5. arthropods 6. nematodes and 7. non-parasitic disorders.

	Causal organism or cause	Name or symptom	Importance[a]
1.	*Phytophthora porri*	white tip	++
	Puccinia allii	leek rust	++
	Alternaria porri	purple blotch	+
	Heterosporium allii-porri	leaf blotch	+
	Pleospora herbarum	black mold	+
	Leptotrochila porri	black stripe	±
	Colletotrichum circinans	smudge	±
	Botrytis spp.	leaf fleck	-
2.	*Fusarium* spp.	basal rot	+
	Pyrenochaeta terrestris	pink root	-
	Sclerotium cepivorum	white rot	-
	Urocystis cepulae	smut	-
3.	*Erwinia* sp.	bacterial rot	+
	Pseudomonas sp.	bacterial rot	+
4.	*Leek yellow stripe (LYSV)*	yellow stripe	±
5.	*Acrolepiopsis assectella*	leek moth	+
	Delia antiqua	onion fly	+
	Thrips tabaci	onion thrips	++
	Lyriomyza spp.	leaf miner	+
	Tetranychus spp.	spider mites	+
6.	*Ditylenchus dipsaci*	twisters	+
7.	Genetic deficiencies	white spots	.
	Herbicide damage	white spots	.
	Ozone injury	white spots	.
	Sun scald	necrosis	.
	Frost damage	necrosis	.

[a] · no estimate given, ++ heavy losses every year, + light losses every year, ± light losses in some years, - no losses in the Netherlands in 1990-1995

Purple blotch (*Alternaria porri*) was present in most leek crops in autumn and winter, but it was generally considered a minor problem, mostly occurring on dying leaves, and only occasionally causing an outbreak.

Fusarium basal rot (*Fusarium* sp.) was a widespread hazard of transplanted seedlings. To prevent seedling death, fungicides were applied to roots or planting holes. Sometimes a mild leaf attack by *Fusarium* sp. could be found. In the course of our research, *Fusarium* basal rot sometimes caused adult plant death when bolting plants were transplanted from field to glasshouse for breeding purposes. Death of potted plants, especially inbreds with little vigour, was often associated with *Fusarium* basal rot.

Black mould (*Pleospora herbarum*; anamorph *Stemphylium herbarum* (Bakker, 1953; Boerema *et al.*, 1993) could be found in almost all leek fields on dying leaves and as a secondary invader of necrotic spots on the leaves, but apparently never caused significant losses.

Leaf blotch (*Heterosporium allii-porri*; synonym *Cladosporium allii* (Boerema *et al.*, 1993) was rarely detected on leek. Sometimes the fungus occurred as a secondary invader, but at least one local epidemic did occur.

Black stripe disease (*Leptotrochila porri*) was virtually absent in 1990-1995, although it appeared to be increasing in 1980-1990.

Smudge (*Colletotrichum circinans*) was reported at least once as a disease on leeks (Alofs, 1995).

White rot (*Sclerotium cepivorum*), smut (*Urocystis cepae*) and pink root (*Pyrenochaeta terrestris*) seem to be absent from leek in the Netherlands, although they are reported on leek in other European countries (Bonnet, 1976; Grill, 1985).

Botrytis spp., although described on leek (Maude, 1990), are not found on leeks in the Netherlands, except sporadically as a secondary invader.

Bacterial diseases, causing characteristic malformations, were regularly seen in Dutch leek crops. Leaf flecks caused by *Pseudomonas* sp. were reported from Germany by Smolka *et al.* (1992).

Leek yellow stripe virus (LYSV) is a nonpersistent, aphid transmitted virus, which used to be an important pathogen, but after ca. 1980 the prevalence of LYSV has declined steadily (Van Dijk, 1993, p. 40). This decline coincides with a shift to cultivars with darker green leaves that are called resistant. The resistance of these cultivars may have been improved through selection (Matthieu *et al.*, 1984). Before the LYSV epidemic in the seventies, selection fields were often far from the areas with intensive leek cropping. After moving the selection activities to locations with high disease pressure, resistance appeared to improve rapidly (pers. comm. T. van der Jagt). Resistance to LYSV was also found in kurrat (Van der Meer, 1990), but because of the decline of LYSV (and also because of very high susceptibility for leek rust in the LYSV resistance source) breeders did not start introgression programs. It is probably not true that the LYSV decline is the result of a time gap in the annual production cycle, as stated in Brewster (1993), although a better geographic isolation of seed beds and production fields, and better aphid control may have contributed to the LYSV decline.

White, necrotic spots on leaves caused by a genetical deficiency may occur in all plants of certain inbred lines of leek. These spots may vary in size and form, and their occurrence may be related to developmental and environmental factors. There are probably several deficiency genes, each with its own characteristics. Berninger and Buret (1967) mentioned 8 different chlorophyll deficiency phenotypes. We suppose that a genetic deficiency may resemble leafspot symptoms. The symptoms sometimes resemble those of ozone damage of onion (Engle & Gabelman, 1966). Usually about 1% of the

plants in commercial cultivars show genetic deficiencies. These plants may give rise to false alarm in supervised disease control programs, as they may be confounded with white tip disease. In most cases, however, genetical deficiencies can be recognized by the typical occurrence of white spots on all similar plant parts, and by the isolated position of affected plants.

1.1.7 Breeding and germplasm sources

Leek breeding is based on mass or half-sib family selection, usually within seasonal crop types (Currah, 1987). Leek is a tetraploid, self-compatible outbreeder with low tolerance to inbreeding. Outbreeding is enhanced by insect pollination and serial flowering within the umbels. Leek cultivars are open-pollinated and should contain enough genetic variation to avoid inbreeding depression. The genetic heterogeneity of leek is undesirable for mechanical harvesting and for cultivar registration. Registration authorities use a very broad cultivar concept for leeks because of the heterogeneity of leek selections (Van Marrewijk, 1988).

The aims of leek breeding vary according to the seasonal crop types. Summer leeks are selected for rapid growth to improve the yield. Winter leeks, on the other hand, are selected for slow growth, as this property is associated with winter hardiness and late flowering ('resistance to bolting'). Autumn leeks are selected for an optimal balance between rapid growth and cold tolerance. All leek types are selected for long shafts and absence of bulbing. Winter leeks are selected for dark leaf colour, which is considered as an indication of winter hardiness and is important for market presentation, and for an upright habit with easily removable outer leaves and closed 'collars', preventing the access of soil to the shafts.

Resistance plays a secondary role in most Dutch breeding programs. Selection is usually based on the natural occurrence of disease in selection fields. It appears that LYSV resistance has increased in winter leek and has contributed to the remarkable decline of this disease in the past decades. Leek rust also occurred regularly in selection fields. Nevertheless, resistance to leek rust is still insufficient. Breeders may select occasionally for resistance to leaf diseases such as *P. porri*, but probably because of annual variation in severity and because the leaf diseases are not distinguished properly, there are no examples of leek cultivars resistant to one of these diseases.

Schweisguth (1970; 1984) described male sterility (ms) genes. He suggested that it is possible to create F_1 hybrid leek cultivars with higher yield and greater uniformity than the traditional open-pollinated (OP) varieties by crossing a male sterile and a fully fertile inbred line. Schweisguth demonstrated that F_1 hybrids may produce yields 19-34% higher than OP varieties. Kampe (1980) found even larger differences (18-79%). The superiority of F_1 varieties over OP varieties may be due to the presence of 10-20% inbreds in the OP varieties rather than to heterosis. Without these inbreds, OP varieties

may be more heterozygous than (two-way) F_1 hybrids. Three- or four way F_1 hybrids are probably needed for combining high heterozygosity with genetical homogeneity (Van der Meer & Hanelt, 1990). Synthetic varieties, as proposed by Schweisguth (1982), may be highly heterozygous, but not very homogeneous because of the inclusion of selfed plant material in the synthetic race.

Recently, Smith (1994; 1995) selected vigorous ms-lines which can be vegetatively propagated in an easy way, either via topsets or by the tissue culture method described by Silvertand *et al.* (1995). Because the male sterility factor is under nuclear control, the ms-line cannot easily be maintained via seed propagation. Production costs for F_1 hybrid seed will be high as long as no cytoplasmic male sterility (cms) is available. Several attempts to find or create cms have failed in recent years, but there are still some unexplored opportunities (Buiteveld *et al.*, 1994, Verbeek *et al.*, 1995).

Leek germplasm collections exist in Bulgaria, Czechia, Germany, France, the Netherlands, Turkey, UK and USA (Astley *et al.*, 1982; Astley, 1992). The largest collection of leek cultivars appears to be the Dutch collection at the Centre for Genetic Resources (CGN), which is a semi-autonomous part of the Centre for Plant Breeding and Reproduction Research (CPRO-DLO) in Wageningen. Apart from the CGN collection, a second collection was created at CPRO-DLO for research purposes. The material of this collection was obtained for a large part from the Pullman Institute in Washington State, USA, and from various other sources, including botanical gardens and individual collectors.

1.2 White tip disease

1.2.1 Biology and distribution

The causal organism of white tip disease is *Phytophthora porri* Foister, a homothallic species of the genus *Phytophthora* (family Pythiaceae, order Peronosporales, class Oomycetes) (Alexopoulos & Mims, 1979), which can grow on natural media like corn-meal agar or V8 agar. Infected leaves show papery white local lesions, typically lozenge-shaped (ca. 3 x 5 cm) and sometimes surrounded by a dark green water-logged zone (Fig. 1.2 and 1.3). The fungus is present in the infected tissue as large non-septate mycelium which is both inter- and intracellular. Sporangia can develop *in vitro* and in wet lesions and may release 10-30 zoospores. Oogonia with both paragynous and amphigynous antheridia are produced in abundance in leaves, while oospores are formed as the leaves dry up. Oospores are sexual spores with a thick wall. They can be considered as resting structures, which may survive at least the crop free period in summer and possibly several years longer. Oospores probably initiate epidemics of *P. porri* in autumn. Subsequent intensification is supposedly due to sporangial infections. Early

infections are typically half-way the leaves or at leaf tips, but later infections are nearer to the leaf bases.

The host range of *P. porri* includes a number of *Allium* species (*A. porrum, A. cepa, A. fistulosum, A. sativum, A. tuberosum, A. bakeri, A. nipponicum*), cabbage (*Brassica spp.*), peach-leaved bell-flower (*Campanula persicifolia*), carnation (*Dianthus caryophyllus*), carrot (*Daucus carota*), *Chrysanthemum* sp. (Baker, 1972), *Gladiolus* sp. and tulip (*Tulipa gesneriana*) (CMI, 1978; Ho, 1983). Only cultivated hosts of *P. porri* are known. Although cross-infection appears to be possible in some cases, there is some degree of specialization, at least in isolates from cabbage and leek (Van Es, 1992; De Cock, 1993). Leek isolates were found to infect onions (Tichelaar & Van Kesteren, 1967; Griffin & Jones, 1977), and onion isolates were found to infect leeks (Tomlinson, 1951).

P. porri has been reported from Western Europe, Italy, Greece, Canada, South Africa, Australia and Japan (CMI, 1990), and recently also from the USA (Heimann, 1994). The disease is regularly causing harvest losses of leek in the Netherlands, Belgium, England and Japan. In France and Germany losses seem to occur only incidentally.

Harvest losses may be severe. In some cases total crop loss is reported. A diseased crop generally gives a lower yield weight, requires more labour for trimming the plants, and obtains lower prices. If the number of lesions is low, they can be removed by trimming, but then the leek is often declassified, because of unequally cut leaves. Moreover, diseased plants tend to wilt more rapid than healthy plants.

Figure 1.2 Leek (*Allium porrum* L.) with a lesion of *Phytophthora porri*

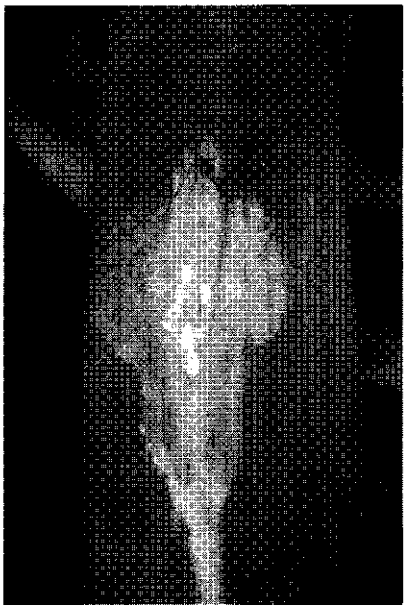

Figure 1.3 *P. porri* lesion with irregular water-soaked halo.

1.2.2 Research history

Foister (1931) described and named the disease, and studied its epidemiology in Scotland and England. A first attempt to find resistance followed soon (Ogilvie & Mulligan, 1932), but no resistance was found in 17 leek cultivars. After Foister's study, the importance of the disease decreased gradually, possibly because of phytosanitary measures like burning or burying of crop debris (Foister, 1961).

Van Hoof (1959) studied chemical control of white tip disease in the Netherlands, which became the standard method of disease control, although the cold and wet weather conditions which are favourable for the disease appear to reduce the effectiveness of chemical control. On heavily infested soils control is often unsatisfactory. Moreover, the use of fungicides is increasingly seen as a health hazard, both for humans and other organisms.

Alofs & Pijnenburg (1988) developed an alternative control method by straw mulching. This method is derived from the theory that splash dispersal of soil particles is usually the first step in a *P. porri* epidemic. From this theory it follows that initial infection is minimized when the soil is covered, e.g. with a layer of straw. Straw mulch is now applied on ca. 10% of the Dutch leek acreage. The mulching method is particularly popular with farmers that hope to get peak prices in winter, as mulched crops can be harvested a few days longer after the start of a frost period. The method is not reliable when the disease is already present in the crop before mulching, usually at the end of August, when plants are big enough for mulching with straw.

Because of the relatively high input of chemical control agents in leeks, the development of alternative control strategies for leek diseases has a high priority in the Dutch Multi-Year Crop Protection Plan (Anonymous, 1990). To control fungal diseases of leek 34,000 kg active ingredient (a.i) was used in 1990 in the Netherlands. This is equivalent to 25 kg a.i./ha.

In 1986 the first steps towards the present research project were taken by Van der Meer (IVT, 1986), having collected leek accessions in the preceding years. Several breeding companies showed interest for an in-depth study of *P. porri*. It was felt that the success of the breeding project would strongly depend on the development of phytopathological techniques and insights. The expertise for the phytopathological research was found in the Department of Phytopathology of the Wageningen Agricultural University (WAU) where oomycetes had been studied for many years. A first draft of a research proposal was prepared in 1990 by E.G.A. Linders. C. Kik (Centre for Plant Breeding and Reproduction Research (CPRO-DLO), the former Institute for Breeding of Horticultural Crops (IVT)) and H.D. Frinking (WAU-Phytopathology) elaborated it and obtained financial support from the Dutch Technology Foundation (STW).

1.3 Outline of this thesis

1.3.1 Research aims and methodology

The ultimate aim of the research presented in this thesis is to control the fungal pathogen *Phytophthora porri* effectively. The derived goals were

- to study the mycology and epidemiology of *P. porri*
- to identify and exploit resistance to *P. porri* in leek.

These two goals are interrelated in various ways. First, fungal growth *in vitro* should be studied to develop screening techniques. Second, the design and interpretation of field experiments for resistance evaluation depends on epidemiological insight concerning natural sources of inoculum and the influence of weather on sporulation, dispersal and infection. Third, the problems and prospects of alternative control strategies may stimulate or, in contrast, discourage breeding for resistance. Effective control may result from the use of partially resistant varieties in combination with other control measures.

1.3.2 Epidemiology

Epidemiological questions were addressed in field and controlled environment experiments. In the field, the major research questions were

- how important is rain for epidemic development?
- what is the velocity of spatial progress of an epidemic?
- are there differences in resistance among leek cultivars and their relatives?

In the controlled environment studies, the major questions were

- what is the incubation period of *P. porri* at different temperatures?
- is early screening for resistance possible?

To achieve our aims, new techniques were developed, among which

- a technique for *in vitro* production of zoospore inoculum
- an inoculation technique to test resistance of young plants

For resistance screening, two inoculation techniques were developed. In 1991-1993 young plants were inoculated with a few ml zoospore inoculum dripped into the shafts of young leek plants. This technique allows a crude resistance evaluation. In 1994 the immersion technique was invented, which allowed a better differentiation for resistance than the earlier drip inoculation method.

1.3.3 Resistance

The research program, aimed at the exploitation of resistance, was organized along two lines: (1) introgression of resistance from selected landraces, and (2) genetic studies of resistance within winter leek.

1. *Introgression strategy*. First, gene-bank accessions were tested in a (drip-inoculated) glasshouse test, with field-tested cultivars as more or less susceptible controls. Next, a number of gene-bank accessions was chosen for a field evaluation, and gene-bank accessions with a high level of resistance in the field test were chosen for crossing with mother plants of winter leek cultivars. The F_1 populations were again tested in a crude glasshouse test. From the best F_1 populations single plants were selected for backcrossing and inbreeding. Finally, the backcrossed and inbred populations were evaluated in an (immersion-inoculated) glasshouse test and in a field test.

2. *Genetic studies*. Differences in field resistance among plants of the same cultivar of winter leek were analyzed through tests of clones and half-sib families. Realized heritabilities were estimated with half-sib families selected for resistance or for susceptibility.

1.3.4 Contents of chapters

The epidemiological part of the study is described in chapters 2-5, the research concerning resistance in chapters 6 and 7. Key words referring to specific subjects addressed in each chapter of this study are given in Table 1.4.

In chapters 2 and 3 most emphasis is placed on indoor experiments. In chapter 2 basic mycological techniques for producing oospores, sporangia, zoospores and mycelium are described. A field experiment was included as an application of these techniques. In chapter 3 two new techniques are described: the immersion method and the oospore germination assay. Both techniques were used to study temperature effects.

In chapters 4 and 5 the temporal and spatial progress of epidemics of *P. porri* in leeks is studied in multi-year field experiments. In chapter 4 the hypothesis that rain is a key factor explaining the course of the temporal progress curve is tested. In chapter 5 the intensification and extensification of artificial disease foci is studied simultaneously.

In chapters 6 and 7 sources of resistance to *P. porri* are identified in landraces, using field tests and immersion tests. Also, genetic variation for resistance in modern leek cultivars is investigated. In chapter 6 resistance is described in landraces and modern cultivars with an emphasis on multi-year field tests. In chapter 7 genetical aspects of resistance are investigated using clonal and half-sib progenies of modern cultivars and cross- and backcross-progenies of modern cultivars and landraces.

General aspects of this thesis are discussed in chapter 8, which finishes with recommendations.

Table 1.4 Key words for subjects treated in the eight chapters of this thesis.

Chapter - Page headlines	Key words
1 - Introduction	*Allium porrum* L.
	leek diseases
	Phytophthora porri Foister
2 - Field inoculation	fungus isolation
	inoculation with zoospores
	inoculation with oospores
	inoculation with diseased tissue
3 - Temperature	immersion inoculation
	incubation period
	degree-day model
	thermal death
	oospore germination
	soil solarization
4 - Rain	epidemic progress curves
	degree-day model
	splash dispersal
	sporulation
	host growth
	disease profiles
5 - Foci	disease maps
	gradient analysis
6 - Resistance	field tests of cultivars
	glasshouse tests of gene-bank accessions
	field tests of gene-bank accessions
7 - Genetics	immersion and field tests
	clones of winter leek
	half-sib families of winter leek
	crosses of winter leek and resistant landraces
8 - Discussion	recommendations

Chapter 2

Field inoculation of leek with *Phytophthora porri*

Summary: Field inoculation of leek with zoospores of *Phytophthora porri* resulted in a high level of infection within a short time. Inoculation with infected leaf tissue resulted in a more gradual increase of disease incidence. Inoculation with oospores was relatively unsuccessful. Zoospores were produced in Petri-dishes by treating fast-growing, young mycelium with a diluted soil extract for at least 2 days, followed by a cold treatment in sterile demineralized water. The successful methods can be used for evaluation of resistance or fungicide performance, and for epidemiological experiments.

2.1 Introduction

Field experiments with *Phytophthora porri* Foister on leek (*Allium porrum* L.) depend on natural infection. The absence of the disease during some years has often hampered field evaluations of leek cultivars and breeding lines, and of fungicide performance (Vanparijs and Bockstaele, 1984; Van Bakel, 1964). Therefore, a reliable inoculation method was needed.

2.2 Materials an methods

During the autumn of 1991 three different inoculation methods were tested in a field experiment, using three different kinds of inoculum: zoospores, infected leaf tissue of leek, and oospores.

Zoospore inoculum was produced as follows: 15 discs (diam. 5 mm) from a *P. porri* culture on leek agar (200 g leek leaf extract in 1 l of demineralized water and 17 g agar) are grown on 10-15 ml of 10% V8 broth in a Petri dish (diam. 9 cm) at 15°C for 2-3 days. Then the V8 medium is decanted and a 10x diluted soil extract is added, which is produced by suspending 500 g of soil (Trios 17) in 1 L of demineralized water, leaving the suspension overnight, and autoclaving the filtrate. After at least 2 days incubation with soil extract at 15°C numerous sporangia should be visible. Soil extract is then removed and 10 ml cold demineralized water is added. After 2-4 h at 3°C this medium will contain about 1000 active zoospores per ml. Usually less than ten percent of the sporangia has germinated after the cold treatment. The described method is a modification of the method of Chen & Zentmyer (1970) and of Hamm & Koepsell (1984).

Table 2.1 Disease incidence (n=600) after inoculation with (1) zoospores, (2) infected leaf tissue, (3) oospores. days after inoculation

	(1)	(2)	(3)
7-13 days	50%	30%	7%
30-60 days	80%	80%	12%

Zoospores will remain active for several hours when the suspension is kept cool. When sporangia are kept at 3°C they will produce fresh zoospores for about 3 months. Inoculation is achieved by dripping one ml of the zoospore suspension into a leaf sheath of a leek plant.

Infected leaf tissue is produced by placing an agar disc with mycelium or one drop of a zoospore suspension on a leaf piece of 5 cm length. After incubaton in a moist chamber for about 4 days at 15-20 °C the leaf pieces are dried. Agar discs are removed. At the seventh or eighth day after inoculation greenish symptoms, typically lozenge-shaped, will develop. For inoculation, the infected tissue is placed into the leaf sheaths of leek plants, where a small quantity of water is often present for long periods.

Oospore inoculum is produced in leek agar. Colonies of *P. porri* are incubated for 2 months at ca. 17°C. To harvest the oospores, agar plates are homogenized in 50 ml sterile demineralized water per plate and incubated for 20 h at 18 °C with 0.5% (w/v) Novozym (Sigma L-2265) solution to digest the mycelium (Spielman *et al.*, 1989). The surviving oospores (ca. 1000 per ml) are used as inoculum.

Each method was used to inoculate 600 leek plants. Five winter leek cultivars were used. Non-inoculated control plants were grown in neighbouring rows at 45 cm distance and in separate plots at 2.4 m.

2.3 Results

Zoospore inoculation caused the highest incidence levels (Table 2.1): seven days after inoculation 50% of the inoculated plants showed disease symptoms. This percentage increased in the next few weeks till about 80%. Inoculation with infected leaves resulted in 30% incidence at the eleventh day after inoculation. In the next two months the incidence increased gradually till 80%.

Oospore inoculation was relatively unsuccessful. Thirteen days after inoculation only 7% of inoculated plants showed symptoms, and one month after inoculation 12% was infected.

Non-inoculated plants in neigbouring rows did not show symptoms until about three weeks after inoculation. Therefore, after three weeks, infection of inoculated plants may

be due to secondary spread. During the reported period, no natural infection occurred in neighbouring control plots. This proves that soil-borne inoculum of *P. porri* was absent in the experimental plot initially.

2.4 Discussion

Advantages of zoospore inoculation are the high infection percentage, the absence of contaminants from the inoculum, and the possibility to quantify the inoculum and to apply it homogeneously. A disadvantage of zoospore inoculation compared with infected leaf tissue inoculation may be the higher sensitivity to weather conditions. Zoospore inoculation was completely unsuccessful when temperatures were too high (>25°C) during inoculation. Cool and wet conditions are supposed to favour the infection process.

Zoospores or infected leaf tissue may be applied to spreader plants in a field trial. Both methods may be useful for field evaluation of resistance and of fungicide performance.

Chapter 3

Effects of temperature on *Phytophthora porri* *in vitro* and *in planta*

Summary: Cardinal temperatures for mycelial growth of *Phytophthora porri* on corn-meal agar were <5 (minimum), 15-20 (optimum) and just above 25°C (maximum). The number of infections after zoospore inoculation of young leek plants was relatively low at supra-optimal temperatures, but was not affected by sub-optimal temperatures. At 0°C plants were infected easily. The incubation periods needed for symptom formation were 36-57 days at 0°C, 13-18 days at 5°C, and 4-11 days at >11°C, and were fitted to temperature between 0 and 24°C with a hyperbolical model (1/p= 0.00812*T+ 0.0243). Oospore germination, reported for the first time for *P. porri*, was strongly reduced after 5 h at 45°C, and totally absent after 5 h at 55°C. Soil solarization for six weeks during an exceptionally warm period in May-June 1992 in the Netherlands raised the soil temperature at 5 cm depth for 17 h above 45°, but did not reduce the initial level of disease in August significantly.

3.1 Introduction

Phytophthora porri Foister is a serious disease of winter leek in Europe. The disease causes white, more or less lozenge-shaped local lesions of ca. 3 x 5 cm cm on leaves, sometimes surrounded by a green water-logged zone. Sporangia are formed readily in water-logged lesions under wet conditions, and may release 10-30 zoospores. Epidemics may destroy the crop before January-April, when winter leek is harvested. The fungus is homothallic. Oospores, and possibly chlamydospores which are very similar in shape, formed in infected leaves, may enter the soil with leaf debris and survive the crop-free period, roughly from February till July, and probably longer. In autumn, the oospores may germinate to form immediately sporangia, which may release zoospores. The zoospores may be transported to leek leaves by rain splash, thus triggering a new epidemic.

Species of *Phytophthora* may be seen as groups of isolates, brought together by taxonomic conventions, but not necessarily separated from other species by natural barriers (Waterhouse, 1983). Non-morphological characters such as host specialization, cardinal (minimum, optimum, and maximum) or lethal temperatures are not considered to be important for the definition of species. Yet these characters are ecologically relevant and may provide important clues for disease control.

Table 3.1 Minimum, optimum and maximum temperatures (°C) reported for *in vitro* growth of *P. porri* isolates from various countries and host plants

Reference	Temperature (°C)				
	Minimum	Optimum	Maximum	Country[1]	Host
Foister, 1931	<8	<25	<35	UK	Leek
Leonian, 1934			27	UK	Leek
Legge, 1951			27	UK	*Campanula*
Tomlinson, 1951			27	UK	Onion
Waterhouse, 1963	<5	25	33-35	UK	Leek
Van Hoof, 1959	3	15-19	26	NL	Leek
Katsura *et al.*, 1969	0	15-20	27	JA	*Allium* spp.
Noviello *et al.*, 1971	<5	20	27	IT	Onion
Semb, 1971	-3	15-22	25	NO	Cabbage
Geeson, 1976	0	15-20	25	UK	Cabbage
Katsura, 1976	<5	15-20	28	JA	*Allium* spp.
Kouyeas, 1977			27	GR	*Gladiolus*
					Carnation
Ho, 1983	0	15-20	31-32	CA	Carrot
Von Maltitz *et al.*, 1983		20		SA	Onion
Kröber, 1985	2	20	23-26	GE	Leek
					Cabbage
Kiewnick, 1985	<5	20	30	GE	Cabbage
Luo *et al.*, 1988	<5	25-30		CA	Leek, Carrot
De Cock *et al.*, 1992	<3	18-21	24-27	various	Leek & others

[1] CA= Canada, GE = Germany, GR = Greece, IT = Italy, JA = Japan, NO = Norway, SA = South Africa, UK = United Kingdom

Cardinal temperatures for mycelial growth *in vitro* of *P. porri* were determined by several authors (Table 3.1). In real life, however, the fungus is growing in living tissue, so it is interesting to compare growth *in vitro* and growth *in planta*.

Lethal temperatures of several *Phytophthora* species have been determined in various studies, mainly in relation to disease control based on thermotherapy (Table 3.2). Several *Phytophthora* spp. appear to be sensitive to temperatures that are associated with solar heating of soil in subtropical climates (Juarez-Palacios, 1991) or composting (Bollen, 1985). The most heat resistant structure of *P. capsici* was the oospore (Bollen *et al.*, 1989). We assume that the same is true for *P. porri*.

Table 3.2 Comparison of lethal temperature dosages for oospores, chlamydospores or mycelium of several *Phytophthora* species

Phytophthora species	Fungal structure[1]	Lethal temperature dosage		Optimum temperature	Reference
		°C	duration	°C	
P. cinnamomi	chl	38	30 min	24-28[2]	Barbercheck & von Broembsen, 1986
P. capsici	chl	42.5-45	30 min	28[2]	Bollen, 1985
P. capsici	oos	47.5-50	30 min	28[2]	"
P. cryptogea	oos	40-45	30 min	22-24[2]	"
P. infestans	oos	40	2 d	20[2]	Drenth *et al.*, 1995
P. megasperma	oos	45	30 min	24	Juarez-Palacios *et al.*, 1991
P. megasperma	oos	>45	30 min	30	"
P. cactorum	oos	45	30 min	25[2]	"
P. fragariae	oos	30	40 d	20-22[2]	Duncan, 1985
P. porri	myc	45	10 min	15-20	Yokoyama, 1976
P. porri	myc	40	20 min	15-20	"
P. porri	myc	25	90 d	15-20	"

[1]chl = chlamydospore, oos = oospore, myc = mycelium
[2]optimum temperature data from Waterhouse (1963)

The aims of this study were, first, to study the effect of temperature on *P. porri* growth *in vitro* and *in planta*. For measurement of *in planta* growth a new inoculation method was developed, and the results of the *in planta* growth experiment are described by a degree-day model which may be applied to field data. Second, the prospects for thermotherapy were explored by determining the lethal temperature for *P. porri* oospores *in vitro* and in a field experiment. As no germination of *P. porri* oospores has been reported in earlier studies, a new germination assay had to be developed for the *in vitro* test. In the field experiment, an attempt was made to eliminate natural inoculum in soil by soil solarization.

3.2 Materials and methods

3.2.1 General

Origin of isolates. All four isolates were obtained from commercial leek crops in The Netherlands. Isolate 1 originates from Gelderland (Lienden), 1991; isolate 2 originates from Noord-Brabant (Rijsbergen), 1987, and is stored at the Centraal Bureau voor Schimmelculture as CBS 141.87; isolate 3 and 4 originate from Limburg (Blerick and Horst-Meterik, respectively), 1992.

Isolation, identification and maintenance of P. porri. Isolates 1, 3 and 4 were obtained from light-green, water-logged margins of fresh lesions. Excised leaf pieces were rinsed for 2 min in 1% NaOCl and cut into pieces of 1 × 0.5 cm which were placed on Petri-dishes with corn-meal agar (17 $g.L^{-1}$; Oxoid, code CM103), amended with *ca.* 200 ppm vancomycin (Tsao, 1983) at 15°C. The fungus was identified using the most recent morphological key (Stamps *et al.*, 1990). The fungus was subcultured at 15°C in Petri-dishes on 1.2% agar (Oxoid Technical Agar No. 3) mixed with 20% leek extract, or occasionally on 1.2% agar mixed with 20% V8 broth. Leek extract was made from 200 g leaf tissue, mixed and boiled with 10 g saccharose in 1 L demineralized water, and then sieved through a plastic sieve (mesh width 0.5 mm).

Mass production of sporangia. Fresh axenic sporangia were obtained by growing the fungus on ca. 30 agar pieces of 5 mm^2 in diluted (2%) leek extract in Petri-dishes, with notches to improve aeration, at 15°C in the dark. The leek extract was decanted after 2-3 d, and sterile soil-extract was added. Sporangia developed on fresh mycelium after another 2-3 days. Then, the plates were used for harvesting zoospores or transferred to 4°C for later zoospore harvesting. Sporangia remained viable for several months at 4°C.

Sterile soil-extract was made by mixing 500 g steamed Trios 17 peat soil with 1 L demineralized water. The mixture was decanted and sieved the next day. The filtrate was autoclaved. Tap water and materials containing copper were avoided, as traces of copper may inhibit sporangium formation (Ribeiro, 1983).

Inoculation of plantlets by immersion in zoospore suspension. Zoospores were harvested shortly before inoculation by decanting the soil extract and adding cold (4°C) sterile demineralized water (SDW) to the dishes. Two to three hours later the dishes contained a suspension of $1\text{-}5.10^3$ active zoospores per ml. This suspension was decanted and poured into sterilized capped glass bottles which were placed on ice for some hours, before transfer to nonsterile plastic containers (40×25×15 cm) and tenfold dilution with SDW of 4°C. The diluted suspension had 100-500 zoospores per ml. For inoculation, 3 months old plants (5-7 leaves) were uprooted and their roots were shortly rinsed to remove most of the adhering soil and to check for the absence of root diseases. Healthy plants were placed horizontally into the container for ca. 20 h, at 15°C in a dark climate chamber or shadowed glasshouses. The plants were completely submerged during incubation.

Oospore germination assay. Oospores were harvested from *P. porri* cultures in a nutrient-rich liquid medium (20% leek extract or 20% V8) in Petri-dishes (Ø 9 cm) after at least one month of incubation at 18-20°C. A semi-sterile enzyme mixture (NovoZym; Sigma L-2265; 0.5 % w/v, incubated overnight at 18-22°C) was used to digest the mycelium and sporangia. NovoZym was removed by three cycles of centrifugation at low speed

(3000 rpm), discarding the supernatant and adding SDW (Spielman *et al.*, 1989). Germination was tested in SDW at 20°C and dimmed artificial light (6 $W.m^{-2}$) in Microtiter plates (Greiner, Ø 1 cm wells).

3.2.2 Experiments

Experiment 1. In vitro growth. To compare the cardinal temperatures of the Dutch isolates to those given in Table 3.1, radial growth of two isolates was measured in Petri-dishes (Ø 9 cm) with 13 ± 0.1 ml corn-meal agar (CMA), incubated in six dark cabinets with constant temperatures (5, 10, 15, 20, 25, 30 ± 0.2°C). Fungal colonies grew from agar pieces (Ø 5 mm) taken from the advancing margin of a *P. porri* colony on CMA, and placed upside down on the agar plates. During the first 24 h Petri-dishes were placed at 15°C to let the fungus grow into CMA. After this first day the Petri-dishes were distributed over the five temperature cabinets in six replicates. Radial growth rates were calculated as the mean of four radii measured along two perpendicular lines, divided by the incubation period, including the first day of growth at 15°C.

Experiment 2. In planta growth. Inoculation 1: On 27 December 1993, 70 leek plantlets were inoculated by immersion at 15°C and divided over eight rooms with constant temperatures at 0, 5, 11, 14, 18, 22, 24, 30 ± 1°C. Ten plants per treatment were used. After inoculation the plants were placed horizontally in a plastic container in a closed polythene bag, with wetted cotton plugs covering the root system. At 0 and 5°C the plantlets were incubated in dark rooms. At ≥11°C plants were incubated in shadowed glasshouses with natural daylight (20-40 $W.m^{-2}$) for ca. 8 h per day.

Inoculation 2: On 17 Jan 1994, 35 plantlets were inoculated at 15°C and submitted to the same seven incubation treatments. Only five plants per treatment were available.

Inoculation 3: On 12 Jan 1995, 18 plants were divided into three groups, which were inoculated by immersion at 0°C, 5°C and 9°C, and subsequently incubated at these temperatures in the described way. This experiment was designed to prove that not only colonization, but also infection of leaves may occur at low temperatures.

Plants were inspected daily. Lesions were counted as soon as they appeared and marked by a plastic peg to prevent double counts. The average numbers of lesions per leaf, and the longest, shortest and average incubation periods were determined.

Experiment 3. Oospore germination. Repeat 1: Oospores of isolate 1 were harvested as described above from 20% leek-broth cultures that were first incubated at 15-17°C for five months, and at 4°C for another five months. The oospores were divided over three temperature treatments. Aliquots of 0.02 ml SDW, containing ca. 280 oospores, were pipetted into wells of three Microtiter plates. Twelve wells per plate were used. Varying volumes of additional SDW were pipetted into each well, as the volume of SDW affected

the germination rate in preliminary experiments. The volume of additional SDW was 0.3, 0.6 or 0.9 ml. Each SDW treatment was applied to four wells per plate. The three Microtiter plates were incubated at 20, 45 or 55°C for five hours. Numbers of germinated oospores per well were counted at 100× magnification with a Zeiss Axiovert light microscope.

Repeat 2: Oospores of isolates 3 and 4 were harvested from 20% leek broth cultures incubated at 15-17°C for four months. Temperature and SDW treatments were as described for inoculation 1. Ca. 350 oospores were pipetted into each well. Two wells were used for each temperature × SDW volume treatment.

Experiment 4. Solarization. Four plots (5 × 5 m) of a field (20 × 5 m) with natural infestation by *P. porri* were used for a solarization experiment. The field was located at CPRO-DLO, 'De Goor', Wageningen. On May 27, 1992, the soil was drenched to field capacity and in two plots the soil was covered with a transparent polythene sheet. These plots were solarized for six weeks. Temperature at 5 cm depth was measured automatically every hour by three thermocouples at 5 cm depth in a solarized plot and one in a control plot. Soil tillage after solarization was restricted to the topmost soil layer. The four plots were planted on 7 July 1992 with three months old plants of cv. Carina. In each plot, 180 plants were planted at 12 cm within rows and 50 cm between rows. The plots were separated from each other by placing straw bales on the borders between plots. On 25 August and 18 September, all diseased leaves were counted per plant.

3.3 Results

Morphological identification. *Phytophthora*-isolates from leek were readily identified as *P. porri* by coiling hyphae in solid media. This character distinguishes *P. porri* from all other *Phytophthora* species. The presence of semi-papillate sporangia and of a mixture of paragynous and amphigynous antheridia leads to the same conclusion concerning the species name (Stamps *et al.*, 1990). The sporangia were ellipsoid or ovoid, seldomly obpyriform, noncaducous, with length/breadth ratios of ca. 1.3 and sizes of 45-75 μ. Twin apices, tapered bases, lateral attachments and distorted shapes were noted occasionally. Oospores were formed readily in host and in nutrient-rich culture media, were markedly aplerotic, had thick walls (2-5 μ) occasionally and varied in size from 25-35 μ. Chains of intercalary hyphal swellings were often observed in water. These characters are in accordance with Stamps's key. Intercalary attached sporangia and aerial sporangiophores on agar, mentioned in the key, were not found. Coralloid hyphal swellings were observed in some isolates, but not mentioned in the key.

Table 3.3 Experiment 1. Radial growth rates (× 0.01 mm.d^{-1}) of 2 isolates of *P. porri* on corn meal agar at six temperatures. Means and their standard deviations are given (n=6).

Temperature	Isolate 1	Isolate 2
5°C	5.0 ± 0.8	3.1 ± 0.5
10°C	4.6 ± 0.9	4.5 ± 0.3
15°C	14.9 ± 2.3	8.6 ± 1.3
20°C	9.3 ± 4.3	5.8 ± 1.4
25°C	0.3 ± 0.2	0.4 ± 0.1
30°C	0	0

These coralloid structures survived the digestion of mycelium with NovoZym which was used for cleaning the oospore culture.

Experiment 1. In vitro growth. (Table 3.3) The optimum temperature for *in vitro* growth of *P. porri* was ca. 15°C for isolates 1 and 2. The temperature minimum is well below 5°C. The variance in growth rates was relatively high at supra-optimal temperatures. The temperature maximum is between 25°C and 30°C, and colonies died at 30°C.

Experiment 2. In planta growth. (Fig. 3.1) The mean incubation period ranged from 5 d at 24°C to 43 d at 0°C. The inverse of the mean incubation period was fitted to the average temperature during the incubation period, including the first day of inoculation at 15°C for inoculations 1 and 2. The equation of the fitted hyperbolic line is given in Fig. 3.1. This equation implies that, on average, a lesion appears at 120 degree-days (base temperature -3.0°C) after infection.

The time between appearance of the first and last lesion was 2 days at 24°C and ca. 20 d at 0°C. In Fig. 3.1 the considerable variation in incubation period is shown by the hyperboles fitted through the observed shortest and longest incubation periods. The shortest incubation period corresponded with on average 92 degree-days, and the longest with 154 degree-days (base temperature -3.0°C).

The average number of lesions per leaf at the end of the experiment was 1.5-2.5 at temperatures below 18°C (Fig. 3.2). The low temperature during inoculation in inoculation 3 did not affect the number of lesions strongly. Temperatures above 18°C were apparently supra-optimal for lesion development. No lesions appeared at 30°C. At all temperatures, lesions were typically 1-3 cm long and increased slowly in size. A semi-permanent kind of latent infections was discovered in plants that were incubated at 24° for 10 d. When the plants were transferred from 24°C to 15°C, new lesions were observed after 6-8 d.

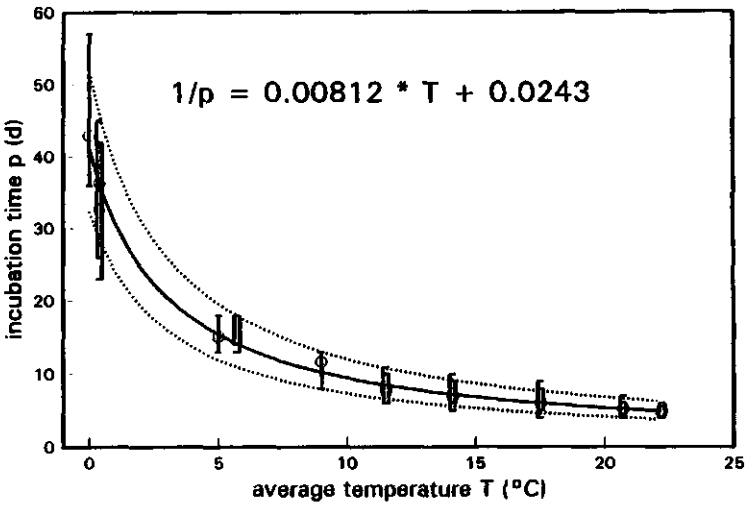

Fig. 3.1 Incubation periods (p) of *P. porri* on immersion-inoculated leek plantlets at various average temperatures. o = mean p, - shortest or longest p. Vertical lines represent the ranges for p within each experiment. Hyperboles were fitted by means of linear regression of the inverse of p on temperature. ___ mean p, ... shortest p or longest p. The equation above the graphs describes the fitted mean p as a function of temperature T.

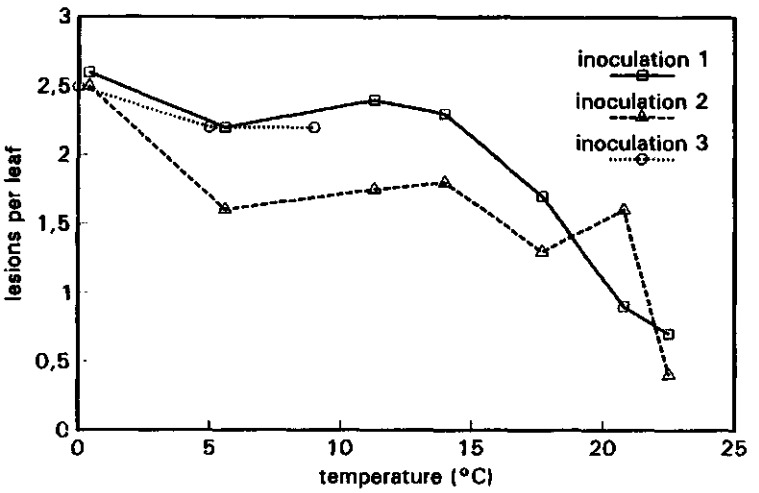

Fig. 3.2 Total number of lesions per leaf developing after immersion-inoculation of leek plantlets at 15°C, followed by incubation at various temperatures ranging from 0 to 24°C.

Table 3.4 Mean numbers of germinated oospores per well in microtiterplates after temperature treatments of 5 h. Differences between 20 and 45°C in each column are statistically significant (P<0.05)

	Isolate 1[1]	Isolate 3[2]	Isolate 4[2]
Oospores.well[-1]	280	360	350
d after inoculation	32	43	43
20°C	62	62	20
45°C	29	14	2
55°C	0	0	1[3]

[1] averaged over 12 wells (4 replicates at 0.3, 0.6 and 0.9 ml SDW)
[2] averaged over 4 wells (2 replicates at 0.6 and 0.9 ml SDW)
[3] short, abortive germination tubes

Experiment 3. Oospore germination. Treatment at 45°C for 5 h reduced germination of all isolates, compared with the control at 20°C (Table 3.4). The contrast between 45°C and control treatment was statistically significant (P<0.05, F-test). Treatment at 55°C prevented germination in both inoculations, except for a few abortive germination tubes from oospores of isolate 4.

An inhibitory effect of high water volumes on germination at 20 and 45°C was found in repeat 1 (P<0.01, F-test). In repeat 2 the control (0.3 ml SDW) failed due to stronger evaporation during incubation.

The first germinated oospore was observed 27 days after incubation in both inocualtions. The highest numbers of germinated oospores were counted 1-2 weeks later. Later countings were impossible because of mycelium growth and lysis. Oospores often formed a sporangium immediately after germination (Fig. 3.3).

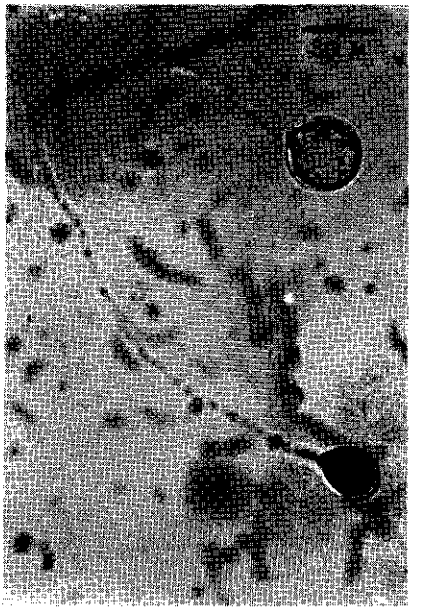

Fig. 3.3 Germinated oospore of *P. porri* with an immature sporangium (dark) at the end of the germination tube.

Experiment 4. Solarization. During the solarization treatment the weather was exceptionally warm. June 1992 belonged to the warmest six months of June since 1900 in the Netherlands. In solarized plots, at 5 cm depth, the average daily maximum temperature was 37°C. The temperature exceeded 40°C during 59 h, 45°C during 17 h, and never exceeded 48°C. In control plots the average daily maximum was 28°C and never exceeded 34°C. The average number of diseased leaves was 0.6 and 1.4 in solarized and control plots, respectively. The solarization effect was, however, not statistically significant ($0.1 > P > 0.15$), which may be partially ascribed to the low number of degrees of freedom. The weak solarization effect had disappeared 3 weeks later, when the number of diseased leaves was 2.5 and 2.2 in solarized and control plots, respectively.

3.4 Discussion

The maximum temperature for mycelial growth of *P. porri* mentioned by Foister (1931) in his original description of the fungus was 'below 35°C'. Leonian (1934), using Foister's isolate, published a more precise maximum of 27°, which was confirmed by several authors (Table 3.1) and in the present work, but not by Waterhouse (1963) and Waterhouse *et al.* (1983) after examining 'authentic material', and Ribeiro (1978), relying on Waterhouse's data. Kouyeas (1977) and Ho (1983) pointed out that Waterhouse's isolate is an extreme variant. De Cock *et al.* (1992) suggest that aberrant isolates do not belong to *P. porri sensu stricto*, as he showed that a *Phytophthora* isolate from leek, morphologically similar to *P. porri* but with higher cardinal temperatures, had mtDNA restriction patterns which were similar to *P. nicotianae*, a species with relatively high cardinal temperatures. The *P. porri* isolate from carrot described by Ho (1983) with maximum temperature above 27° also had a mtDNA restriction pattern that differed from *P. porri sensu stricto*.

The optimum temperature for *in vitro* growth of *P. porri* is between 15 and 20°C according to most authors listed in Table 3.1. This is confirmed in the present work. At supra-optimal temperatures the number of lesions in inoculated plantlets appears to be relatively low, although the *in planta* growth rate increased with temperature until 24°C. It may be speculated that at supra-optimal temperatures young *P. porri* colonies may have two different fates: they either grow very fast or they remain latent until lower temperatures return. A similar kind of semi-permanent latent infection was reported by Sutton (1989) for *Botrytis squamosa* on onion.

The extrapolated minimum temperature of *P. porri* growth *in planta* is -3°C, which is in accordance with Semb (1971). It may be assumed that ice formation limits the infection capacity of zoospores, and therefore the minimum temperature for infection is probably 0°C. Remarkably, the infection capacity of zoospores is not reduced at 0°C, as was convincingly shown in experiment 2, inoculation 3.

Low temperatures apparently slow down infection and colonization in a regular, predictable way, in spite of the relatively large stochastic variation of incubation periods at each treatment. The base temperature for growth of adult leek plants is 0°C (Hay & Brown, 1988). Leek leaves appear at intervals of 132 degree-days. Thus, the growth rate of the pathogen at low temperatures is relatively fast in comparison to the growth rate of the crop.

Oospores of *P. porri* did not germinate until they were 4-5 months old, although they were visually mature after 1 month. After the 4-5 month period, they still needed an incubation period of ca. 1 month in the liquid medium, although in some instances (data not shown) spores germinated in 1-2 weeks. These results indicate that oospores may become internally dormant. External dormancy may further regulate a timely germination in the field. It may be speculated that oospore germination was inhibited in the wells with more water, because of reduced aeration of the medium. If this is true, oospores may be activated by rain, as rain will improve the degree of aeration of soil water. Similar effects of aeration have been reported for formation and germination of sporangia of various *Phytophthora* species (Ribeiro, 1983).

The lethal temperature for oospores of *P. porri* is between 45 and 55°C, which is higher than for mycelium of *P. porri* (Yokoyama, 1976), and comparable to a *P. megasperma* isolate with a temperature optimum at 30°C (Table 3.2). This indicates that *P. porri* is adapted to high temperatures for oospore survival, in spite of the relatively low optimum and maximum temperatures for mycelial growth.

P. porri of leek is one of the few soil-borne *Phytophthora* species that do not infect roots. Therefore, the inoculum in the topmost layer of soil is probably indispensable for the completion of the infection cycle. Solar heating will mainly affect the top layer. Nevertheless, soil solarization is not a useful control option in the Netherlands, because the climate is too cold. Soil-solarization may be useful in a temperate climate if it can be combined with other disinfecting treatments, and if it can be targeted to infested leaf debris, as proposed by Entwistle (1990) in England for white rot of onion.

Composting of leaf debris may be a more effective thermotherapy. Composting temperatures are 50-70°C (Bollen *et al.*, 1989), and may therefore kill oospores of *P. porri*. A more radical solution is mentioned by Foister (1961) who speculates that the recommendation to burn or bury crop debris instead of ploughing it into the land has contributed to the gradual decrease in importance of the disease in Scotland after 1931.

Acknowledgements

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Chapter 4

Rain-driven epidemics of *Phytophthora porri* on leek

Summary: White tip disease of leek (*Allium porrum*), caused by *Phytophthora porri*, was studied in field experiments. On fields infested by soil-borne inoculum (oospores), relatively short periods of explosive disease increase alternated with periods in which apparently no new infections occurred. The analysis of rain data and disease data, using a degree-day model for incubation periods at various temperatures, confirmed the hypothesis that disease increase of *P. porri* is significantly correlated with rain; R^2_{adj} was 0.91, 0.41 and 0.51 in 1992, 1993 and 1994, respectively. Correlations were highest early in the season. Lack of correlation later in the season may be ascribed to the effect of lesion death, which may be caused by total or partial leaf death, by desiccation or by other fungi overgrowing *P. porri*, and to the effect of secondary infection by zoosporangia, which appears to be not so strongly rain-driven as primary infection. Zoosporangia were observed in fields on water-logged light-green lesions. High lesion densities of leaf tips and leaf units at 10-20 cm above the leaf axils indicated that most infections depend on free water, either in puddles or in a water basin near the leaf axils. Although disease correlates well with rain data, disease forecasts will be unreliable as long as rain forecasts are unreliable.

4.1 Introduction

White tip disease of leek (*Allium porrum* L.) is a soil-borne leafspot disease, caused by *Phytophthora porri* Foister. The disease, first recorded in Scotland in 1928 by Foister (1931) as a common and disastrous disease, steadily decreased in importance in Scotland during 1931-1948, until a resurgence took place in 1949 (Foister, 1961). In the Netherlands it was first described by Van Hoof (1959), mentioned by Van Bakel (1964) as a minor problem, but later by Alofs (1986) as one of the most prominent diseases of leeks during winter. The Dutch leek area has increased from 1285 ha in 1975 to 4250 ha in 1994 (De Kraker & Bosch, 1993; CBS, 1994) and epidemics of *P. porri* have become common, especially in the southern provinces of the Netherlands, where 80% of the Dutch leek production is concentrated.

Foister (1931) observed that the onset of epidemics of *P. porri* in Scotland may be early in August or as late as December. He could not explain this variation, partly because of his theory that sporangia, growing saprophytically on soil and discharged by wind,

were the most important infection agents. He did not find sporangia on leaves under field conditions. Legge (1951) demonstrated that a *Campanula*-isolate of *P. porri* may produce sporangia in non-sterile soil. Saprophytic growth in non-sterile soil was, however, limited. Van Hoof (1959) did not find sporangia on leaves at all, and suggested that oospores are the main source of infection. Taylor (1965), however, reported the occurrence of sporangia on wet foliage after prolonged dews for a few days. Ogilvie and Walton (1941) observed that from a few initial attacks the disease spread outwards in a circle, most infections taking place during wet weather. Yokoyama (1976) studied epidemics of *P. porri* on winter-grown onion (*Allium cepa* L.) in the south of Japan and concluded that disease was most severe in March when rainfall and temperature were relatively high in previous December and February. Grill (1985) reported that the onset of *P. porri* on leek in France occurred between September 5 and October 15 in 1980-1984, and observed that humid conditions and relatively high temperatures in winter favour the disease.

The initial (oospore-) inoculum of *P. porri* is soil-borne, but infections occur only on the plant parts above the ground. Secondary inoculum (sporangia) is only found on wet leaves. The wet sporangia are not discharged easily by wind only, so the main discharge mechanism could be rain splash. Spore discharge by rain splash may therefore be a limiting factor for disease progress. This crucial role of splash dispersal has been described in other pathosystems, e.g. black pod (*P. palmivora* (Butler) Butler) of cocoa (Thorold, 1975), leather rot (*P. cactorum* (Lebert & Cohn) Schröter) of strawberry (Madden *et al.*, 1992), and other plant diseases (Fitt *et al.*, 1989). In analogy with these pathosystems, we hypothesize that for *P. porri* rain is the main factor explaining disease progress, because of the direct effect on the discharge and dissemination of spores, and the indirect effects on sporulation and infection. The direct and indirect effects cannot be separated from each other in field experiments.

In the present study, epidemic progress curves of *P. porri* were obtained in three successive years. These curves probably describe rain-driven epidemics, i.e. epidemics for which rain is the main factor for disease progress. Several problems related to the interpretation of these curves were studied. First, the degree of correlation between rain and disease was quantified, and periods with low correlation were identified. Sporulation was observed qualitatively. Second, the turn-over of leaves was studied, as the continuous appearance and death of leaves might disturb the correlation between rainfall and disease increase. Third, lesion dynamics were investigated by direct observation of the fate of individual lesions, and by a study of disease profiles.

This study aims at a better understanding of the relative importance of the various processes affecting epidemics of *P. porri*, and may contribute to an effective research strategy for disease control.

Table 4.1 Summary of experiments

Purpose	Planting date	Observed variables[1]	Interval (days)	Plants total	Plants plot[1]	Plots	Blocks[1]	Cvs[2]
Epidemic curves	9-7-92	D,T,R	7	135	45	3	-	1-3
Turn-over of leaves	"	N,N_{new}	30	360	15	8	-	1-8
Epidemic curves	7-7-93	D,T,R	7	120	10	12	1-4	1-3
Turn-over of leaves	"	N,N_{new}	30	200	10	20	1-4	1-5
Turn-over of lesions	"	U_{new},X_{new}	7	45	5	9	5-7	1-3
Disease profiles	"	U(i),X(i)	"	"	"	"	"	"
Causes of lesion death	"	D,D_{new}	5-14	600	300	2	-	1
Epidemic curves	14-7-94	D,T,R	7	540	180	3	-	1-3

[1] Cf. Table 4.2

[2] Block codes 1-7 indicate seven different blocks

[3] Cultivar codes 1: Carina, 2: Wintina, 3: 91021, 4: Derrick, 5: Platina, 6: Gavia, 7: Portant, 8: Porino

4.2 Materials and methods

General. A series of field experiments was performed in 1992-1994 (Table 4.1). The experiments were located at CPRO-DLO ('De Goor'), Wageningen. Plants were sown in April in seedbeds and transplanted to fields in the first week of July, in planting holes of 12 cm depth and at planting distances of 12 x 50 cm. Fertilizer was applied according to professional advice based on soil samples (De Kraker and Bosch, 1993). Weather data were recorded in a weather station placed at the border of the field in 1992 and 1993. In 1994 these data were obtained from the meteorological station 'De Haarweg' at ca. 3 km distance. In all experiments the crop was initially infected by soil-borne inoculum, produced by infested plants at the same field in previous years.

Epidemic curves. To obtain epidemic progress curves of *P. porri*, the number of diseased leaves per plant (D) was determined in naturally infested leek crops in 1992/3, 1993/4 and 1994, mostly at weekly intervals, from planting in July till the end of the experiment in February, March and December, respectively. Three cultivars were used in 1992 (in plots of 45 plants, with three rows of 15 plants) and in 1993 (in four single-row plots of 10 plants). In 1994, three epidemic curves were obtained from three plots of 180 plants with different cropping histories (no, one or two previous years with a diseased crop), supposedly leading to different levels of natural infestation from soil. In 1992 plots were planted adjacently; in 1993, they were separated by a single row of plants (cvs. Carina or Derrick), and in 1994 they were separated by fallow strips of 2 m width.

Table 4.2 Parameters used for epidemiological studies. t = observation time, δ = length of previous observation interval, i = leaf number (leaves counted from top to bottom), max =maximum leaf number

Weather variables		
T	Daily mean temperature	[°C]
RR	Average daily rainfall	[mm.d^{-1}]
Disease variables		
D	number of diseased leaves	[#.plant^{-1}]
DR	average daily increase of D = [D(t)-D(t-δ)]/δ	[#.(plant.d)$^{-1}$]
D_{new}	number of leaves, healthy at t-δ, diseased at t	[#.plant^{-1}]
$D_{removed}$	number of leaves, diseased at t-δ, removed at t	[#.plant^{-1}]
LD	average lifetime of diseased leaves	[d]
X	number of diseased leaves = x(max)	[#.plant^{-1}]
X_{new}	leaf units, healthy at t-δ, diseased at t	[#.plant^{-1}]
$X_{removed}$	leaf units, diseased at t-δ, removed at t	[#.plant^{-1}]
LX	average lifetime of diseased leaf units	[d]
x(i)	number of diseased leaf units on leaf ≤i	[#.plant^{-1}]
x'(i)	standardized x(i) = x(i) / x(max)	[-]
Crop variables		
N	number of leaves	[#.plant^{-1}]
N_{new}	number of leaves that appeared since t-δ	[#.plant^{-1}]
$N_{removed}$	number of leaves that died since t-δ	[#.plant^{-1}]
LN	average lifetime of leaves	[d]
U	number of leaves = u(max)	[#.plant^{-1}]
U_{new}	leaf units that appeared since t-δ	[#.plant^{-1}]
$U_{removed}$	leaf units that died since t-δ	[#.plant^{-1}]
LU	average lifetime of leaf units	[d]
u(i)	number of leaf units on leaf ≤i	[#.plant^{-1}]
u'(i)	standardized u(i) = u(i)/u(max)	[-]

Correlation of disease and rain. From the epidemic progress curves, the degree of correlation was determined for the average daily increase of the number of diseased leaves (DR) and the average daily rainfall (RR) in an infection period (the period in which the fungus probably penetrated the leaf) one incubation period before the observation interval of DR. The infection period was calculated from measured daily mean temperatures during the incubation periods. First, at each time of observation the shortest and longest incubation time were calculated as 92 and 154 degree-days above -3°C, respectively, multiplied with the daily mean temperatures during incubation. The degree-day model was derived from infection experiments in growth cabinets at various temperatures (cf. Chapter 3). Second, for each observation interval the corresponding infection period was calculated by subtracting the longest incubation time from the

observation interval's first day, and the shortest incubation time from the observation interval's last day. Third, the average rainfall ($mm.d^{-1}$) in the calculated infection period was determined. Regression analysis of DR on RR yielded the R^2_{adj} values and their levels of significance.

Turn-over of leaves. To measure the turn-over of leaves, the total number of leaves (N) and the numbers of new leaves (N_{new}) were determined at monthly intervals from October to February, in 1992/3 in the middle rows (15 plants) of plots of 45 plants of 5 cultivars, and in 1993/4 in 4 plots of 10 plants of 8 cultivars. The same plants were also used for monitoring epidemic progress, as desribed above. N_{new} was determined with the help of 1-cm plastic pegs that were placed on the second leaf (top leaf = first leaf) at each time of observation.

The average lifetime of leaves (LN) was calculated as the inverse of the relative growth rate of leaves ($N.\delta/N_{new}$, with δ = the observation interval for N_{new}; cf. Table 4.2). In principle, relative death rates of N could also be used to calculate LN, but considering that growth rates were less influenced by short-term weather and disease fluctuations than death rates, and that full-grown plants have a rather constant number of 8-10 leaves, growth rates were preferred.

Turn-over of lesions. The distribution of lesions over leaves was determined from 6-9-93 till 15-11-93, using diagrams (Fig. 4.1) for recording the length of each leaf and the position of each lesion on 10-cm sections of leaves graphically at weekly intervals.

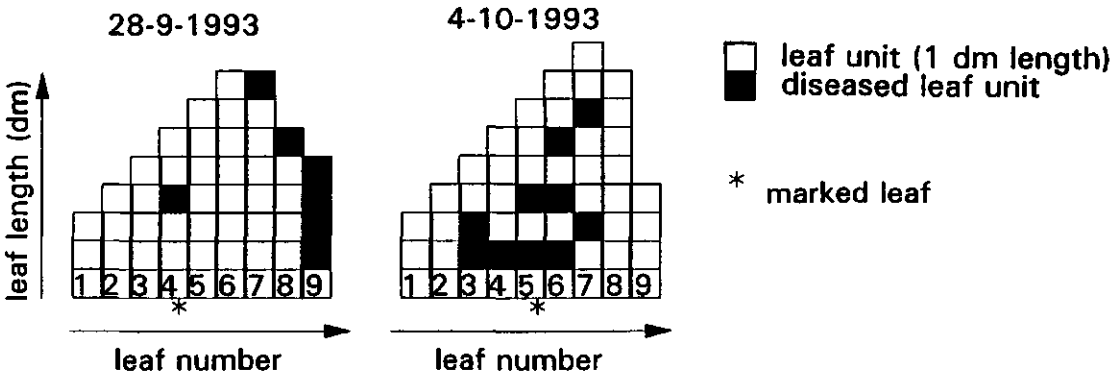

Fig. 4.1 Two plant diagrams of the same plant before (left) and after (right) week 40 of 1993, illustrating the method of observation of disease profiles. During week 40 one new leaf appeared and one old leaf died. Therefore the leaf numbers of individual leaves were raised by one. Leaf 4(→5) was marked with a peg, leaf 7 (→8) lost a lesion, and leaf 8 (→9) lost a diseased leaf tip.

A total of 45 plants was used, representing 3 cultivars and 3 blocks. From plots with 10 plants each, five plants were chosen randomly for observation. The plants were not used in another experiment. The turn-over of lesions was studied through comparison of diagrams of successive observation times. One leaf of each plant was marked with a peg to make this comparison possible.

The average lifetime of diseased leaf units LX was calculated as the inverse of the relative decay rate of diseased leaf units ($X.\delta/X_{removed}$, with δ = the observation interval for $X_{removed}$). For calculating the average lifetime of leaf units LU the inverse growth rate ($U.\delta/U_{new}$) was used, because growth rates of leaves were less sensitive to environmental factors than decay rates.

Disease profiles. Two types of disease profile were studied from the diagrams mentioned above: disease as a function of leaf number and disease as a function of distance along the leaf, measured from the soil surface.

For the disease profile over leaf numbers, the standardized number of lesions at the i^{th} and younger leaves x'(i), and the standardized number of leaves u'(i) for all leaf numbers i were calculated for each plant, following Daamen (1989). Averages of x'(i) and u'(i) were taken over groups of ten values in a column of x'(i) or u'(i) values sorted on size for each cultivar. Plots of x'(i) against u'(i) were constructed with these averages.

For the disease profile over distances along the leaf, the lesion density was plotted as a function of distance along the leaf. The lesion density was calculated as the number of lesions divided by the total number of leaf sections at each distance along the leaf, and was expressed as a percentage.

Causes of lesion death. To study the causes of lesion death, diseased leaves of 600 plants were marked with a plastic peg from 17-9-93 till 5-11-93 at intervals of 5-14 days. At each observation time new diseased leaves (D_{new}) were marked with a peg and counted; pegs were removed from dead leaves (without green leaf area) and leaves that had lost signs of *P. porri* without dying off totally. In this way the effect of leaf death, which could be an important reason for disappearance of lesions, was distinguished from other causes of lesion death.

Data from this experiment were also used for the calculation of the lifetime of diseased leaves LD (= $D.\delta/D_{removed}$).

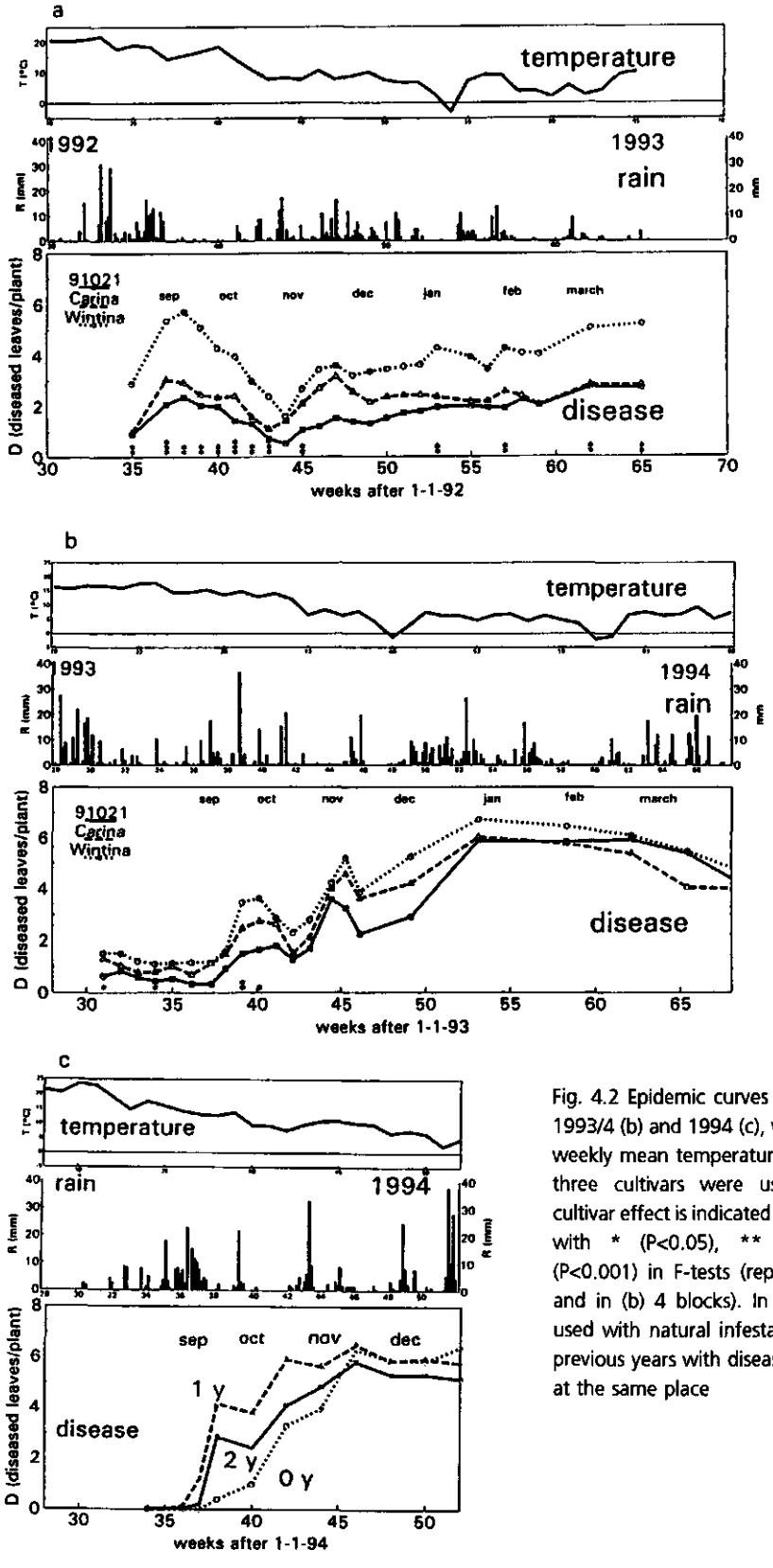

Fig. 4.2 Epidemic curves of in 1992/3 (a) and 1993/4 (b) and 1994 (c), with daily rainfall and weekly mean temperature data. In (a) and (b) three cultivars were used. Significance of cultivar effect is indicated below disease graphs with * ($P<0.05$), ** ($P<0.01$) and *** ($P<0.001$) in F-tests (replicates in (a) 3 rows and in (b) 4 blocks). In (c) three plots were used with natural infestation from 0, 1, or 2 previous years with diseased leek crops grown at the same place

4.3 Results

Epidemic curves. In Fig. 4.2, epidemic curves, rain- and temperature data are presented for three seasons. The curves fluctuated strongly from August till November, indicating (1) that infection occurs during short periods, alternated with longer periods without new infections, and (2) that the lifetime of individual lesions and diseased leaves is short relative to the total period of epidemic build-up.

Statistical significance of cultivar effects on D for single time points is indicated in Fig. 4.2. In 1992 the cultivar effect is possibly overestimated because of a location effect which is confounded with the cultivar effect. It appears that cultivar differentiation is most pronounced before November, during or shortly after an infection wave. In 1993, differences between cultivars were generally smaller than in 1992, possibly because of the smaller plots and the proper separation of cultivar and location effects. Cultivar x time interactions are further analyzed in Chapter 6.

The onset of the epidemic in 1994 was latest in the plot without a previous leek crop. The inoculum for this late onset probably originated in the other plots. The plot with one previous diseased leek crop had more disease initially than the plot with two such crops, indicating that the amount of initial inoculum was not limiting for initial infection in either plot. Plot scores converged in November, and in December the differences between plots became negligible. This indicates the increasing importance of secondary inoculum.

Correlation of disease and rain (Fig. 4.3). To investigate the possibility that infection periods are associated with rainfall, DR and the corresponding RR were plotted against time. In '91, '92 and '93, R^2_{adj} was 0.91, 0.41 and 0.51, respectively, for August-November. These values were statistically significant at $P<0.001$, $P<0.01$ and $P<0.05$, respectively.

The onset of the epidemics in week 35 (1992), week 31 (1993) and week 37 (1994), was preceded by >15 mm rain on a single day 8-12 d earlier. Each infection wave (2 in 1992, 3 in 1993, 2 in 1994) was preceded by >20 mm rain divided over 2-3 days, 8-21 d earlier.

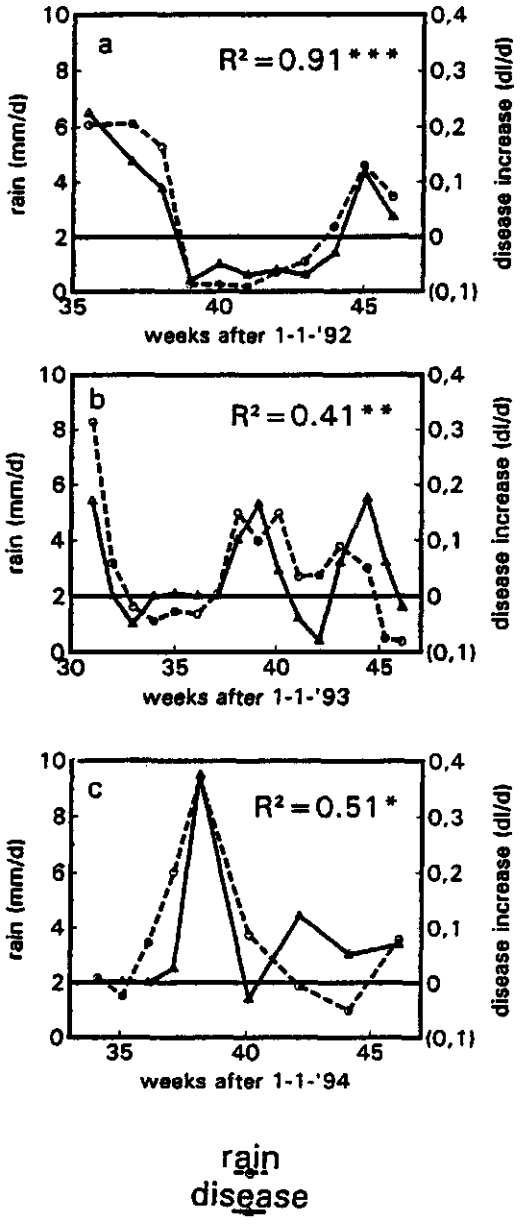

Fig. 4.3 Transformed disease and rainfall curves in 1992 (a), 1993 (b) and 1994 (c), illustrating the relation between disease increase (DR) in observation intervals and average daily rainfall (RR) in calculated infection periods, one incubation perod before the observations. A degree-day model was used to calculate infection periods from observation intervals (see text). dI/d = diseased leaves per day

Sporulation. Sporangia and zoospores were observed on and in fresh leaf material through a binocular microscope at 50x magnification in autumn and winter (Fig. 4.4). Sporulating lesions were often found on old, dying leaves or leaf tips of long leaves when these were lying on wet soil or in standing water. The sporulating lesions were light-green and often short-lived. White lesions (Fig. 4.5) did not sporulate, unless a water-logged zone developed around the white lesion, as occurs in a humid environment.

Fig. 4.4 Sporangia of *P. porri* on the surface of a wet leek leaf, stained with cotton blue.

Fig. 4.5 Naturally infected leek plant, with a white lesion of *P. porri* at a characteristic position, ca. 20 cm above the leaf axils

Table 4.3 Total number of leaves (N) at the end of different time intervals, new leaves (N_{new}) and removed leaves ($N_{removed}$) for each interval, and the average lifetimes of leaves (LN) for full-grown plants

Interval (weeks)	(days)	N (#)	N_{new} (#)	$N_{removed}$ (#)	LN (days)
1992 (n=120, 5 cvs)					
41		-	9.9		
41-46	35	9.9	1.3	1.3	
46-50	30	9.5	0.8	1.2	
50-59	63	9.1	1.9	2.3	
41-59	128	9.4	4.0	4.8	303
1993 (n=200, 8 cvs)					
31		-	5.1		
31-36	34	8.0	4.5	1.6	
36-42	39	8.0	3.7	3.7	
42-47	35	8.5	1.2	0.7	
36-47	74	8.2	4.9	4.4	124

Table 4.4 Total, new and removed leaf units (U) and diseased leaf units (X) in week 37-47, 1993 (n=45), and the average lifetime of leaf units (LU) and diseased leaf units (LX) before and after week 40

Interval (weeks)	(days)	total (#)	new (#)	removed (#)	lifetime (days)
leaf units		U	U_{new}	$U_{removed}$	LU
37		-	45		
37-40	22	47.5	12.8	10.3	82
40-47	48	46.2	13.1	14.4	169
diseased leaf units		X	X_{new}	$U_{removed}$	LX
37		-	1.1		
37-40	22	3.3	4.1	1.9	38
40-47	48	6.0	15.8	13.1	21

Turn-over of leaves and lesions. In 1992 and 1993, plants grew from 5 leaves in July to 8-10 leaves in September (Table 4.3). In 1992, the full-grown plants had more leaves than in the same month of 1993, probably because plant growth was retarded stronger by the earlier onset of the epidemic in 1993. From September onwards, the number of leaves decreased slowly, in spite of some fresh growth.

The average lifetime of leaves LN was 303 d in October 1992-February 1993, and 124 d in September-November 1993. The average lifetime of diseased leaves LD was 32 d in September-November 1993 on 600 other plants (data not shown). The average lifetime of leaf units LU was 169 d in September-November 1993, whereas the average lifetime of diseased leaf units LX was 21 d, on 45 plants (Table 4.4)

Disease profiles over leaves. (Fig. 4.6) In week 40 of 1992, the curves were very close to the line y=x, indicating that lesions were randomly distributed over leaves (Daamen, 1989). The deviation from this line in week 44 of 1992 indicates that the youngest leaves had relatively few lesions.

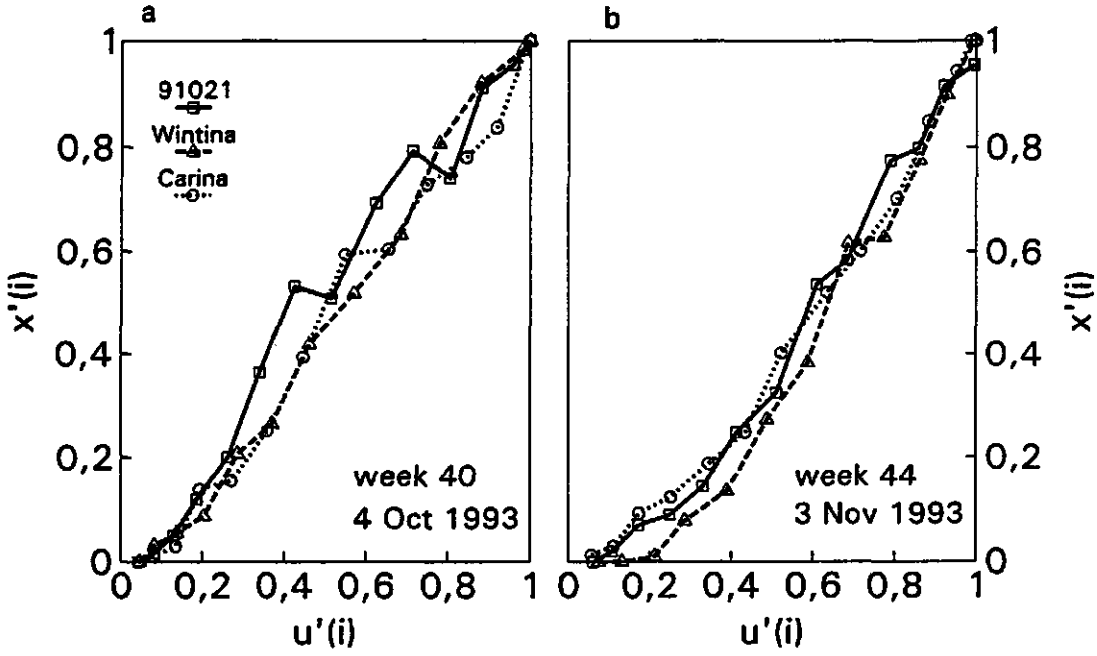

Fig. 4.6 Disease profiles over leaves in week 40 (a) and 44 (b), 1993. The standardized cumulative number of diseased leaves x'(i) is plotted against the standardized cumulative number of leaves u(i) from i = 1 (top leaf) to i = max (bottom leaf)

Disease profiles over distances along the leaf. (Fig. 4.7) At 10-20 cm above the soil surface, measured along the leaf, the lesion density was relatively high, especially during an infection wave. This indicates that many infections start in the water basin that is usually present near the leaf axils. Lesions will appear at some distance above this water basin (Fig. 4.4) because of leaf growth during the incubation period.

Relatively high disease frequencies were also found at leaf tips of leaves longer than ca. 75 cm, indicating that infections may result from direct contact between infested soil and leaf tips.

Causes of lesion death. The causes of lesion death were observed for 1379 newly diseased leaves on 600 plants in September-November 1993. At the end of the five week period 569 of these newly diseased leaves either disappeared totally (284 leaves) or just lost the *P. porri* lesion (285 leaves), either through partial leaf death (mainly through loss of necrotic leaf tips), or through invasion by other fungi, (mainly *Pleospora herbarum* (Fries) Rabenhorst), or through desiccation of the lesions.

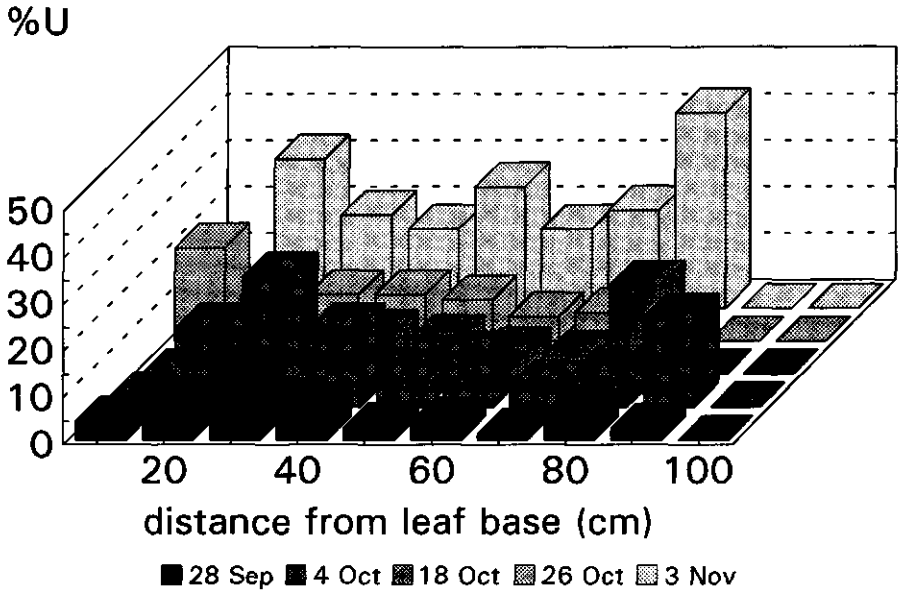

Fig. 4.7 Disease profiles over leaf distances in weeks 39-44, 1993. The lesion density, expressed as percentage leaf units with disease, is given as a function of distance along the leaf, measured from the soil surface.

4.4 Discussion

Inoculum sources and effectiveness. Oospores are supposedly the main source of initial infection in autumn, as they are probably the only fungal structures that can survive in leek-free soil for some months. Oospores are formed abundantly in some, but not in all infected leaves, and are always found in pure cultures on nutrient-rich media. The requirements for oospore production under field conditions are still unknown. As long as oospores are embedded in host tissue they cannot cause new infections, so it is unlikely that oospores play a major role for the build-up of secondary inoculum within one cropping period.

Sporangia on the surface of infected leaves may outnumber the perennated oospores and become the most important infection agents as soon as the first light-green symptoms appear in wet weather. The dominant role of foliar inoculum for intensification of disease was demonstrated in 1994, when the delayed infection in the plot without soil-infestation probably stemmed from foliar inoculum generated in the other plots at >2 m distance.

The observed absence of sporangia on dry leaves does not preclude a dominant role for these spores, as prolonged dry conditions were scarce. Nevertheless, we suppose that in exceptional cases lack of sporulation may limit disease progress and that then oospores may be needed to restart an epidemic.

Sporulation may be poorly related to the number of diseased leaves, since many diseased leaves may never produce spores, whereas the leaves with the most abundant sporulation may easily pass unnoticed. The water status of leaf debris and soil may prove to be better estimators of sporulation than leaf wetness or air humidity (Weste, 1983). Unfortunately, direct measurement of soil-borne inoculum of *P. porri* is difficult, as standard selective media, containing the *Pythium* inhibitor hymexazol, inhibited growth of *P. porri* strongly (Ho, 1987).

High lesion densities of leaf units at 10-20 cm above the soil surface measured along the leaf and of leaf tips indicate that most infections depend on free water, which is almost continuously present in the water basin at the leaf axils or in puddles on the soil surface. Thus, after heavy rainfall the conditions for sporulation and infection will be good. This indirect effect of rainfall may improve a correlation between disease and rain caused by primarily by rain splash.

Shape of the epidemic curves. The epidemic curves obtained in our experiments typically showed a sudden, strong and spatially homogeneous ('inundative') onset of disease and a further epidemic development characterised by short periods of explosive disease increase, alternated by periods of marked disease decrease 3-5 weeks after an infection wave. Because of the inundative onset, the epidemic skipped the exponential phase which is normally expected in a polycyclic epidemic (Zadoks and Schein, 1979) almost

totally. Only in 1992 there appears to be some evidence of inoculum build-up, as the rain rates causing an infection wave in weeks 31, 39 and 45 were ca. 8, 5 and 4 $mm.d^{-1}$, respectively, indicating that later in the season less mm rain is needed per new infection than earlier in the season.

The infection waves are supposedly associated with earlier rain events. Our analysis of the rain data, using a degree-day model to determine a putative infection period corresponding to an observed disease increase, confirms this hypothesis, but cannot give information about the relative importance of possible causes of this correlation, which include spore discharge and dissemination by rain splash, and better conditions for sporulation and infection.

Our analysis allows the identification of factors that disturb the relation between rainfall and infection. We conclude from Fig. 4.3 that the correlation between rainfall and disease is relatively high at the onset of an epidemic and relatively low later in the season. Apparently, rainfall is necessary for initial infection, but less important for subsequent auto-infection.

Correlation between rain and disease was also poor 3-6 weeks after infection waves e.g. in week 40, 1993, when a high rainfall rate produced a rather small disease increase. Considering that shortly after infection waves most lesions are of the same age, and that the average lifetime of lesions was 21-38 d, lack of correlation between rain and disease may be due to a wave of lesion death 3-6 weeks after a rain event. Thus, leaf and lesion dynamics should be studied to get a better insight in the epidemic.

Although our study of leaf and lesion dynamics was limited, it highlighted the fact that the disappearance of diseased leaves was not only caused by leaf death but also by several processes causing lesion death or disappearance (leaf tip necrosis, secondary infection, desiccation). As these processes are all related in their own way to weather and plant factors, prediction of lesion death seems to be difficult.

In their terminal phase, epidemics of *P. porri* never approached the theoretical maximum of 8-9 diseased leaves, in spite of the inundative onset. It is not clear what factor determines the apparent maximum number of diseased leaves. Possibly, the epidemic just slows down at lower temperatures. This retardation may explain the slow but steady increase from 1-4 diseased leaves in weeks 43-65 after 1-1-1992, and is also illustrated by the fact that resistance appears to have a constant effect during this period. The maximum disease level of ca. 6 diseased leaves in the exceptionally wet winters of 1993 and 1994 cannot be explained in this way. The difference with the theoretical maximum may be explained by the relatively small size and protected position of the youngest leaves. In spring the disease level declines, probably because plants start to grow faster under the influence of higher temperatures.

Disease control. Control of *P. porri* by preventive spraying with protectant chemicals is not economically justified, because of the unpredictable onset of epidemics, according to Van Bakel (1964). He advises to postpone the first spray treatment until the appearance of the first symptoms. In this way the costs of spraying may be minimized, but control of *P. porri* will be insufficient when the onset of disease is explosive, as in 1992. Unnecessary treatments may be applied when white leaf tips are caused by drought stress or by genetical disorders which are mistaken for symptoms of *P. porri*. Therefore, extension workers often recommend to spray only when rain is expected in the next week (W. Alofs, pers. comm.), except when rain is expected within 24 hours and rain would wash the fungicide from the leaf surface. We can add tentatively that a forecasted 20 mm rain in one day may justify a preventive spray as long as symptoms of disease are absent in an infested field. After observation of the first few symptoms 20 mm rain in 2-3 days may be enough to initiate an infection wave. However, local showers are hard to predict, even on the short term, so a spraying-scheme based on weather forecasts will never be reliable. Moreover, the effect of chemical control is, in general, limited when the onset of an epidemic is explosive. Other control methods, based on prevention of infection through mulching (Alofs & Pijnenburg, 1988) or host plant resistance (chapters 6 and 7) are therefore needed.

Chapter 5

Exponential growth and focus expansion of a splash-borne pathogen *(Phytophthora porri)* in leek crops.

Summary: The spatial and temporal development of *Phytophthora porri* on leeks was studied in artificially infected fields. Disease sources of various shapes and sizes were created in eight adjoining plots of 10 x 10 m in 1991 and 1993. An exponential model was fitted to disease progress curves. The doubling time of disease was ca. 20 days in December. The disease progress curves converged, and uninfected control plots became infested at the end of the season, indicating interplot interference. In spite of severe infestation at the end of the season, epidemics did not enter the saturation phase. Low temperatures in December did not slow down the epidemics.

Disease gradients were analysed by the negative exponential model and the inverse power model. The gradients were initially steep and gradually flattened. The half-distances of the gradients increased from <35 cm in October to >140 cm in December. The displacement velocity of the focal front, which could only be estimated early in the epidemic, was ca 3 $cm.d^{-1}$. Breeders planning field trials to select for resistance under climatic conditions as occur in the Netherlands are recommended to initiate epidemics on at least 2 % of the plants in spreader rows at most 3 m apart, in the first half of September. Such trials should be laid out on well-drained, homogeneous fields.

5.1 Introduction

White tip disease of leek (*Allium porrum* L.), caused by *Phytophthora porri* Foister, is a leafspot disease which causes severe economic losses in northern Europe (Foister, 1931; Van Hoof, 1959; Grill, 1985; Dobson & Clarkson, 1989; Kautny & Köhler, 1994). A similar leafspot disease is known in winter-grown onions in the southern part of Japan (Katsura, 1969; Yokoyama, 1976). The water-logged zones which frequently appear around the characteristic papery white foliar lesions may sporulate profusely after prolonged wetness periods. Most infections occur during rainy periods (Chapter 4), arising either from perennating oospores or fresh sporangia formed on diseased leaves. Oospores are probably the most important survival structures during the crop free period. Disease foci of a circular (Ogilvie & Walton, 1941) or 'odd' shape (Foister, 1931) were reported from naturally infested fields. An irregular, shallow disease gradient extending

over ca. 9 m was recorded by Yokoyama (1976). In Dutch cultivar and mulching trials the disease is notorious for its irregular, patchy development (J. Jeurissen, pers. comm.).

Splash dispersal of plant diseases has been studied in increasing detail since about 1979 (Madden, 1992), but field studies of spatial and temporal aspects of splash-dispersed pathogens are still scarce. The insight obtained from such studies may be relevant for estimating the effect of interplot interference e.g. in breeders' trials with partially resistant genotypes, for identification of sources of natural inoculum, for the development of mulching techniques, for disease prediction from rain parameters and for phytosanitary recommendations e.g. on isolation distances (Fitt *et al.*, 1989).

In the present study the temporal and spatial development of *Phytophthora porri* was investigated. The field data were analysed assuming either a negative exponential model (Van den Bosch *et al.*, 1988) or an inverse power model (Ferrandino, 1993) for the gradient tails. Line, area, and point sources were used to study the effect of source size and shape on the gradients. The results are discussed in relation with the design and interpretation of factorial field trials.

5.2 Materials and methods

Fields. Experimental fields consisting of eight plots of 10 x 10 m were laid out in 1991 and 1993. In each plot 15 rows of 60 plants were planted at a distance of 15 cm within rows, 50 cm between rows within beds and 75 cm between beds of three rows. Most plots were planted with cv. Carina, sown in April and transplanted in June. One plot was planted with the partially resistant breeding line PI 368351 (cf. Fig. 5.1, 'Line 3'). Because of a shortage of April-sown plants, two plots were planted with plants that were sown in May and transplanted in August ('Control 1' and 'Control 2', 1993).

The soil was assumed to be free of inoculum because no diseased leek had been grown in previous years on the same and nearby fields in 1991 and because the soil was sterilized by steam shortly before the experiment in 1993. The risk of cross-contamination between plots was reduced by means of a dense row of Brussels sprouts and a 3 m wide path along the long axis of the field in 1991, and by placing 0.5 m high straw bales along the borders of each plot in 1993.

Inoculation. In 1991, plants were inoculated on 26 August with a zoospore suspension injected into the shaft with a syringe, and on 2 and 9 September by dripping a zoospore suspension into the shafts of the plants. Repeated inoculation was considered necessary because of the poor result of the first inoculation. In 1993, a single inoculation on 2 September with zoospores dripped into the shafts was sufficient to initiate the epidemic.

Disease foci of different shapes ('line','area' and 'point') and sizes (60, 9, 5 or 1 plants) were created by inoculation of source plants in the centre of the field in six of the

eight plots per field (Fig. 5.1, Table 5.1). Two plots per field were not inoculated and served as controls.

Gradient analysis. The development of disease was monitored at intervals of 1-3 weeks by counting the number of diseased leaves per plant, usually on one day, but in December and January the assessment of the whole experiment extended over several days.

Disease gradients from point and area sources were calculated as the average number of diseased leaves per plant for a series of contiguous rings with a width of approximately 50 cm and mean radii of 32, 86, 125, 165, 234, 279, 345 and 390 cm. The source area was considered to be part of the central rings located at an average of 32 cm from the source. The number of plants per ring increased from a minimum of 12 plants in the central ring to a maximum of 142 plants at 345 cm distance.

Disease gradients from line sources were derived from the average number of diseased leaves per row of 60 plants at 50, 125, 175, 225, 300, 350 and 400 cm distance from the source row in west-southwest (WSW) downwind direction, and east-northeast (ENE) upwind direction.

Disease gradients, averaged over the replicates, were calculated for the point, area and line sources in 1991 and point and line sources in 1993. The WSW and ENE gradients of line source plots were pooled before averaging the gradients over replicates.

Two models were fitted to the individual gradients and averaged gradients with disease scores at four or more distances: the inverse power model $y=a.s^{-b}$ and the negative exponential model $y=a.e^{-b.s}$, in their linearized form (Campbell & Madden, 1990; McCartney & Fitt, 1985)

$$\ln(y) = \ln(a_P)-b_P.\ln(s) \qquad \text{(inverse power)}$$

$$\ln(y) = \ln(a_E)-b_E.s \qquad \text{(negative exponential)}$$

where y = mean number of diseased leaves per plant, s = distance from source in cm; a_P and a_E are intercept parameters and b_P and b_E are slope parameters, estimated by linear regression. The subscripts P and E refer to the inverse power model and negative exponential model, respectively. The exact interpretation of a and b differs among the models: a_P = number of diseased leaves at 1 m from the source, a_E = number of diseased leaves in the source, b_P = dimensionless slope parameter (independent of scale of measurement), $b_E = \ln(2)/s_h$, with s_h = half-distance; the dimension of b_E is length^{-1}. Larger values of b describe steeper gradients in both models. Flattening of gradients is described by a time-series of b-values approaching zero.

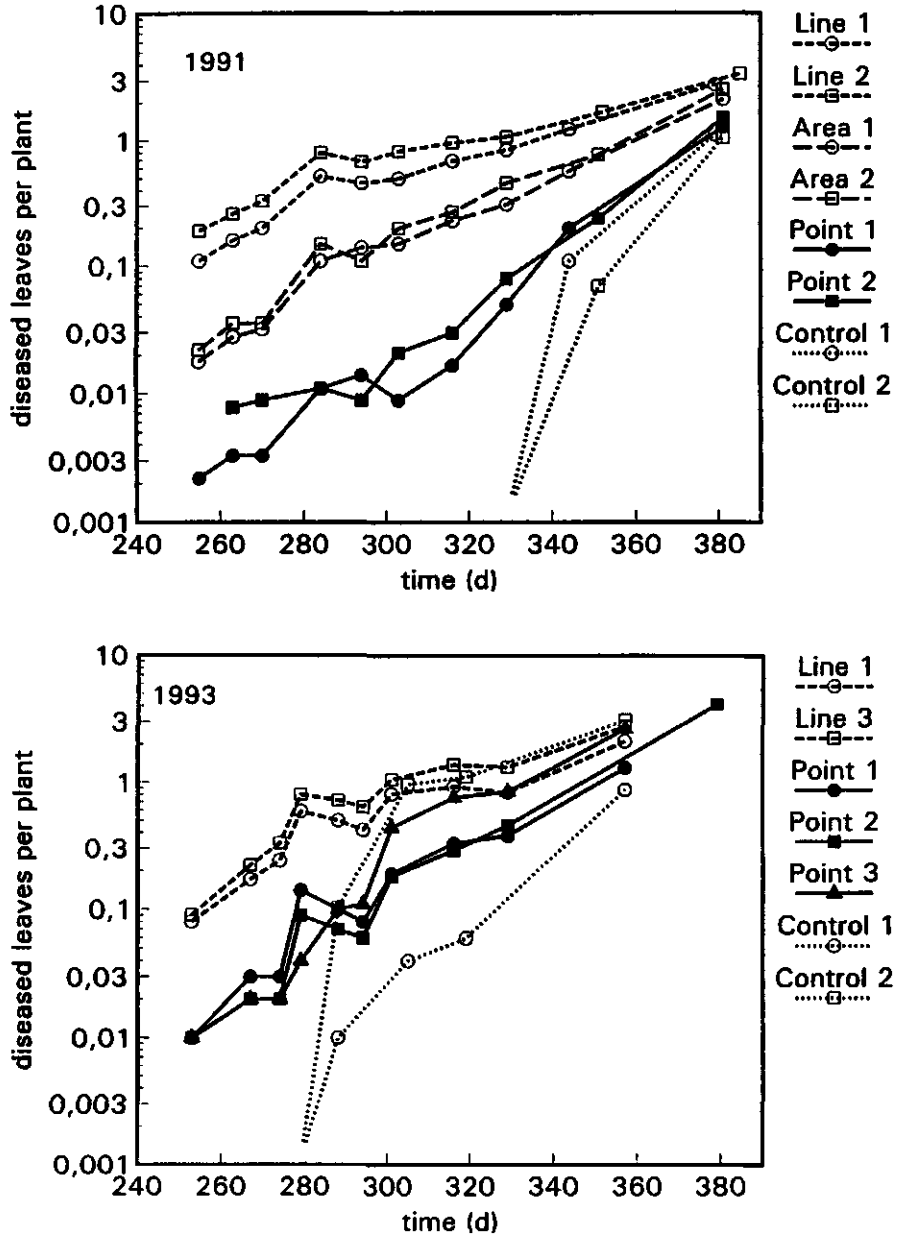

Fig. 5.1 Disease progress curves of *Phytophthora porri* per plot in 1991 and in 1993. The onset of disease in control plots is estimated by assuming that disease was just below the detection level (0.001 diseased leaves per plant) at the time of the last zero-observation. Time is expressed in days from January 1 of the first year

Table 5.1 Mean exponential growth rates (r_E) and their standard deviations (σ_r) after 25 November in plots with disease sources of different shapes, number of inoculated source plants, and number of replicated fields.

Year	Plot	Shape of source	Source plants	Replicates	$r_E \pm \sigma_r$ ($10^{-3}.d^{-1}$)
1991	Line	10 x ½ m	60	2	22 ± 2
"	Area	1 x 1 m	9	2	35 ± 2
"	Point	0.2 x 0.1 m	1	2	59 ± 2
"	Whole field	-	140	1	35
1993	Line	10 x ½ m	60	2	32 ± 5
"	Point	0.3 x 0.3 m	5	3	43 ± 3

Regression analysis of the linearized models was done with the statistical package GENSTAT 5.2. The models were compared by means of graphs of the fitted gradients and by means of the R^2 value adjusted for degrees of freedom, which is an estimate of the percentage variance accounted for by the model (Genstat 5 Committee, 1987).

5.3 Results

Temporal analysis of epidemic growth. The log-transformed growth curves of the epidemics of all plots with cv. Carina are given in Fig. 5.1. In spite of initial fluctuations in disease level, the curves conform reasonably well to an exponential model ($y=a.e^{x.t}$) even at the end of the season, as is indicated by their apparent linearity.

In the first part of the epidemic season, before day 320 (November 15) in 1991 and before day 275 (October 1) in 1993, the curves ran parallel, while later they converged. The epidemic growth rates in point source plots were higher than in line source plots (Table 5.1). The convergence of the curves suggests interplot interference.

The epidemic progress was also analysed for the whole field, including control plots. The whole-field epidemic was initiated on ca. 2% of the plants (140 of 7200 plants) in 1991. The exponential growth rate of the whole field describes disease progress without inflow and with relatively little outflow of inoculum. This parameter was estimated as r_E = 0.035 diseased leaves per diseased leaf per day in December 1991 (Table 5.1). The corresponding doubling time was ln(2)/0.035 = 20 d.

A saturation effect, leading to a slow-down of the epidemic above a certain disease level, was not detected. The highest disease level observed was ca. 3 diseased leaves per plant, which is far below the theoretical maximum (the average total number of leaves per plant) of 9 leaves through autumn and winter, and well below the observed maximum of ca. 6 diseased leaves per plant in heavily infested small plots (Chapter 4).

Saturation effects are probably more important when more than 3 leaves per plant are infected, but this did not happen.

Low temperatures in December-January must have caused longer incubation periods, and could have caused a decreasing epidemic growth rate. Latency periods were 7-14 d in September-November, and 14-28 d in December-January (Chapter 3). Other conditions being equal, one would expect that r_E in September is twice as high as in December. However, other conditions seem to have compensated this effect, leading to a constant r_E.

Control plots. In 1991, relatively little disease was recorded in the non-inoculated control plots. The infection of the control plots apparently originated from the neighbouring inoculated plots (Fig. 5.2). This was especially clear in Control 1, where an invasion of disease coincided with a gap in the row of cabbages along the border of the plot. In Control 2 two disease foci arose between 25 November and 10 December. In January, both control plots were almost as much infested as the plots with point sources.

In 1993, both control plots were heavily infested from October onwards. In Control 1, disease increased in a more or less regular way until it reached almost the same level as the nearby plots with point sources. In Control 2 the level of disease increased explosively, and eventually grew higher than in all other plots. In the southwest corner of Control 2, where the tractor which punched the planting holes had made a double rut, disease was most severe. Here, and to a lesser degree in the other plots of the southern half of the fields, the paths were regularly flooded for weeks. These observations provide anecdotal evidence that local variation in the draining capacity of the soil is responsible for the formation of odd-shaped foci. Obviously, the extent and behaviour of such foci cannot be modelled with dispersal models that presuppose a homogeneous space.

Effect of resistance. In November 1993, only three diseased leaves were observed in the plot planted with PI 368351. These diseased leaves were found at 50 cm distance from the source. As the plants of PI 368351 turned out to be sensitive to frost, many plants died in the first week of December 1993. At the same time a general epidemic of *P. porri* appeared in the plot, suggesting a loss of resistance after frost. The epidemic growth curves and disease gradients of PI 368351, which could not be analysed in the same way as those of cv. Carina, were therefore discarded.

Disease gradients. Fig. 5.2 shows the disease maps based on the assessments of December 10-18, 1991, and November 25, 1993. The log-transformed average disease gradients, fitted to the inverse power model, are plotted in Fig. 5.3. The negative exponential model would yield straight lines on the same axes. Parameter estimates for both models are given in Tables 5.2 and 5.3.

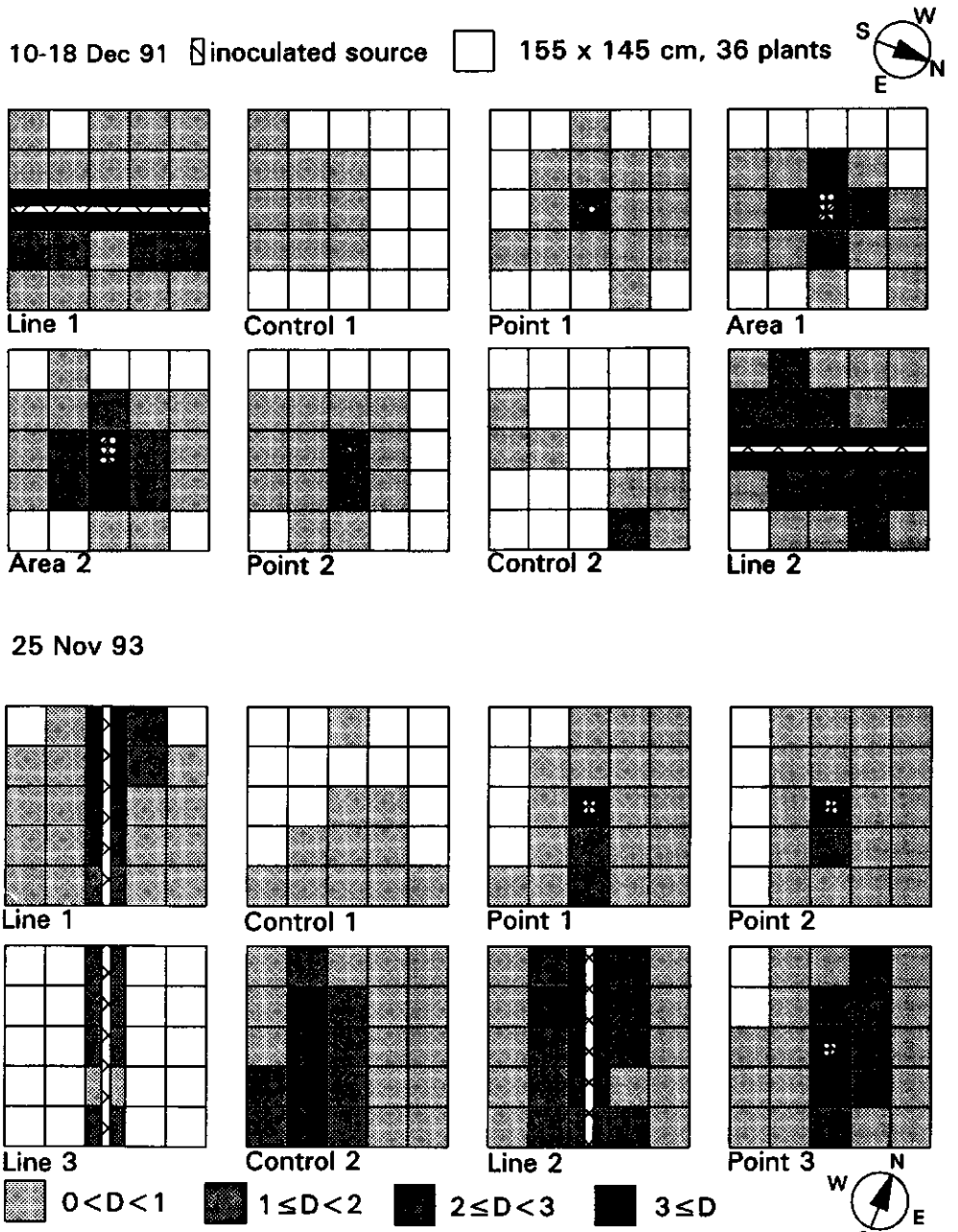

Fig. 5.2 Disease maps of *Phytophthora porri* in 40 x 20 m leek fields, divided into eight 10 x 10 m plots. a. Situation on 10-18 December, 1991. b. Situation on 25 November, 1993. The location of inoculated source plants is indicated in the plots. D = average number of diseased leaves of 36 plants. Line = line source of 60 plants; area = area source of 9 plants; point = point source of 1 (1991) or 5 (1993) plants

Table 5.2 Estimated parameters for the linearized inverse power and negative exponential models, fitted to the mean gradients per source type and time of observation; a= intercept; b= slope; R^2_{adj} values, adjusted for degrees of freedom, indicate the percentage of variance accounted for; ns = non-significant slope parameters (P>0.05); df = degrees of freedom for regression

Source	Dates	Inverse power model			Negative exponential model			df	Best Model[a]
	dd.mm.yy	ln(a)	-b	R^2_{adj}	ln(a)	-b	R^2_{adj}		
1991/92									
Line	30.10.91	-1.22	-3.23	90	1.40	-2.24	79	3	P
	12.11.91	-0.74	-2.41	70	0.71	-1.35 ns	48	4	P
	25.11.91	-0.04	-2.38 ns	63	0.46	-1.01 ns	52	3	P
	10-18.12.91	1.06	-2.00	75	1.62	-0.91	76	3	(E)
	14-20.01.92	1.55	-0.74 ns	36	1.79	-0.35 ns	42	3	(E)
Area	11.10.91	-0.33	-1.38 ns	53	1.85	-1.95 ns	79	2	E
	12.11.91	1.06	-4.71	95	3.35	-2.52	96	4	(E)
	25-26.11.91	1.34	3.48	97	2.81	-1.72	99	5	(E)
	11-17.12.91	1.53	2.25	94	2.45	-1.10	93	5	(P)
	16.01.92	1.72	-0.76	93	2.03	-0.37	94	5	(E)
Point	11.10.91	-2.89	-2.48 ns	81	0.77	-3.25	92	2	E
	12.11.91	-2.46	-2.62	71	-0.25	-1.75	56	5	P
	25-26.11.91	-0.84	-2.64	93	0.07	-1.21	80	5	P
	11-17.12.91	0.13	-1.79	85	0.74	-0.82	73	5	P
	15-17.01.92	1.19	-0.67	84	1.43	-0.31	72	5	P
1993									
Line	15.10.93	-1.10	-2.35 ns	68	1.81	-2.55 ns	86	1	E
	28.10.93	0.05	-1.08	95	1.00	-0.79	97	3	(E)
	12.11.93	1.05	-2.11	77	1.62	-0.93	89	4	E
	25.11.93	1.03	-1.88	74	1.54	-0.83	86	4	E
	21.12.93	1.56	-1.12 ns	50	1.93	-0.52	66	4	E
Point	28.10.93	-0.49	-2.58	90	0.38	-1.17	76	5	P
	25.11.93	0.73	-1.18	90	1.13	-0.54	76	5	P
	21.12.93	1.56	-0.89	95	1.92	-0.43	93	5	(P)

[a] The best model is the model with the highest R^2_{adj}. P = inverse power model , E = negative exponential model. Brackets indicate that the R^2_{adj} values of the two models differ less than 10 percentage points.

Table 5.3 Estimated parameters for the linearized negative power and negative exponential model, fitted to all gradients observed at 10-18 December 1991 and 25 November 1993; ENE = east-northeastern direction; WSW = west-southwestern direction

	Inverse power model			Negative exponential model			Best Model[a]
Gradient	ln(a)	-b	R^2_{adj}	ln(a)	-b	R^2_{adj}	
1991							
Line 1-ENE	0.35	-1.69	69	0.68	-0.71	54	P
Line 1-WSW	1.10	-2.28	79	1.62	-0.98	68	P
Line 2-ENE	0.99	-1.65	50	1.56	-0.80	61	E
Line 2-WSW	1.68	-2.79	51	2.57	-1.32	58	(E)
Area 1	1.42	-2.53	94	2.53	-1.26	100	E
Area 2	1.64	-2.15	88	2.48	-1.03	84	(P)
Point 1	-0.09	-1.70	68	0.44	-0.76	52	P
Point 2	0.26	-1.89	88	0.95	-0.89	79	(P)
1993							
Line 1-ENE	2.91	-5.94	69	4.55	-2.64	80	E
Line 1-WSW	1.14	-3.67	33	2.49	-1.76	51	E
Line 2-ENE	2.34	-4.00	36	3.71	-1.88	52	E
Line 2-WSW	1.12	-1.34	60	1.40	-0.56	60	-
Point 1	0.65	-1.70	97	1.27	-0.79	88	(P)
Point 2	0.52	-1.19	49	0.79	-0.49	30	P
Point 3	0.96	-0.96	95	1.34	-0.46	92	(P)

[a] The best model is the model with the highest R^2_{adj}. P = inverse power model , E = negative exponential model. Brackets indicate that the R^2_{adj} values of the two models differ less than 10 percentage points.

The figures and tables show that the disease gradients were usually not parallel. In 1991 they were initially steep (b_P<-2 and b_E<-1.5 m^{-1}), and became gradually flatter at the end of the season (b_P>-1 and b_E>-0.5 m^{-1}). Half-distances at 1-4 m from the source increased from <35 cm in October to >140 cm in December. In 1993 the gradients were less steep than in 1991 at the same dates, but the gradients also flattened between October and December 1993.

Both models fitted well to most gradients. For the point sources the inverse power model was slightly superior, but for the line sources in 1993 the negative exponential model was better. Line source gradients were not consistently flatter than point or area source gradients. In 1991, the downwind (ENE) gradients from line sources were less steep than the upwind (WSW) gradients, but in 1992 the reverse was true.

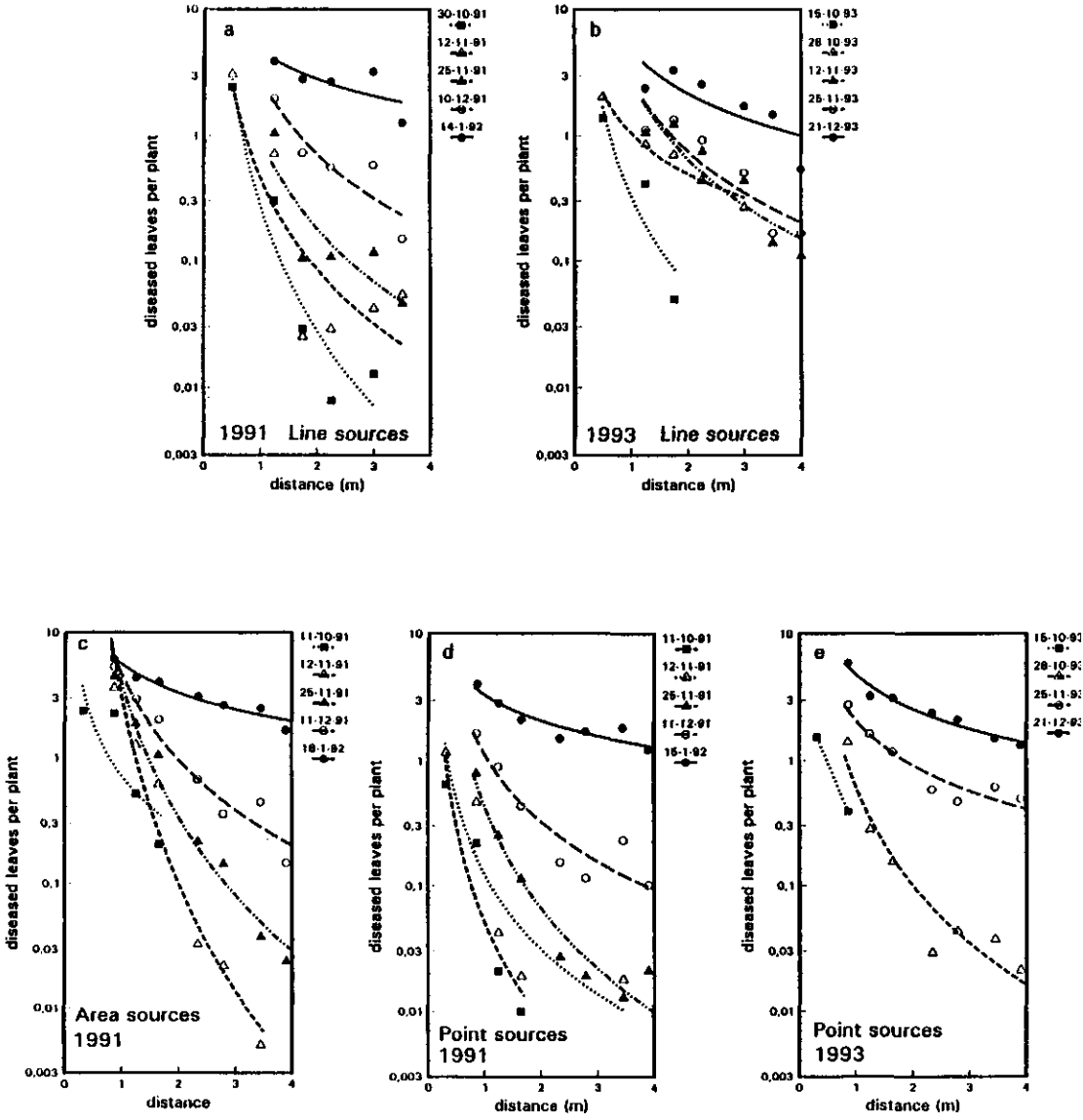

Fig. 5.3 Averaged disease gradients in fields with a. a point source of 1 plant in 1991 (two fields) b. a point source of 5 plants in 1993 (three fields), c. an area source of 9 plants in 1991 (two fields), d. a line source of 60 plants in 1991 (two fields), e. a line sources of 60 plants in 1993 (two fields).

5.4 Discussion

Epidemic progress of *P. porri* epidemics appears to be determined mainly by splash dispersal. The strong fluctuations in disease level in early autumn are correlated to rain events, as was shown in a field study with natural inoculation (Chapter 4). If splash dispersal is the key to understanding temporal development, then this mechanism should also give a clue to understanding spatial development. The splash mechanism is probably responsible for detaching spores from infectious tissue or soil. Horizontal spread of splash dispersed diseases may be limited to a relatively small area near a source, as the ballistic trajectories of splash droplets will carry the diaspores not further than ca. 1.5 m, but a turbulent air stream may carry splash droplets much further, especially the smallest droplets, which form a splash-incited aerosol (Gregory *et al.*, 1984)

Spatial development of splash-borne diseases may be approached mathematically as a diffusion process (Yang *et al.*, 1991). Several authors have elaborated models for diffusion-like dispersal of air-borne pathogens in combination with logistic growth (Zadoks & Kampmeijer, 1977; Minogue & Fry, 1983; Van den Bosch *et al.*, 1988; Ferrandino, 1993). In general, it is difficult to validate these models with field data. For splash-borne diseases modelling may be easier than for air-borne diseases because only the two horizontal dimensions are important, and field studies may be easier because of the shorter dispersal distances. On the other hand, models of splash-dispersal may be more complex, and field studies more difficult, because of the greater influence of spatially or temporally heterogeneous processes, e.g. canopy drip and rain showers.

In our experiments, the flattening of gradients may be ascribed to interplot interference: a net transfer of inoculum from the plots with more disease to the plots with less disease, ultimately leading to equal amounts of disease in all plots of a field. This explanation agrees with the observation of converging epidemic growth curves and dispersal to control fields over 5-10 m distance.

The theory of Van den Bosch *et al.* (1988), postulating gradients moving outward at a constant velocity with a constant slope was not confirmed. Only in the first part of the epidemic in 1991 (before day 320) the inoculum exchange between the plots might have been small enough to allow an estimation of the constant displacement velocity of the focal front, which is approximately 3 $cm.d^{-1}$ (Fig. 5.3). Our results suggest that the dispersal mechanism that is responsible for this relatively low focal expansion rate becomes less important later in the season when 'blanket' infection from more distant sources occurs, in the form of interplot interference.

It is likely, but not proven, that some inoculum escaped from a short-distance mechanism (e.g. ballistic dispersal), and was transported by a second mechanism (e.g. wind dispersal) with a flatter dispersal gradient. This so-called dual dispersal mechanism (Zawołek & Zadoks, 1992) implies gradient flattening. Gradient flattening also follows from the theory of Ferrandino (1993), assuming dispersal by atmospheric turbulence

which increase in size with distance from the source (Sutton, 1932). To examine the influence of wind or, more generally, of atmospheric turbulence on dispersal of splash-borne disease, large plots are required to prevent outflow of spores; these plots must be free of inflow of spores and have a spatially homogeneous soil structure. Splash-borne diseases appear to have a spatial scale of focus formation of the same order of magnitude as air-borne diseases.

The described field experiments reflect the natural development of epidemics from relatively few infections in large fields in the absence of control measures. The observed epidemics had a focal pattern initially, and gradually developed towards a pattern which was probably determined by the draining capacity of the soil and the susceptibility of the crop. Initial infection of ca. 2% of the plants in September resulted in severe epidemics in December, and to total crop loss in economic terms in January. It should be understood that leek plants with one or more *P. porri* lesions near the centre of the plant cannot be auctioned. If the lesions are near the leaf tips they should be removed manually before selling the plants.

In December, the initial gradients had become shallow, but they were still visible. This observation demonstrates the importance of an even distribution of initial inoculum in breeding trials. For breeding trials under climatic conditions as prevail in the Netherlands it is recommended to initiate an epidemic on at least 2 % of the plants in September, e.g. in spreader rows at 3 m distances at most. Disease should not be assessed early in the season, as ca. 2 months are needed for the development of an evenly distributed epidemic. Usually, early December will be the best time for disease assessment, provided that no severe frosts occur earlier. The precision of resistance evaluation will depend then on the homogeneity of the soil structure. Therefore, it is recommended to use only well-drained fields for resistance evaluation.

Chapter 6

Resistance to *Phytophthora porri* in leek and some of its wild relatives

Summary: Winter leek and related plant material was evaluated for resistance to white tip disease (*Phytophthora porri*) in the Netherlands. Significant differences in partial resistance were found between winter leek accessions in field tests with zoospore and natural inoculation. These differences were consistent over three years, and indicate that resistance can be improved through mass selection in winter leek cultivars. Some accessions of wild or cultivated relatives of winter leek with higher levels of resistance were found in a glasshouse test with zoospore inoculation. These sources of resistance were also highly resistant in field experiments over a two year period.

6.1 Introduction

Phytophthora porri Foister is a splash-dispersed leaf pathogen which causes considerable losses in leek (*Allium porrum* L.), especially in the United Kingdom (Foister, 1930), Belgium, and the Netherlands (Van Hoof, 1959), where the crop is grown intensively. Severe epidemics occur in autumn and winter, when cold and wet weather conditions favour the disease. The disease causes an increased handling time at harvest and reduces the quality of the crop. In winter, chemical control is often ineffective because of low temperatures, and expensive because of long cropping periods. Resistant varieties can provide an alternative to chemical control.

Reliable data on resistance to *P. porri* are scarce. No information is available about resistance to *P. porri* in relatives of leek. Ogilvie & Mulligan (1931) were the first to screen a group of 17 cultivars, indicating that no resistance was present in leek. More recently, Vanparijs & Bockstaele (1984) described different levels of attack by *P. porri* in Belgian cultivar trials with severe natural infestation, without drawing any conclusions about resistance. In Germany, cultivar trials also failed to reveal differences in resistance (Heine, 1990). The Dutch cultivar list indicates resistance to leaf fleck diseases in general, but not to *P. porri* in particular (Anonymous, 1993).

Leek is a self-compatible, outbreeding tetraploid with little tolerance to inbreeding (Pink, 1993). Because of this, leek varieties cannot meet strict requirements of uniformity, distinctness and stability, and legal authorities in the EU only recognize so-called umbrella-varieties (Van Marrewijk, 1988). These umbrella-varieties consist of a group of

named selections, maintained by breeders, delimited by the broad description of the umbrella variety. In this article named selections will be called cultivars.

Leek breeding is based on mass and family selection (Van der Meer, 1990). Breeders do not generally make crosses between umbrella varieties, especially when they aim for slow growing late winter types. Thus, all cultivars of the late winter umbrella-variety 'Blauwgroene Winter' can be considered as genetically narrow populations stemming from the same original population, though each still contains enough variation to avoid inbreeding depression.

The purpose of this study was to assess the perspectives for resistance breeding against *P. porri* with and without broadening the genetic base of modern Dutch winter leek. During this study, only partial resistance was studied, i.e. a type of resistance which reduces the susceptibility of plants, but still allows infection. Complete resistance has not been observed.

6.2 Materials and methods

Resistance to *P. porri* was evaluated in glasshouse and field experiments over a three year period (Table 6.1).

Glasshouse experiments were conducted at temperatures between 10 and 20°C. Plants were sown 3-6 months before the first inoculation and were grown in plastic pots (11 x 11 cm).

For field experiments plants were sown in April in sowing beds and transplanted into plant holes of ca. 15 cm depth in fields at Wageningen (CPRO-DLO, 'De Goor') in the first week of July. Fertilizer was applied following professional advice based on soil samples taken in June. Leek rust (*Puccinia allii*) and onion thrips (*Thrips tabaci*) were controlled by spraying two or three times with Topaz (penconazol) and Decis (deltamethrin), respectively. Plant distance within rows was 12 cm, between rows within plots 50 cm, and between rows between 3-row plots 75 cm.

Plant material. All tested accessions (Table 6.2) belong to the CPRO-DLO *Allium* collection, obtained from gene banks, botanical gardens, and individual taxonomists. All tested species, except *A. tuberosum*, belong to the section *Allium* of the genus *Allium* (Traub, 1968). CGN numbers were obtained from the Centre for Genetic Resources, Wageningen, the Netherlands; PI numbers from the Western Regional Plant Introduction Station, Washington, USA. Most of the tested PI numbers belonging to *A. ampeloprasum* were originally collected in the southern part of the former Yugoslavia in 1971 (USDA, 1972) and multiplied through seed at CPRO-DLO in 1990.

Two accessions (*A. commutatum* 92024 and *A. ampeloprasum var. lussinense* 92018) were included (Table 6.6) because of their resistance against leek rust (unpublished data).

Table 6.1 Summary of the experiments. Inoculation methods: zo=zoospore-, oo= oospore-, dt=diseased tissue, nat= natural

Experiment no.	Accessions x plants	Blocks x plants	Sowing times	Inoculation /Observation	Inoculation method
Glasshouse					
1.	48 x 60	3 x 20	Aug '91	Dec-Feb	zo
2.	50 x 32	2 x 12-20	Apr '92	Nov-Dec	zo
Field					
3.	5 x 360	4 x 90[a]	Apr '91	Sep/Nov	zo, oo, dt[b]
4.	8 x 180	4-8 x 45[a]	Apr '92	Aug-Jan	nat
5.	5 x 40	4-8 x 10[c]	Apr '93	Aug-Jan	nat
6.	16 x 60	5 x 12[a]	Apr '92	Sep	nat
7.	18 x 60	5 x 12[a]	Apr '93	Nov	nat

[a] three-row plots

[b] only in middle rows

[c] one-row plots, alternated with spreader rows (Carina or Derrick)

All cultivars, 91021 and 93001 were obtained from breeders. 91021 is a selection from Carina, and 93001 is an unselected progeny of 91021. Wintina is relatively susceptible (pers. comm. T. v.d. Jagt). Portant belongs to 'Blauwgroene Herfst' and Platina is a separate umbrella variety. The other tested cultivars belong to 'Blauwgroene Winter'. Cultivar seeds were taken from the same seed lots throughout the experiments.

Inoculum production. The *P. porri* isolate (E) used throughout was taken from a commercial leek crop in Lienden (NL) in 1990. The fungus was grown on 20% (w/v) leek agar with 1% saccharose at 15°C. Leek agar was made by blending 200 g fresh leek leaves and shafts in demineralized water, boiling with 1 L water, sieving (mesh width 1 mm), diluting the filtrate to a volume of 1 L, and adding the sugar and leek extract to a 1.2% (w/v) agar suspension.

Zoospore inoculum was generated from fresh sporangia (Chapter 2). The sporangia developed in 9 cm Petri-dishes after 3 days of rapid mycelial growth from colonized 4 mm agar discs in a 10% (v/v) V8 broth at 15°C, followed by 2-3 days incubation of the discs with fresh-grown mycelium in a 10x diluted soil extract. Soil extract was obtained by suspending 500 g of soil in 1 l of demineralized water, leaving the suspension overnight and autoclaving the filtrate. Indirect germination of sporangia was induced by a cold treatment at 5°C for 3 h. The density of zoospores in the spore suspension was 10^3-10^5 ml^{-1}.

Oospores were produced by growing *P. porri* in 20% leek agar for 2 months at ca. 17°C. They were harvested shortly before inoculation (Spielman *et al.*, 1989). In the field trial in 1991 the oospore inoculum density was 10^3 ml^{-1}.

Diseased tissue was produced by placing an agar disc with mycelium or one drop of a zoospore suspension on a leaf piece, 5 cm in length, and incubating this at 15 ± 1°C at high humidity for 7 days.

Inoculation. In glasshouse experiments, each plant was inoculated at least once with 1 ml of zoospore suspension, dripped into the shaft of the second or third leaf. When no symptoms were visible after 3 weeks, plants were inoculated a second time.

In 1991, the field experiment was artificially inoculated in three ways: by placing diseased tissue into the standing water at the leaf bases, or dripping a fresh zoospore or oospore suspension (ca. 1 ml) into the shafts. Later field experiments became infected naturally.

Disease assessment. Incidence: in glasshouse experiments each plant was scored daily for the presence or absence of disease symptoms, until no new lesions appeared. The final scores were used for data analysis.

Leaf incidence: in field experiments disease was assessed by counting the number of leaves with at least one *P. porri* lesion per plant. The total number of leaves per plant varied from eight to ten.

Glasshouse experiments. A total of 91 different *Allium* accessions, belonging to 14 different species, was tested in experiments 1 and 2 (Table 6.2).

In experiment 1, 49 different accessions of cultivated and wild relatives of leek were tested in three sequential replications with 20 plants each. In experiment 2, 47 different accessions of leek and relatives were tested, in two sequential replications with 20 plants each. Four leek cultivars and 27 different *A. ampeloprasum* accessions from Yugoslavia were included in this experiment. Some accessions, indicated in Table 6.2, were tested in experiments 1 and 2.

Data from experiments 1 and 2 were analyzed with GENSTAT according to a generalized linear model with a binomial variance function (GENSTAT 5 Committee, 1987). Sequential replications were used as blocks.

Field experiments. Leek cultivars were tested in experiments 3-7 (Table 6.4 and 6.6). Seeds of each cultivar were taken from the same seed lots in different years. Experiments 4 and 5 were planted in 8 blocks, but only four blocks were observed at regular intervals. These data were analyzed by repeated-measures ANOVA (Table 6.5), applied to sequential observations of disease. Homogeneity of variances at different times was checked by Bartlett's χ^2 test (Gomez & Gomez, 1984)

In experiments 6 and 7, 14 different accessions of relatives of winter leek were tested (Table 6.6). Most of these accessions were tested earlier in experiment 1 or 2. Comparisons were made with Tukey's test.

Comparison of field experiments. The precision of field experiments was compared by the LSD (P=0.05) expressed as a percentage of the difference between the most susceptible (Wintina) and the most resistant cultivar (91021). For comparisons (Table 6.7), the LSD was calculated for the same set of five leek cultivars that were included in all field experiments.
This procedure was considered to be better than comparison by coefficients of variation, because the variance of disease scores was more or less constant over the measured range of disease levels (1-4 diseased leaves per plant).

6.3 Results

Glasshouse Experiments. In experiments 1 and 2 disease incidence varied from 15% to 100% (Table 6.2). In Table 6.3 the scores of potential sources of resistance (incidence <50 %) are listed, together with all accessions that were later included in experiments 6 and 7. Standard errors of mean scores per accession did not exceed 7% in experiment 1 (n = 3) and 10% in experiment 2 (n = 2). In both experiments, the mean score was significantly lower in the last replication (Table 6.3, bottom line), probably because of random variation in inoculation success.

All cultivars were susceptible, in spite of their different resistance in field tests. In the last time replicate of experiment 2 less than 80% of 91021 and Wintina was infected, so the precision of this test was relatively low.

An Egyptian land race (CGN 873243) of *A. porrum* was moderately resistant in two replicates of Experiment 1 (Table 6.3). Because of the relatively high disease level in these replicates, this accession was classified as highly resistant in the overall analysis.

Of 47 different accessions of *A. ampeloprasum* tested in both experiments, 38 originated from the southern part of the former Yugoslavia, of which 22 were highly resistant, and 16 not highly resistant. Nine other accessions of *A. ampeloprasum* were not highly resistant.

One of the six tested accessions of *A. commutatum* was highly resistant and one moderately resistant. *A. bourgeaui, A. pyrenaicum, A. heldreichii,* and *A. atroviolaceum* were not resistant. *A. polyanthum* and *A. sphaerocephalon* were moderately resistant. *A. babingtonii* was highly resistant. One accession of *A. scorodoprasum* was resistant, another was not. Less than 50% of plants of *A. guttatum* ssp. *sardoum* and *A. tuberosum* became infected, probably due to the unkeeled leaves and shafts being too small to hold the inoculum.

Table 6.2 Number of different *Allium* accessions per species in three resistance classes in experiments 1 (n=60) and 2 (n=40)

Resistance class:	High[a]	Moderate[a]	Low[a]	Total
Experiment 1				
A. porrum	1	4	11	16
A. kurrat	-	-	5	5
A. ampeloprasum	5	7	6	18
A. commutatum	1	-	4	5
A. bourgeaui	-	-	1	1
A. babingtonii	1	-	-	1
A. pyrenaicum[b]	-	-	1	1
A. heldreichii[b]	-	-	1	1
A. tuberosum[b]	1	-	-	1
Sum				49
Experiment 2				
A. porrum	-	-	6	6[c]
A. ampeloprasum	19	1	-	32[d]
A. bourgeaui	-	-	1	1[e]
A. commutatum	-	1	1	1
A. polyanthum	-	1	-	1
A. atroviolaceum	-	-	1	1
A. sphaerocephalon[b]	-	2	-	2
A. scorodoprasum[b]	1	-	1	2
A. guttatum[b]	1	-	-	1
Sum				47

[a] resistance classes: 'High': incidence < 50%; 'Moderate': incidence 50-79%; 'Low': incidence ≥ 80%

[b] not belonging to the group of species that is narrowly related to *Allium ampeloprasum* (B. Mathew, pers. comm.).

[c] 1 accession also tested in experiment 1

[d] 3 accessions also tested in experiment 1

[e] also tested in experiment 1

Table 6.3 Disease incidence of some *Allium* accessions in each replicate of experiments 1 and 2. Scores in italics indicate plants from multiplication at Centre for Plant Breeding and Reproduction Research (CPRO-DLO)

	Experiment 1			Experiment 2	
Inoculation date:	Dec 23	Jan 24	Mar 12	Nov 10	Jan 18
Carina	95	100	95	95	95
Derrick	-	-	-	90	92
Wintina	-	-	-	95	67
91021 (selected from Carina)	-	-	-	90	58
A.ampeloprasum PI 357195[a]	*40*	*50*	*50*	-	-
A.ampeloprasum PI 357199	*50*	*25*	*20*	16	18
A.ampeloprasum PI 357201	*90*	*85*	*65*	75	75
A.ampeloprasum PI 368348	*50*	*35*	*25*	-	-
A.ampeloprasum PI 368349	-	-	-	39	27
A.ampeloprasum PI 368350	-	-	-	46	17
A.ampeloprasum PI 368351	30	30	40	37	18
A.ampeloprasum PI 368353	-	-	-	38	25
A.ampeloprasum PI 368353	-	-	-	*45*	*25*
A.babingtonii 91038	20	25	15	-	-
A.bourgeaui 84417	100	95	80	95	75
A.commutatum 92024	-	-	-	73	77
A.commutatum 85194	45	55	45	-	-
A.porrum CGN 873243[b]	45	60	-	-	-
Mean incidence (49/47 acc.)	80	78	69	62	47

[a] PI numbers from Western Regional Plant Introduction Station, Pullman, Washington, USA

[b] CGN number from Centre for Genetic Resources, Wageningen, the Netherlands

Table 6.4 Mean number of diseased leaves per plant in experiments 3-5. Data from 10 consecutive weeks were used for experiment 5

	Experiment 3	Experiment 4	Experiment 5
Observation time	Nov	Sept	Jul-Oct
Blocks observed	8[a]	8	4
Plants per block	90	45	10
91021 (selected from Carina)	1.5 a[b]	1.8 a	0.7 a
Blauwgroene Winter-Carina	2.1 b	2.6 b	1.3 bc
Blauwgroene Winter-Porino		2.7 b	
Blauwgroene Winter-Derrick	2.5 bc	2.9 b	1.5 bc
Blauwgroene Winter-Gavia		2.7 b	
Platina	2.8 c	3.0 b	1.1 b
Blauwgroene Herfst-Portant		3.7 c	
Blauwgroene Winter-Wintina	4.5 d	4.0 c	1.7 c
LSD (P = 0.05)	0.51	0.44	0.45

[a]Zoospore- and diseased-tissue-inoculated blocks

[b]Cultivars with the same letter in a column are not significantly different (two-sided t-test, P = 0.05)

Table 6.5 Repeated Measures ANOVA of four observations of eight cultivars in experiment 4 and of ten observations of five cultivars in experiment 5

	Experiment 4[a]		Experiment 5[b]	
Source of variation	d.f.	MS	d.f.	MS
Block	3	12.45	3	0.63
Cultivar	7	5.60***	4	5.81**
Residual	21	0.64	12	0.87
Time	3	6.107***	9	11.21***
Cultivar x time	21	0.155	36	0.26***
Residual	72	0.196	135	0.12
Total	127		199	

,*significant at P≤0.01 and 0.001 respectively

[a] Observation dates 21-09, 21-12, 1-02, 22-03; χ^2 for 4 residual variances = 1.09 < 9.4 = critical value

[b] Weekly observations starting from 26-8; χ^2 for 10 residual variances = 13 < 18.3 = critical value

Field Experiments. In experiments 3-5, the ranking order of cultivars was almost equal in different years (Table 6.4). Breeding line 91021 was significantly less affected by *P. porri* than the other four cultivars in three consecutive years. Wintina was significantly more affected in those years.

The same ranking order of cultivars was also found at different times of observation in experiments 3-5. In experiment 3 the ranking order was the same in October and November. In experiment 4, no cultivar x time interaction was detected in a repeated-measures ANOVA for the four observations (Table 6.5). In experiment 5 the variance of ten repeated measures (weekly observations July-October) was homogeneous. Nevertheless, a significant cultivar x time interaction was detected in this experiment, which did not affect the ranking of Wintina and 91021. The variance of 17 repeated measures (weekly observations July-December) was not homogeneous, and therefore could not be analysed in the same manner.

In experiments 6 and 7 the tested accessions could be assigned to four groups (Table 6.6). The most resistant group consists of almost all PI accessions, *A. ampeloprasum* var. *lussinense* (92018), *A. commutatum* (92024) and the Egyptian land race (CGN 873243). An intermediately resistant group comprises two accessions (PI 357201 and PI 357195) that produced inconsistent results over years. All cultivars except Wintina belong to the third group of intermediately susceptible accessions. Wintina belongs to the most susceptible group.

Zoospore inoculation produced a higher disease level than diseased-tissue inoculation (Table 6.7), but the oospore inoculation in experiment 3 was unsuccessful. The mean level of disease in the oospore-inoculated middle rows was 0.19 diseased leaves per plant in November (not shown in table). No significant effect of cultivars was found in these plots.

The variance of leaf incidence was homogeneous over the relevant range of one-four diseased leaves in all field experiments. Therefore, no data transformation was needed before application of ANOVA and coefficients of variation could be relatively low when disease levels are high. This prompted the idea that the precision of experiments at different disease levels should be compared by the LSD, expressed as a percentage of the difference between the two extreme cultivars (Table 6.7).

From a comparison of the field experiments it appears that no increase in precision is gained by making the test plots much larger than 10 plants; the number of blocks should be increased instead. Repeated observations did not consistently increase precision.

Table 6.6 Number of diseased leaves in the field evaluation of leek cultivars and relatives in Experiments 6 and 7. Observation dates are indicated

	Experiment 6 Sept 18, 1992	Experiment 7 Nov 3, 1993
Blauwgroene Winter-Wintina	3.44 e[a]	5.39 e
Blauwgroene Winter-Carina	2.37 de	3.58 d
Blauwgroene Winter-Derrick	2.11 d	3.68 d
Blauwgroene Winter-Platina	1.96 d	3.11 cd
91021 (selection from Carina)	1.74 cd	3.66 d
91021 "	-	3.44 d
93001 (progeny of 91021)	-	3.10 d
93001 "	-	3.40 d
A. ampeloprasum PI 357201	2.40 de	1.64 bc
A. ampeloprasum PI 357195	1.36 bcd	0.39 ab
A. ampeloprasum PI 357194	0.75 abc	-
A. ampeloprasum PI 368341	0.72 abc	0.89 ab
A. ampeloprasum PI 368348	0.39 ab	0.42 ab
A. ampeloprasum PI 368349	0.71 abc	0.58 ab
A. ampeloprasum PI 368350	0.51 ab	0.48 ab
A. ampeloprasum PI 368351	0.60 ab	0.32 ab
A. ampeloprasum PI 368353	0.19 a	0.13 a
A. ampeloprasum PI 370335	0.12 a	0.15 ab
A. commutatum 92024	0.55 ab	-
A. ampeloprasum var. *lussinense* 92018	-	0.42 ab
A. ampeloprasum PI 357199	-	0.81 ab
A. ampeloprasum CGN 873243	-	0.60 ab

[a] Letters indicate significant differences (Tukey-test; P = 0.05)

Table 6.7 Comparison of several data sets from experiments 3,4 and 5. Numbers of blocks, numbers of repeated measures in those blocks, number of plants per plot, mean number of diseased leaves per plant (dl/p) of Wintina (susceptible) and 91021 (resistant) and the LSD value (P=0.05) expressed as a percentage of the difference between Wintina and 91021

Month	No. Blocks	Repeats in time	Plants /plot	Wintina (dl/p)	91021 (dl/p)	LSD (%)
Experiment 3						
Nov	4(zo)[a]	1	90	5.0	2.0	24
"	4(dt)[b]	1	90	4.0	1.0	30
"	8[c]	1	90	4.5	1.5	17
Oct/Nov	4(zo)[a]	2	90	3.8	1.5	31
"	4(dt)[b]	2	90	2.8	0.7	31
"	8[c]	2	90	3.3	1.1	19
Experiment 4						
Sept	4	1	45	4.4	2.0	32
"	8	1	45	4.0	1.8	21
Sept/Mar	4	4	45	3.5	1.7	30
Experiment 5						
July	4	1	10	1.6	0.6	55
Sept	4	1	10	3.6	1.6	54
Dec	4	1	10	5.3	2.9	73
"	8	1	10	5.5	3.5	51
July/Oct	4	10	10	1.7	0.7	45
July/Dec	4	17	10	2.5	1.4	65

[a] zoospore inoculated blocks

[b] diseased tissue inoculated blocks

[c] zoospore and diseased tissue inoculated blocks

6.4 Discussion

In the present work we have identified partial resistance within the gene-pool of winter leek and stronger partial resistance in leeks of a different type. The value of the off-type sources of resistance depends not only on the level of resistance, but also on crossing barriers, genetic linkages between resistance and other traits, and on the perspectives of breeding strategies without outcrossing.

CGN 873243 and PI 368351 have been successfully used in a crossing programme and have produced F_1 plants of intermediate colour and height. Both sources of resistance are early-flowering, tall leek types. CGN 87324 is a very early flowering Egyptian landrace, clearly distinct from kurrat. PI 368351 is a local cultivar from the former Yugoslavia, named Visok (USDA, 1972), and bears a visual resemblance to the variety 'Bulgaarse Reuzen', which is commercially available but rarely grown in The Netherlands.

A. babingtonii was discarded as a crossing parent in spite of the high level of resistance, because only a few sterile flowers were produced by the tested accession. Other accessions of *A. babingtonii* with more abundant flowering exist, and are potential sources of resistance.

From a scientific point of view, it is also interesting to find resistance to *P. porri* in distantly related species of leek, such as *A. scorodoprasum*, but for practical purposes crosses with this material are not desirable, if at all possible, as many generations of backcrossing would be needed for introgression of the desired gene or genes.

Selection for resistance within cultivars of winter leek may be a good alternative to introgression of resistance from wild relatives of winter leek. In the present work it has been proven that a selection from Carina performed better than Carina. It is not yet clear if further selection for resistance is possible in winter leek. If further selection is possible there is no need to cross winter leek with the early-flowering sources of resistance.

Similar differences in resistance were detected in glasshouse and field tests. Most accessions that were highly resistant in glasshouse experiments were also highly resistant in field experiments. It follows therefore that field resistance is not based on an escape mechanism, as for example has been claimed for resistance to the splash-dispersed *Septoria tritici* in wheat (Eyal *et al.*, 1987). In that pathosystem resistance may be caused by a tall growth type. The same mechanism could have explained the field-performance of our sources of resistance if glasshouse tests had not confirmed the field test results. As resistance is expressed well in the glasshouse test, in which plants were inoculated by dripping a spore suspension into the shafts, a true physiological resistance must be present.

Partial resistance is often considered as a factor that reduces the polycyclic growth rate of epidemics, or the effective amount of initial inoculum, or both (Parlevliet, 1979). In this view, partial resistance is not clearly expressed in the field at the start and end of

the epidemic, and the time of observation is critical for selection. Our results point in another direction. Disease did not progress to 100% diseased leaves, in spite of the apparent polycyclic build-up, and differentiation for resistance was possible at different disease levels throughout the epidemic in relatively small plots. Therefore, in our field experiments resistance to *P. porri* affected infection and colonization rather than sporulation.

The largest plots in our experiments (90 plants) are still relatively small, compared with growers' fields. In such plots, the level of resistance to a polycyclic disease may be underestimated. For barley leaf rust, this underestimation varied from 5 to 16 times if the adjacent plots were 4.5 m wide, and up to 130 times for single row plots (Parlevliet & van Ommeren, 1984). For splash-dispersed pathogens such as *P. porri* this underestimation will be less important, but possibly still significant, as a shorter dispersal gradient may be expected. A detailed study of the epidemiology of *P. porri* is needed before final conclusions about the desired level of resistance and the right trial size can be drawn.

The inoculum levels and the environmental conditions are apparently more critical in glasshouse than in field tests, probably because of the small area of contact between inoculum and plant, and because of the observation of plant incidence instead of leaf incidence. Although the glasshouse test was suitable for identifying sources of resistance with 20 plants, no differentiation between cultivars was possible in the glasshouse test. However, the inoculation and observation methods can be improved. The combination of resistant breeding material, early screening methods and an improved insight into leek genetics allows faster progress in leek breeding.

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Chapter 7

Genetical studies of resistance to *Phytophthora porri* in *Allium porrum*, using a new early screening method

Summary: A new screening method was developed to evaluate resistance of leek (*Allium porrum*) to *Phytophthora porri*, based on inoculation by 24 h-immersion of leek plantlets in the 3-6 leaf stage in a suspension of ca. 100 zoospores.ml^{-1}. The immersion test was used for identifying new sources of resistance and to determine the genetic basis of resistance, (1) in winter leek and (2) in landraces. 1. Within winter leek, five resistance classes were defined on the basis of average field scores of 21 plants. Clones from these plants were tested with the immersion technique. The average scores per resistance class in immersion and field test were significantly correlated ($P<0.01$). The correlation of single-date field scores with the immersion test scores was better in the second half of the epidemic season, after November. A selection experiment yielded a strong response to selection for resistance but no response to selection for susceptibility. This may indicate that resistance is due to 2-4 recessive genes in the studied winter leek. 2. Inbred lines of partially resistant landraces differed for resistance. Crosses between landraces and winter leek were analyzed by means of F_2 (selfed F_1) and BC_1 progenies. This analysis indicated the presence of 2-4 loci with dominant genes for resistance in accession CGN 873243 and additive polygenes in accession PI 368351.

7.1 Introduction

In an earlier study (Chapter 6), high levels of partial resistance to white tip disease (*Phytophthora porri* Foister) of leek (*Allium porrum* L.) in field- and glasshouse tests were described. The highest levels of resistance were found in accessions from the eastern Mediterranean area. These accessions can be characterized as light-green, long-shafted, early-flowering landraces. In the Netherlands, the most important leek cultivars are dark-green, short-shafted and late-flowering. Therefore, it is not possible to select improved cultivars for commercial use directly from the partially resistant landraces. For exploiting the partial resistance to *P. porri* observed in the landraces, crosses and repeated backcrosses to commercially interesting cultivars are needed. With this breeding method undesired genes may enter the breeding material, which cannot be eliminated easily due to the tetraploid nature of the crop and the low tolerance to inbreeding (Pink, 1993).

Introgression of resistance may become more attractive if new sources of resistance could be identified which resemble winter leek more than the sources that were identified earlier.

Leek cultivars are, just as landraces, open-pollinating and genetically heterogeneous populations of self-compatible plants, with a proportion of up to 20% inbred plants (Berninger & Buret, 1967). Within broad limits, cultivars may change over the years, due to random genetic drift and selection (Van Marrewijk, 1988). The proportion of inbreds depends on plant density, umbel size and the activity of pollinators during flowering of the seed parents (Gray & Steckel, 1986; Currah, 1986). Leeks are considered to be autotetraploids (2n=4x=32) (Schweisguth, 1970; Potz, 1987) in spite of the low number of tetravalents during meiosis (Stack, 1993).

In our earlier study we demonstrated differences in field resistance for some modern Dutch winter leek cultivars. Because current breeding schemes are based on mass selection or half-sib family selection within cultivars or cultivar groups, differences among modern cultivars probably stem from selectable differences within cultivars. The level of resistance found in landraces was higher than in the most resistant selections of winter leek.

The aim of this study was to gain knowledge about the genetic basis of resistance, both in landraces and in winter leek, in order to improve breeding strategies based on winter leek alone or on backcrosses of winter leek and highly resistant landraces.

In the present study, we developed a new glasshouse test for resistance screening (Experiment 1). The new test is called 'immersion test', after the method of inoculation by immersion of plants in a zoospore suspension. Potential new sources of resistance were tested in the course of this work. For this purpose cultivars of the cultivar group 'Bulgaarse Reuzen', visually resembling and geographically related to partially resistant landraces, were chosen. Subsequently, we studied the genetic variation within cultivars of winter leek by tests of clones (Experiment 2) and half-sib families (Experiment 3), and the genetic variation within landraces by tests of inbred lines (Experiment 4). Finally, the prospects for introgression of resistance from landraces into winter leek were studies through examining F_2 (selfed F_1) and BC_1 (backcrossed F_1) populations (Experiment 5).

7.2 Materials and methods

Immersion test

Immersion in a zoospore suspension is an efficient method to inoculate leek plantlets for resistance screening. In our earlier study on resistance (Chapter 6) the indoor screening tests were based on another method, which we will call drip-inoculation.

At least seven days before inoculation the production of zoospore inoculum from mycelial cultures in Petri-dishes was started, following Chapter 2 and 6. The method is based on gradual starvation of fresh mycelium to produce zoosporangia in liquid medium, followed by a cold treatment to synchronize the germination of sporangia. The germination of sporangia was verified under a binocular microscope at 50 × magnification. The undiluted inoculum had on average a density of 4.10^3 zoospores ml^{-1}, but sometimes varied considerably between Petri-dishes.

The inoculum was harvested from Petri-dishes some hours before inoculation, transported in glass bottles at 0°C, and shortly before inoculation diluted with a ten- to twenty-fold volume of sterile demineralized water at 4°C.

For inoculation, plants in the 3-6 leaf stage, raised in greenhouses under natural light conditions and temperatures above 10°C, were uprooted and placed horizontally in non-sterile plastic containers of 40 × 25 × 15 cm (blocks) with 4-5 liter of the diluted inoculum. The plants were totally submerged for ca. 24 hours at 15°C in a dark room. After inoculation the plants were usually replanted in pots with peat soil and incubated for at least two weeks at 18°C and a 16/8 h day/night regime. In experiment 4, plants were incubated in a horizontal position in closed (humid and dark) plastic containers at 12°C for 10 days before replanting in the field.

Disease was scored as number of lesions per plant, 10-14 days after immersion-inoculation. Coalescing lesions were counted separately by using small plastic pegs to mark lesions each day from the first appearance of lesions at about the seventh day after inoculation. In field tests the number of diseased leaves per plant was scored.

The size of plantlets was recorded in experiment 2 by counting the number of visible leaves, and by measuring the length of the longest leaf of each plant just before immersion.

Experiment 1: Immersion test, 'Bulgaarse Reuzen'

Experiment 1 consisted of three inoculations on three different dates (Table 7.1). Five accessions with known levels of field resistance and one or four cultivars of the cultivar group 'Bulgaarse Reuzen' were included in each inoculation. The 'Bulgaarse Reuzen' cultivars were included as potential new sources of resistance. The plants were randomized over five plastic containers (blocks). Experimental units (plots) consisted of

2 plantlets in inoculation 1a and 1b, and 10 plantlets in 1c. In a side experiment of 1c, a sixth plastic container was used for testing the effect of tap water dilution of the inoculum.

Experiment 2: Clones

Eighty-eight plants of cvs. Carina or Derrick were observed 25 times at weekly intervals in a field trial in 1992-1993. The mean of the 25 sequential disease observations was taken to estimate the phenotype for resistance of each plant. Forty plants were taken from the field just before the bolting stage in March 1993, and multiplied through *in vitro* stalk explants (Silvertand *et al.*, 1995). Only 21 plants survived the *in vitro* phase. The original set of 40 plants was grouped according to the resistance level of each plant (Fig. 7.1). After multiplication there were still four clones in the resistant (R), medium (M), moderately susceptible (MS) and susceptible (S) group, and 5 clones in the moderately resistant (MR) group. The 21 clones were grown in pots in a cool greenhouse from December 1993 until they reached the 3-6 leaf stage in March 1994, when they were tested, using the immersion technique with five plants per clone x block. Inoculated, repotted plantlets were incubated at 18°C and a 16/8 h day/night regime.

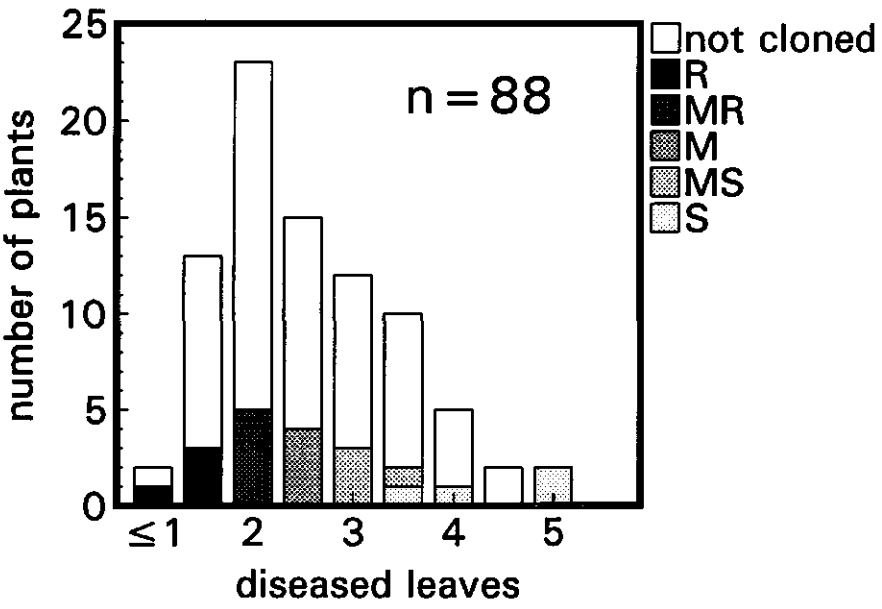

Figure 7.1 Frequency distribution of average field scores (numbers of diseased leaves per plant averaged over 25 observations between August 1992 and March 1993) of 88 plants. Clones were obtained from five groups of plants (R, MR, M, MS and S), representing five resistance levels. Groups of clones consist of four or five plants.

Experiment 3: Half-sib families

In the autumn of 1992, a disease nursery was created with cv. Carina planted in 6 rows of 60 plants at 12 × 50 cm distance. Disease assessments of the 360 heavily infested plants were made on five dates (27-10-92, 23-11-92, 16-12-92, 27-1-93, 4-3-93), and averaged per plant. In March 1993, two groups of plants were selected from the field, one group (30 'resistant' plants) with a high and the other group (36 'susceptible' plants) with a low number of diseased leaves. 'Resistant' and 'susceptible' half-sib families were produced by separating the two groups during flowering in July-August 1993. Blowflies were used to enhance cross-fertilization within the two isolation chambers. Seeds were harvested from five plants in each chamber.

The ten half-sib families were tested for resistance in an immersion test in May 1994 (plants sown in December; 25 plants per family) and in a field test in autumn 1994 (plants sown in April; 40 plants per family tested). The field trial was planted in July in a randomized block design with five blocks, 24 plots per block and ten plants per plot on a field (7 × 11 m) infested by a previous field test. Carina, Wintina, Bulgina, 91021, Olifant-S_1-ms, PI 368351 and CGN 873243 were used as controls in the immersion and the field test of the ten half-sib families. Moreover,
7 or 8 inbred lines were included (described below as Experiment 4). Half-sib families, controls and inbreds were randomized over the 24 plots.

Experiment 4: S_1 lines of landraces

In the spring of 1993, PI 368351 and CGN 873243 were tested in a glasshouse, using the drip inoculation method. S_1 lines were obtained by selfing of plants that had one or more lesions and plants that showed no disease in this test. In 1994 ten S_1 lines were evaluated in either an immersion- or a field test. This experiment was performed in combination with experiment 3 in order to improve the comparability of experiments.

Experiment 5: F_2's and BC_1's

In the summer of 1992, F_1 populations of Carina × PI 368351 and Carina × CGN 873243 were produced. The F_1 populations were harvested from individual Carina plants, which were pollinated by flies with pollen from one or more plants of the paternal population. In the summer of 1993, seven F_1 plants (four of Carina × PI 368351, and three of Carina × CGN 873243) were selfed to produce F_2's, while pollen of the same seven plants was used for manual fertilization of emasculated Carina plants to produce BC_1's. The F_2's and BC_1's were harvested individually from their mother plants. In summer 1994, the F_2's and BC_1's were tested in an immersion test, and subsequently in a field test of all plants that survived the immersion test. In the field test no blocks were used.

Realized heritability

The response to selection was used to calculate $h^2_{realized}$, the realized heritability in one selection cycle. To calculate $h^2_{realized}$ the scaled selection response R was divided by the scaled selection differential S, assuming normal distributions of disease scores for the disease scores in the immersion test (the response environment) and in the field test (the selection environment):

$$h^2_{realized} = R/S$$

with $R = (\mu_{HS}-\mu_{Car})/\sigma_{Car}$, μ_{HS} = mean of half-sib families in the response environment, μ_{Car} = mean of Carina in the response environment, and σ_{Car} = standard deviation of Carina in the response environment, and $S = (\mu_{sel}-\mu'_{Car})/\sigma'_{Car}$, μ_{sel} = mean of selected mother plants in the selection environment, μ'_{Car} = mean of Carina in the selection environment, σ'_{Car} = standard deviation of Carina in the selection environment.

Correlation between field and immersion tests

To evaluate the degree of correlation between field and immersion tests, the coefficient of determination, adjusted for degrees of freedom (R^2_{adj}) was calculated. R^2_{adj} is equal to the proportion variance explained by regression: $R^2_{adj} = 1 - MS_{res}/MS_{total}$ with MS_{res} = residual mean square in regression analysis and MS_{total} = total mean square in regression analysis (GENSTAT 5 Committee, 1987). When regression is not justified because there is no distinction between dependent and independent variables, R^2_{adj} can still be used as a correlation coefficient, as for each number of data pairs there is a fixed mathematical relation with Pearson's correlation coefficient.

7.3 Results

Experiment 1: Immersion test, Bulgaarse Reuzen

For each of the three inoculation dates of Exp. 1 a statistically significant ($P<0.05$) block effect was detected, so blocking with separate containers for the inoculum effectively reduced the experimental error. The precision of the experiment was considered to be rather low. Therefore, in experiments 2 and 3, 25 instead of ten plants were used.

Five accessions which were tested earlier for field resistance to *P. porri* had significantly correlating disease scores in field and immersion tests 1a (R^2_{adj} = 0.77), 1b (R^2_{adj} = 0.99) and 1c (R^2_{adj} = 0.83) (Table 7.1). Wintina and Carina were the more susceptible accessions, whereas 91021, PI 368351 and CGN 873243 were more resistant.

Table 7.1 Disease scores of five control accessions and four cultivars of the cultivar group 'Bulgaarse Reuzen', in a field test[a] (number of diseased leaves per plant) and in three immersion tests (number of lesions per plant). Within columns, scores followed by the same letter are not significantly different (Tukey-test, $P<0.05$)

Experiment	Field[a]	1a	1b	1c
Date of observation	3-11-93	22-3-94	28-3-94	21-3-95
Number of plants per accession	n=60	n=10	n=10	n=25
Inoculum density (zoospores.ml^{-1})	-	250	70	100
Control accessions				
Wintina	5.4 a	3.1 a	3.9 a	6.4 a
Carina	3.6 b	1.1 b	2.8 a	5.6 ab
91021	3.7 b	0.9 b	2.5 ab	4.1 bc
PI 368351	0.3 c	0.2 b	0.4 c	2.8 c
CGN 873243	0.6 c	0.3 b	0.3 c	-
'Bulgaarse Reuzen'				
Bulgina	-	0.3 b	0.8 bc	3.0 c
Lincoln	-	-	-	3.1 c
Kamush	-	-	-	3.6 c
Starazagorsky	-	-	-	2.8 c

[a] data from Table 6.6 included for comparison

The four cultivars of the cultivar group 'Bulgaarse Reuzen' had a relatively high level of resistance.

The degree of infection varied considerably between inoculation dates: in experiment 1a the degree of infection was relatively low (1.1 lesion per Carina plant. At this low level Carina and PI 368351 were not significantly different ($P>0.05$). In experiment 1c the degree of infection was relatively high (5.6 lesions per Carina plant). Carina and Wintina did not significantly differ ($P<0.05$). In experiment 1b, with intermediate infection, the differentiation between cultivars was best. The average disease level for each inoculation date was not related to the zoospore density, nor to the motility of the zoospores as observed under the microscope. From the side experiment of 1c, it appeared that tap water does not strongly inhibit infection by zoospores, as Carina had on average 4.9 lesions per plant (n=10) after inoculation in tap water diluted inoculum, and 5.6 in sterile demineralized water.

Table 7.2 Analysis of variance of disease scores in an immersion test of 5 groups of clones (R, MR, M, MR and S in Fig. 7.1). Twenty-five plants per clone and four or five clones per group were used ("***": P<0.001)

Source of variation	df	MS
Blocks	4	6.64
Groups of clones	4	14.20***
Error	16	0.72

Experiment 2: Clones

In the field test, the average number of diseased leaves per plant was 2.8 and 2.5 for Carina and Derrick, respectively, over the whole 25 week period. In the immersion test the corresponding scores were 5.0 and 4.6 lesions per plant, respectively. The variances of Carina and Derrick were similar. Therefore, data of Carina and Derrick were pooled for variance analysis. Differences in resistance between the five groups of clones (R, MR, M, MS and S) were highly significant in the immersion test (Table 7.2).

Length of the longest leaf and total number of leaves were significantly different ($P<0.01$) among clones (data not shown). The length of the longest leaf per clone varied from 42 to 69 cm and the total number of leaves per clone varied from 4 to 5.5. These variables were not significantly correlated with disease score ($P>0.1$).

The mean scores of the five groups of source plants averaged over all observation dates in the field test and the five groups of clones in the immersion test were significantly correlated ($R^2_{adj} = 0.99$; $P<0.01$) (Fig. 7.2). Most of the single-date group scores of the five groups of source plants in the field test were also significantly correlated to the scores of the five groups of clones in the immersion test. Values of R^2_{adj} varied from 0 to 100% (Fig. 7.3). It appears that after week 46 (mid-November) R^2_{adj} was higher than in the earlier part of the season, but also that R^2_{adj} varied greatly among weeks for unknown reasons.

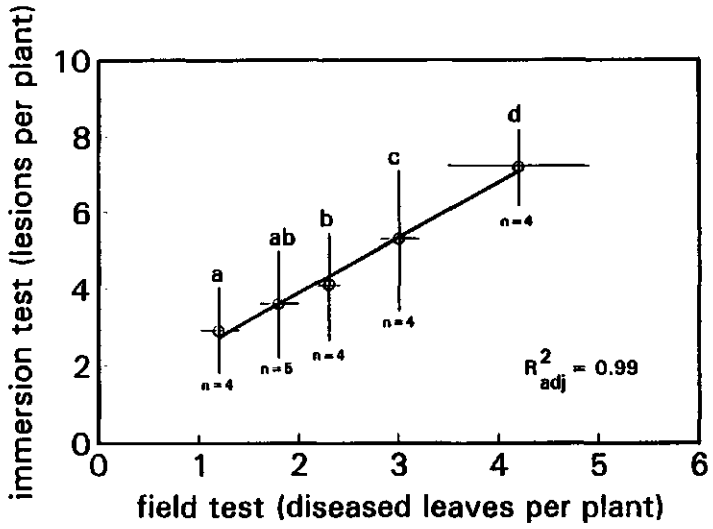

Figure 7.2 Correlation between scores of groups of clonal source plants tested in a field test (average of 25 observation dates; horizontal axis), and groups of clones tested in an immersion test (vertical axis). For each group of clones and both tests the standard deviation is indicated by a horizontal or vertical line. Groups of clones with the same letter code (ad) were not significantly different in the immersion test (vertical axis). n= number of plants or clones.

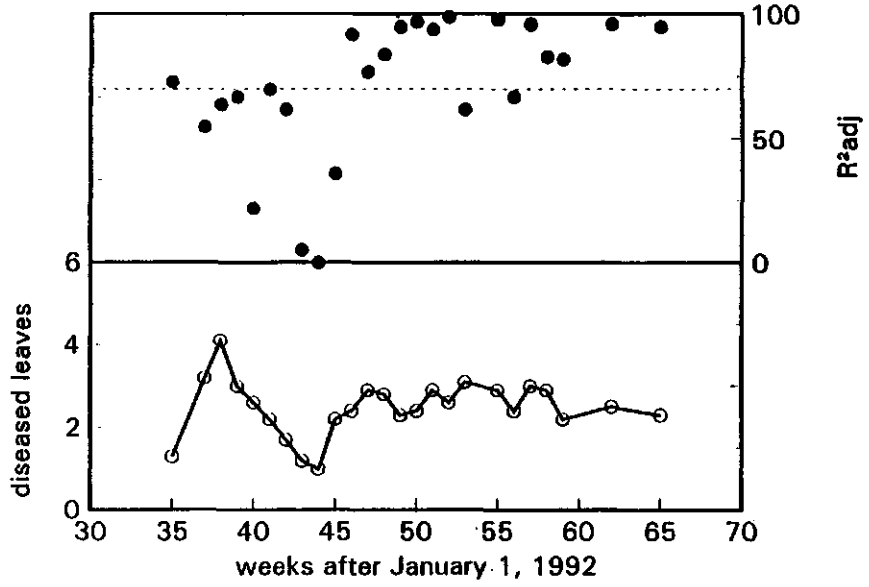

Figure 7.3 Upper graph: time series of R^2_{adj} values for the correlation of single-date scores of five groups of plants in a field test in 1992/3 and the scores of the corresponding groups of clones in an immersion test. Lower graph: the corresponding time series of disease scores in the field test.

Experiment 3: Half-sib families

The results of the immersion test of ten half-sib families and controls are given in Table 7.3. Some half-sib families from the resistant sub-population were significantly different from each other in the immersion and field tests (Tukey's test $P<0.05$). The half-sib families of the susceptible sub-population did not differ significantly from each other, except HS-s-3 which was more resistant than the other 'susceptible' families in the field test (28-9-94) (Tukey's test $P<0.05$).

Diverging selection of winter leek cv. Carina for susceptibility and resistance to *P. porri* is illustrated in Fig. 7.4. The disease scores shown in Fig. 7.4 were corrected for the strong row effect, leading to negative disease scores for the most resistant plants in the least infested rows. In Fig. 7.5 the disease scores of Carina and half-sib progenies of the two opposite selections are given for the immersion test.

The mean scores of Carina in the selection environment and in three response environments, and their standard deviations calculated from the corrected scores, were used for calculating the selection differential and three selection responses (Table 7.4). The $h^2_{realized}$ for immediate response to selection in resistant and susceptible directions were 0.53 and -0.02 respectively for the immersion test. The field scores of the ten half-sib families generally confirm the conclusions that can be drawn from the immersion test scores (Tables 7.3 and 7.4). The $h^2_{realized}$ for selection for resistance was even higher than in the immersion test (0.71-0.97). The field test confirmed that selection for susceptibility was ineffective.

For the five 'resistant' half-sib families, R^2_{adj} for the regression of field test scores on immersion test scores was 0.96 and 0.98 for 28 September and 1 November, respectively (data not shown in a table). Both values are highly significant ($P>0.001$). This indicates that there is little family x test interaction; in other words, that the immersion test is an accurate tool for predicting field performance for the 'resistant' half-sib families. For the five 'susceptible' half-sib families the same R^2_{adj} was not significant. This may be due the limited amount of phenotypic variation in this group.

Experiment 4: Inbred lines of landraces

Inbred lines differed significantly (Tukey, $P<0.05$) for resistance in the immersion- and in field test (Table 7.3). Selection after drip-inoculation of CGN 873243 seems to be effective (F-test, $P<0.001$).

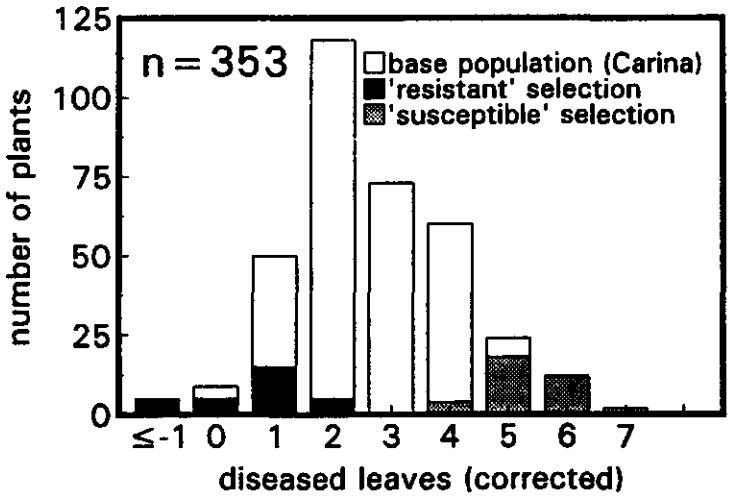

Figure 7.4 Frequency distribution of disease scores (corrected for row effect) in a field test of a population of Carina plants, comprising two sub-populations, selected for resistance or susceptibility.

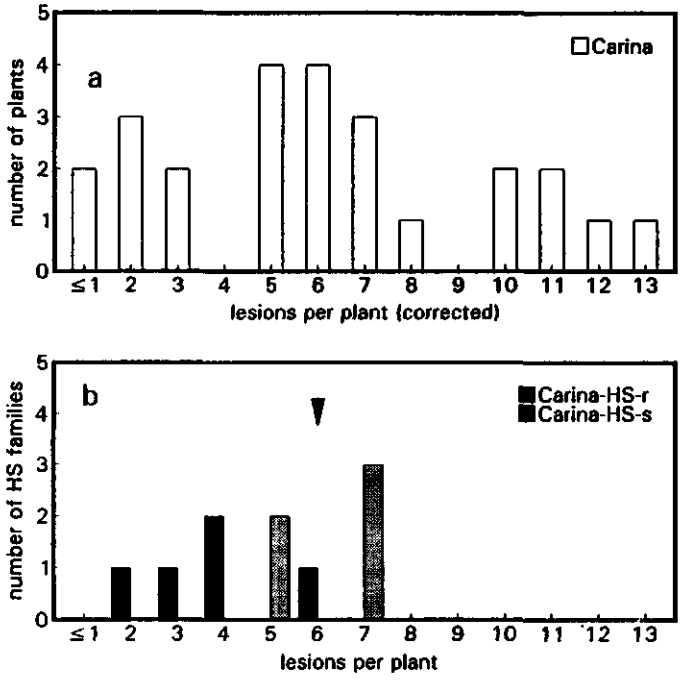

Figure 7.5 Frequency distribution of disease scores of (a) Carina plants (n=25), corrected for block effects, and (b) the means of five 'resistant' and five 'susceptible' half-sib families in the same immersion test. ▸ = mid-parent value.

Table 7.3 Disease scores in immersion- and field tests of control accessions, half-sib families from two groups of Carina plants, selected for resistance (HS-r) or susceptibility (HS-s) to *P. porri* in a field test in 1992-1993, and inbred lines (S_1-r and S_1-s) from landraces. Within the two experiments and three columns, scores followed by the same letter are not significantly different from each other (Tukey, $P<0.05$).

Accession	Immersion test lesions.plant^{-1} 9-5-94	Field tests diseased leaves.plant^{-1} 28-9-94	1-11-94
Experiment 3			
Control accessions			
PI 368351	1.0 a	0.2 a	0.6 a
CGN 873243	1.5 ab	0.2[a]	0.5[a]
Bulgina	1.6 ab	0.8 ab	0.9 ab
91021	3.6 abcde	3.4 defg	3.7 d
Olifant-S_1-ms	5.7 cdef	3.0 cdef	3.7 d
Carina	6.0 def	3.7 efg	3.8 d
Wintina	7.8 f	5.5 h	5.8 e
Half-sib families			
HS-r-1	1.6 ab	0.9 ab	0.9 ab
HS-r-2	2.2 abc	0.8 ab	0.9 ab
HS-r-3	3.2 abcd	1.5 abc	1.9 bc
HS-r-4	3.4 abcd	1.9 bcd	2.0 bc
HS-r-5	6.0 def	3.7 efg	3.6 d
HS-s-1	4.6 abcdef	4.3 fgh	4.1 d
HS-s-2	4.9 bcdef	4.4 fgh	3.7 d
HS-s-3	6.1 def	2.5 cde	2.9 cd
HS-s-4	6.8 def	4.1 fgh	4.0 d
HS-s-5	7.0 ef	4.7 gh	4.1 d
Experiment 4			
Inbreds of landraces[b]			
PI-S_1-r	1.3 ab	-	-
PI-S_1-s	0.7 ab	-	-
CGN-S_1-r-1	-	0.2 ab	0.4 ab
CGN-S_1-r-2	-	0.0 a	0.8 ab
CGN-S_1-r-3	0.2 a	0.0 a	0.2 a
CGN-S_1-r-4	0.3 ab	0.4 ab	0.3 a
CGN-S_1-r-5	0.4 ab	-	-
CGN-S_1-s-1	1.6 b	1.0 b	0.9 ab
CGN-S_1-s-2	1.7 b	0.7 ab	1.5 ab
CGN-S_1-s-3	1.7 b	1.1 b	1.8 b

[a] not used for ANOVA because only ten of the 50 plants had survived

[b] PI = PI 368351; CGN = CGN 873243

Table 7.4 Mean disease scores and their standard errors (m ± sem), calculated from scores corrected for block effects, of Carina and a resistant and susceptible selection. For each standard error the number of observations (n) is given. For three response environments $h^2_{realized}$ for selection to the resistant (r) and susceptible (s) side is given. dl.p^{-1} = number of diseased leaves per plant, les.p^{-1} = number of lesions per plant

1 - in selection environment

	Immersion test	
Date	1992-3	
Unit	dl.p^{-1}	
	m ± sem	n
Carina	2.2 ± 1.4	353
HS-r	0.1 ± 0.8	30
HS-s	4.7 ± 0.7	36

2 - in response environments

	Immersion test			Field test			Field test		
Date	9-5-94			28-9-94			1-11-94		
Unit	les.p^{-1}			dl.p^{-1}			dl.p^{-1}		
	m ± sem	n	$h^2_{realized}$	m ± sem	n	$h^2_{realized}$	m ± sem	n	$h^2_{realized}$
Carina	6.0 ± 3.4	25		3.7 ± 1.3	47		3.8 ± 1.8	46	
HS-r	3.3 ± 0.8	5	0.53	1.8 ± 1.3	5	0.97	1.9 ± 0.5	5	0.71
HS-s	5.9 ± 0.8	5	-0.02	4.0 ± 0.9	5	0.13	3.8 ± 0.5	5	0

Experiment 5: F_2's and BC_1's

For Carina x CGN 873243 the F_2 scores were far below the mid-parent value (Table 7.5). For Carina x PI 368351 the F_2 scores were near or somewhat below the mid-parent value. Thus, the resistance of CGN 873243 and PI 368351 seems to be mainly due to dominant genes and additive genes, respectively.

The BC_1's were on average less resistant then the F_2's. F_2's of different F_1 plants from the same parents, and BC_1's with a common F_1 father plant showed different levels of resistance.

The immersion test is non-destructive. Nevertheless, about 30% of the F_2 and BC_1 plants did not survive the immersion test (Table 7.5, bottom line). The main cause of plant death was infection of the roots by *Fusarium* sp., which was probably favoured by the immersion of uprooted plants. Wintina, the accession with most *P. porri* lesions, survived best. The landraces PI 368351 and CGN 873243 were relatively susceptible for *Fusarium*.

The field test again confirmed the findings in the immersion test. R^2_{adj} of F_2's and BC_1's in the immersion and the field test was 0.85.

Table 7.5 Disease scores of F_2 (selfed F_1 plants) and BC_1 (Carina × F_1) progenies of F_1 (Carina × PI 368351) and F_1 (Carina × CGN 873243) in immersion (lesions per plant) and field tests (diseased leaves per plant). F_1's and BC_1's are grouped according to their common F_1-mother plants. For each disease score, the number of plants tested is given in the right hand side of the table. Units: les.p^{-1} = lesions per plant, dl.p^{-1} = diseased leaves per plant

		Mean disease score		Number of plants	
		Immersion 28-6-94 les.p^{-1}	Field 30-9-94 dl.p^{-1}	Immersion 28-6-94	Field 30-9-94
Control accessions					
Wintina		1.8	6.0	25	24
Carina		1.3	4.7	25	12
PI 368351		0.4	0.3	25	9
CGN 873243		0.6	0.4	25	5
F_1 (Carina × PI 368351) progenies					
Father	*Progeny*				
F_1-1	F_2	0.5	0.6	200	148
	BC_1	0.8	1.9	335	178
F_1-2	F_2	1.0	1.9	42	30
	BC_1-1	1.1	3.4	47	45
	BC_1-2	1.5	4.2	78	67
F_1-3	F_2	1.1	2.1	22	15
	BC_1-1	1.0	3.2	120	111
	BC_1-2	1.2	2.7	99	73
F_1-4	BC_1	1.0	3.6	117	106
midparent value		0.9	2.5		
mean of 3 F_2's		0.9	1.6		
mean of 6 BC_1's		1.1	3.2		
F_1 (Carina × CGN 873243) progenies					
Father	*Progeny*				
F_1-1	F_2	0.6	0.4	10	10
F_1-2	F_2	0.4	0.2	19	13
	BC_1	1.0	2.6	60	52
F_1-3	F_2	0.5	1.4	15	14
	BC_1	0.5	1.9	40	28
midparent value		0.9	2.5		
mean of 3 F_2's		0.5	0.6		
mean of 2 BC_1's		0.8	2.3		
Total for F_2's and BC_1's				1204	890

7.4 Discussion

The new disease resistance test

The immersion test appears to be a useful tool for selection of resistance to *P. porri* in an early plant stage. The correlation of early scores with later field scores was good. The test is independent of weather conditions and the presence of the disease in selection fields. The precision of the immersion test is higher than the drip-inoculated tests. With drip inoculation, at least 60 plants were needed for differentiation of cultivars for resistance, while with immersion inoculation 25 plants were sufficient. The higher precision of the immersion test appears to be related to the higher number of infections, allowing a severity assessment instead of an incidence assessment.

The immersion test is non-destructive, and therefore may be used to select young plants shortly before planting in a selection field. The immersion method is labour-intensive because of the time consuming production of inoculum in Petri-dishes, re-potting of plants (not necessary when plants will be planted in the field), and the careful observation of disease symptoms. If the plants are infected with *Fusarium* sp. before immersion takes place, many plants may die soon after the test. Unfortunately, the infection success is still variable. The average disease level is sometimes too high or too low for a good differentiation. The variation in disease level was not correlated with zoospore density of the inoculum, and attempts at standardization of the procedure as yet failed to reduce the variation in infection success. The factor responsible for this variation is still unknown, but we speculate that the quality of the water used for dilution of the inoculum is important.

Genetics of resistance

In the present study, it is demonstrated that genetic variation for resistance is present within winter leek cultivars (Carina and Derrick; Exp. 2) and landraces (CGN 873243; Exp. 4). There is no reason why the tested accessions would be exceptionally variable with respect to resistance, so we suppose that genetic variation for resistance is commonly present both in landraces and in advanced cultivars. This variation may be conserved because of the tetraploid nature of leek and its low tolerance to inbreeding.

The performance of clones in the immersion test (Exp. 2) indicated that intermediately resistant phenotypes in a field test represent intermediately resistant genotypes. Thus, the resistance factors in winter leek are on average additive. If some of them are dominant, these are counterbalanced by others which are recessive.

According to quantitative genetic theory for tetraploids (Bradshaw, 1994) $h^2_{realized}$ for the immediate response overestimates h^2_n, the narrow sense heritability (defined as the

proportion genetic variance). Non-additive genetic effects may improve the immediate response. Bradshaw (1994) derived (for chromosomal segregation):

$$h^2_{realized} = \frac{V_A + \frac{1}{3}V_D + \frac{1}{2}V_{AA} + \frac{1}{6}V_{AD} + \frac{1}{18}V_{DD}}{V_T}$$

with V_T = total variance and V_A = additive genetic variance, V_D = dominance (di-allelic) genetic variance, V_{AA} = epistatic (non-allelic) interaction variance, V_{AD} = interaction variance due to two alleles at one and one allele at another locus, V_{DD} = interaction variance due to two pairs of alleles at two loci. The non-additive effects will lessen when the selected population is allowed to reach a new genetic equilibrium through random mating for a sufficient number of generations. The final equilibrium response is expected to be lower than the immediate response, because only V_A contributes to the final equilibrium response, for unlinked loci. In our study the various components of genetic variation could not be separated.

For plant breeding purposes it is important to know the proportion of additive genetic variance (= h^2_n), as h^2_n may be used to predict the final equilibrium response to selection, which is solely based on additive polygenes (Wright, 1975). This proportion is expected to be below the proportion total genetic variance, because of the bias included in the immediate $h^2_{realized}$, due to non-additive genetic variance components. A potential bias included in h^2_n is the genotype × environment interaction (Falconer, 1981). Considering the high correlations between field and immersion tests, we assumed that this variance did not contribute to our estimates of genetic variances.

Non-additive genetic interactions may be important components of leek performance. This follows from the strong heterosis (Schweisguth, 1970; Kampe, 1980) and inbreeding effects (Gagnebin & Bonnet, 1979; Smith & Crowther, in press) in combination with the cytological observation that chiasmata are typically localized near the centromeres, probably resulting in tight gene linkage groups or 'super-genes' (Gohil, 1984; Potz, 1987). The linkage groups may stabilize favourable non-allelic gene interactions (Bingham *et al.*, 1994). Because of the expected importance of the non-additive interactions, the overestimation of h^2_n by $h^2_{realized}$ may be considerable.

The response to selection for resistance was 50-97% for resistance and 0-13% for susceptibility. The high responses indicate that there was a high covariance of genotype and phenotype for the relatively resistant plants. The low responses, on the other hand, indicate that this covariance was low for the relatively susceptible plants. This asymmetric response to selection agrees with the presence and absence, respectively, of a significant family effect for 'resistant' and 'susceptible' half-sib families. As selection for a recessive trait is in general more effective than for a dominant trait, and as a considerable effect of one selection cycle indicates oligogenic inheritance, the simplest explanation for this

asymmetry is that resistance is based on 2-4 recessive genes with independent quantitative expression. This implies that for example the genotypes A...B... have the same susceptible phenotypes, while the resistant phenotypes of A...bbbb, aaaaB... are intermediate and possibly different, while the phenotype of aaaabbbb is more resistant than A...bbbb and aaaaB... . However, if non-additive interactions between the loci are important this simple explanation may be misleading.

Another potential cause for asymmetry is scalar asymmetry (Falconer, 1981, p. 190). This phenomenon cannot be very important in the present case, as this would imply a non-linear relationship between field-tested parents and immersion-tested progeny. The relationship, however, appeared to be linear in Exp. 2.

It should be noted that the possibilities for further selection for resistance are probably limited when only few genes are involved in resistance, and that attempts at fixation of recessive resistance factors may be complicated by inbreeding depression.

Resistance in the landraces CGN 873243 and PI 368351 may be different from the resistance found in the cultivars. In CGN 873243 resistance appears to be based on dominant genes. The loss of resistance in the BC_1 may indicate a relatively low (2-4) number of genes responsible for resistance. For PI 368351 the evidence suggests the presence of additive polygenes for resistance. It seems probable that resistance in Carina, CGN 873243 and PI 368351 is governed by different, independent factors, and it seems reasonable to expect some kind of additivity between the resistance factors of different parentage.

Breeding strategy

Partial resistance to *P. porri* can be selected from winter leek cultivars in one generation. It is, however, doubtful whether a second selection cycle will give further improvement of resistance, and if resistance will be stable over later generations. Thus, the genetic system of leek may be the main obstacle in breeding for resistance. This predicament will be overcome when recent attempts to develop commercial F_1 hybrids will be successful (Smith & Crowther, in press). Dominant resistance genes like those in CGN 873243 can be exploited relatively easy in F_1 hybrids, as they need not be present in both parents.

Introgression of resistance may be necessary for obtaining cultivars with a satisfactory level of resistance. The main obstacle for introgression is the simultaneous introduction of undesirable alleles on other loci. Bos (1980) pointed out that in a tetraploid with $x = 8$, as is the case with leek, seven backcross generations are needed to eliminate 90% of the undesired genes in the absence of selection. In practice, however, some degree of selection against undesired genes will be possible and less backcross generations will be needed.

The success of an introgression program will depend on the careful choice of both parents. In the present study, cultivars of the group 'Bulgaarse Reuzen' are identified as potential sources of resistance that have less undesirable traits than PI 368351 and CGN 873243. Both the recipient cultivar and the donor landraces will be genetically heterogeneous for resistance. Therefore, the parent populations should be selected for resistance for at least one generation before making crosses. This may result in a higher degree of resistance in the progenies of the crosses, which should be repeatedly backcrossed and selected to obtain a desirable plant type.

Acknowledgements

Thanks are due to dr J.W. van Ooijen and dr I. Bos for critical comments on this chapter.

Chapter 8

General discussion

8.1 Epidemiology

8.1.1 Temporal disease progress

Disease progress was studied in field experiments described in chapter 4 and 5. In chapter 4, in naturally infested fields, the disease progress curves did not conform to an exponential model, as expected for polycyclic epidemics (Zadoks & Schein, 1979), but had an irregular shape (Fig. 8.1). The curves did not only show progress but also regress, because diseased leaves may recover, for example when lesions desiccate in dry weather. The irregular shape may be explained by the effect of rain, which appeared to be a dominant factor explaining disease progress, in conjunction with irregular rainfall patterns.

Paradoxically, the disease progress curves in chapter 5 had the expected exponential shape (Fig. 8.2). The paradox may be explained by pointing out that the initial disease levels discussed in chapter 5 were lower than in chapter 4. The initial disease level realized in the experiment in chapter 5 was ca. 0.01 diseased leaf per plant, while in the experiment in chapter 4 this was 1-3 diseased leaves per plant.

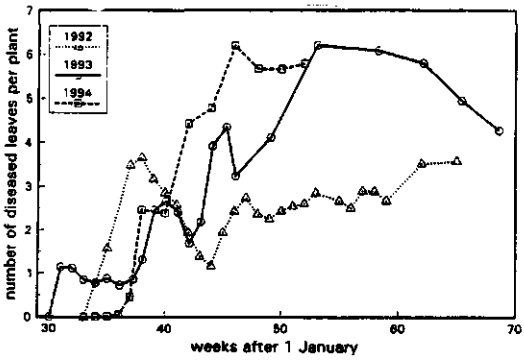

Figure 8.1 Disease progress curves of *P. porri* in naturally infested leek fields in 1992-1994. For 1992 and 1993 the data from cv. Carina,. Wintina and 91021 were averaged. For 1994 the data from three fields with different cropping histories were averaged (cf. Chapter 4).

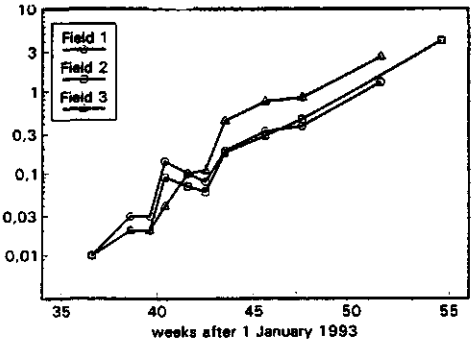

Figure 8.2 Disease progress curves of *P. porri* in three artificially inoculated fields.
The fields were 10 x 10 m and had 900 plants each. In week 35 five plants were inoculated by dripping a zoospore suspension into the shafts (cf Chapter 5).

In the range between 0.01 and 1 diseased leaf per plant the disease progress curves conformed to an exponential model, so in this range the expected exponential build-up of inoculum was less influenced by other factors such as rain and lesion death. When the disease level is above 1 diseased leaf per plant, these other factors have more influence.

In the experiments of chapter 5 the epidemic progressed with the same speed in autumn and winter. The added effects of sporulation, dispersion and infection apparently compensated for the relatively slow fungal colonization at the low temperatures of winter. This contradicts chapter 4, in which the disease level was more or less constant during winter. We suppose therefore that at the higher disease levels in chapter 4 the relatively slow colonization rate is no longer compensated by improved sporulation, dispersion or infection.

In the experiments of chapter 4 the disease increase stopped when, on average, 3-6 leaves per plant were diseased. This is below the average total number of diseased leaves, which was 8-10 leaves per plant. Thus, on average, plants were not 100% infected in terms of diseased leaves. The plants appear to resist further infection after being infected a few times. This may be partially due to differences among leaves with respect to the probability of infection, the top leaves being protected by the lower leaves, but then it is still not clear why differences in resistance remained more or less constant throughout winter. We suggest that the phenomenon is caused by a physiological response, which might be called induced resistance. The phenomenon of induced resistance has attracted much attention in a few model pathosystems in the last decade (Hammerschmidt & Kuć, 1995). It would be interesting to know whether induced resistance occurs in leek. One could start to test this hypothesis using the immersion inoculation technique.

The disease progress curves were based on counts of the numbers of diseased leaves per plant. This unit is not often used for plant diseases, but it appears to be a good choice for *P. porri* on leek. It offers a compromise between a time-consuming and often somewhat subjective severity estimation and a less labour intensive but also less informative plant incidence score. By choosing this unit we could take advantage of the typically stable plant architecture and the relatively constant number of leaves of leek.

8.1.2 Spatial disease progress

Spatial progress of disease was measured in artificially inoculated 10 x 10 m fields, containing 900 plants each. The theory of Zadoks & Kampmeijer (1977), and Zadoks & Van den Bosch (1994), postulating that successive disease gradients will be parallel and move outwards at a constant velocity, could be confirmed only within a limited span of time, in the first month of epidemic development. Within this span of time the speed of the focal front was ca. 3 $cm.d^{-1}$, which is not unlike the speed of pathogens such as *Puccinia striiformis* in wheat (9 $cm.d^{-1}$), *Peronospora farinosa* on spinach (2 $cm.d^{-1}$; Van den Bosch *et al.*, 1988), and *Uromyces appendiculatus* in beans (3-16 $cm.d^{-1}$; Habtu *et*

al., 1995), when these are measured in fields of more or less the same size. For *Phytophthora infestans* on potato, Minogue & Fry (1983) found a much higher velocity (3.7 $m.d^{-1}$) in (linear) fields of 30 m length. It should be noted that dispersal velocities appear to be faster when epidemics are considered on a bigger scale, e.g. 6-15 $km.d^{-1}$ for first-order epidemics (between fields, within a growing season) of *P. infestans* (Zadoks & Van den Bosch, 1994). Estimates of dispersal velocities are scale-dependent, and cannot be easily extrapolated from one scale to another. The apparent increase of the velocity of focal fronts at increasing distance of a source can be handled by dual dispersal models (Zawołek & Zadoks, 1992) or dispersive wave models (Ferrandino, 1994; Scherm, 1995), or by stochastic simulation of dispersal (Shaw, 1994), using a contact distribution or primary gradient which is not exponentially bounded.

After the first month of epidemic development the velocity of the front appeared to accelerate, and the gradients appeared to flatten. The process of acceleration could not be studied properly, because of the small distances between fields and the limited field sizes, and because of the disappearance of sharp disease fronts in the acceleration phase.

Our study on focus expansion, although conceptually simple, proved to be difficult in practice. In 1991 no complications were encountered. In 1992 an experiment was discarded because natural infection had occurred before the intended initiation of artificial foci. In 1993 we succeeded in preventing natural infection by steam sterilisation of the soil, but in this case the regular development of foci was hampered by the heterogeneity of the soil drainage capacity. Another practical limitation concerned the time and circumstances required for field observations. In December, observations required more time than earlier in the season because of the high disease levels. At the same time the daylight periods were relatively short. Thus, it was often impossible to do the observations according to a predetermined scheme.

8.1.3 Links between outdoor and indoor work

Throughout our study we combined the results of indoor (laboratory and glasshouse) experiments with outdoor (field) experiments. An important issue in chapters 6 and 7 was the comparison of resistance expressed in a glasshouse test and in a field test. Resistance rankings turned out to be similar in both environments.

An 'indoor' degree-day model for incubation periods was developed in chapter 3 and used to interpret field data in chapter 4. Because of the large variability of incubation periods, not only the average, but also the 'longest' and the 'shortest' incubation period was used. These 'longest' and 'shortest' periods proved to be very useful for identifying rain as the key factor for infection. Our results would have been less convincing if only the average incubation period were used. We think that this way of dealing with variable incubation times may be applicable to other pathosystems.

In our study of thermal death of oospores *in vitro* and in a solarization experiment outdoors, (chapter 3), thermal death occurred in the same temperature range (>45°C) in both experiments, in spite of the greatly differing conditions for oospore formation and incubation.

8.1.4 Oospores

Infection by *P. porri* oospores (chapter 2), and in vitro germination (chapter 3) were described. We discovered that germination of *in vitro* grown oospores started after 5 months of incubation. We tried several methods to break the apparent dormancy of the oospores (Van Es, 1992).

The longevity of oospores of *P. porri* is unknown. They clearly can survive at least one season. The longest period reported for oospore survival is 25 years for onion downy mildew (*Peronospora destructor*; McKay, 1957). For *Phytophthora* spp. hard evidence is available for *P. cactorum* oospores surviving for one year (Legge, 1953). It is generally believed that oospores may survive for many years (Populer, 1981; Stegmark, 1994).

The disease cycle of *P. porri* depends on oospore survival in summer, followed by leaf infection in autumn. In temperate climates there are few plant diseases that are so ill-disposed to grow in summer. *P. porri* does not infect roots or hypocotyls, as many *Phytophthora* spp., nor does it grow systemically in a plant, as many *Peronospora* spp. These characteristics give clues towards disease control, as they reveal weak points in the disease cycle. The disease cycle may be broken if the build-up of the inoculum in the soil, or the dispersal of inoculum to the leaves can be prevented.

8.1.5 Straw mulch

An important recent development in leek cropping is the use of straw mulch to prevent splash-dispersal of *P. porri* (Alofs & Pijnenburg, 1988). The method is based on the insight that splash-dispersal is crucial for epidemic progress. We conducted several field trials to see the effect of straw mulch on epidemic development. The results of these experiments are not published yet. Straw mulching is an effective method to prevent infection, provided that straw can be introduced before a crop is infected in autumn. However, in very wet seasons, or on highly infested soils, or on soils with poor drainage the straw effect will be small or absent.

8.2 Resistance

8.2.1 Durability of resistance

Host plant resistance can be viewed as an elegant method to minimize damage to a crop, without necessarily eliminating the disease. Complete resistance may be ineffective in the long run, because of selection pressure for virulence in the pathogen population (Parlevliet, 1993). Partial resistance, broadly defined as a trait that reduces disease severity by 1-99% in properly chosen test circumstances, is thought to be more durable. However, some researchers state that also complete resistance may be durable, because the durability of a resistance factor is essentially unpredictable (Johnson, 1981). Only in some well-studied pathosystems in which a range of similar resistance factors broke down soon after introduction, a safe prediction of durability may be possible. Such a prediction cannot be extrapolated to other pathosystems. Genetic and epidemiological differences may play an unexpected role.

In our project we did not encounter complete resistance, so we had to exploit partial resistance, even if we would wish something else. Actually, we feel that too little is known of leek genetics and of the epidemiology of *P. porri* to predict the durability of any kind of resistance.

Throughout all experiments we used one isolate of the pathogen. This implies a risk of selecting a resistance factor which is isolate-specific and not expressed in most naturally infested crops. We do not believe this risk to be high, as no single-spore isolates were used for resistance screening. Moreover, we suspect that natural infection was already present in the soil before we started our experiments, so the field population of *P. porri* might have been genetically more diverse than the introduced isolate *pur sang*.

Figure 8.3 Intercellular growth of *Phytophthora porri* in a cotton blue-stained epidermal strip of a colonized leek leaf.

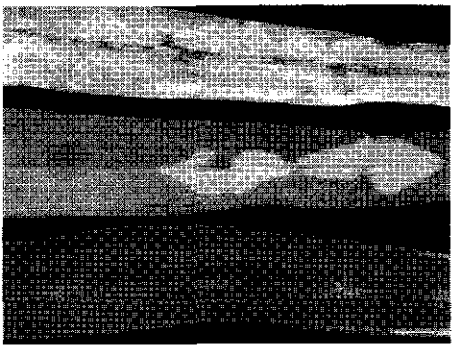

Figure 8.4 *P. porri* lesions showing irregular, map-like patterns.

8.2.2 Resistance mechanism

Microscopic observations suggest that *P. porri* may grow in the intercellular space of the leaf (Fig. 8.3). Macroscopic observations indicate that infections may be resisted by a rapid response, leading to local necrosis (Fig. 8.4). The speed of the response varies, even on one plant and one leaf, and is probably highly sensitive to environmental factors.

In none of our experiments we noticed root infection by *P. porri*. Especially in the immersion test such infections would have been clear if they had occurred. It is not known why leek roots are resistant to *P. porri*. Possibly, there is a physiological cause for the root resistance. It would be interesting to know if the fungus can grow on leek root agar, as this would support this hypothesis. It should be noted that leek roots have been studied extensively on a biochemical level because of their synergistic relations with mycorrhizal fungi (Peretto *et al.*, 1995). These studies may help to understand and exploit root resistance.

The macroscopic symptoms of *P. porri* are the result of necrotic responses to infection. Within the necrotic zone not only the plant but also the fungus, or at least the mycelium, dies. The necrotic flecks may arise within a few hours in tissue that has been colonized for some days. Sometimes fungal growth is effectively blocked by the necrotic response, but the fungus often survives in the margins of the lesions, especially of the larger ones, and colonizes fresh adjacent leaf tissue. This colonization is followed by a second necrotic response, with similar chances of success, and so on, until either the plant or the fungal colony dies. Unlike many other leaf pathogens, *P. porri* lesions often have irregular 'map-like' patterns on the leaves.

Resistance may be seen as the effect of a factor that regulates the speed of the necrotic response, or, more precisely, the probability of an effective response. This implies on the one hand that extremely high partial resistance is identical to hypersensitivity, and on the other hand that extremely low partial resistance would lead to absence of a necrotic reaction and unrestricted lesion growth. We did not find examples of either extreme. For practical purposes highly susceptible plants of cv. Wintina may be chosen as an arbitrary reference point to define a total lack of resistance. However, from a theoretical point of view all leek plants studied, even the most susceptible, can be characterised as partially resistant.

8.2.3 Genetics of resistance

The genetic basis of *P. porri* resistance in leek is still unclear, in spite of some significant results which prove that a considerable amount of phenotypic and genotypic variation is present within and among cultivars. We found that resistance from landraces can be transferred to winter leek, but indisputable conclusions about numbers of genes or

dominance effects were impossible, because of the very complex and largely unknown genetic system of the tetraploid leek.

Useful sources of resistances were found in quite a few leek accessions. Even within the common cultivars the variation for resistance was large enough to start breeding without backcrossing to sources of higher resistance. Apparently not the material, but the selection method was the missing condition for achieving progress in resistance breeding. The selection problem is solved in our study by the demonstration that field selection is possible (chapter 3), and by a new early screening method, the immersion test (chapter 7). Rapid progress may be expected when breeders start to use these tools for selecting *P. porri* resistance.

We do not know at what resistance level the gene-pool of winter leek will be exhausted. Obviously, resistant landraces will broaden the genetic base of winter leek and thus will increase the chances of success of resistance breeding progams. Selected plant material from two backcrosses with a source of resistance (landrace PI 368351) was therefore offered to breeders. The performance of this material is illustrated in Fig. 8.5.

We do not know yet what level of resistance is required to achieve satisfactory disease control. This level will depend on the season of harvesting of a particular crop, quality requirements at auction houses, and the use of other disease control measures. For the time being, we assume that every increase in resistance is worth the effort.

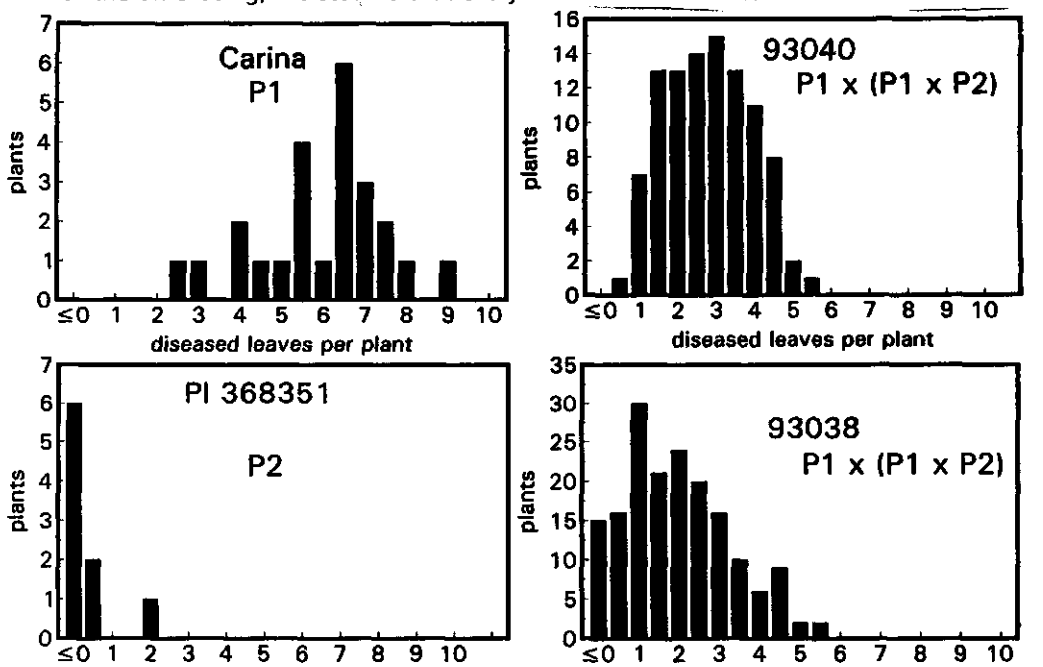

Figure 8.5 Distribution of field scores (diseased leaves per plant; average of two observations (28-9-94 and 1-11-94)) of cv. Carina, a partially resistant landrace of leek (PI 368345), and two backcross progenies of Carina and the landrace.

8.3 Recommendations

The ultimate aim of the project, effective control of *P. porri*, can only be achieved when the current cropping method is revised. It appears that phytosanitary measures are needed. The common practice of leaving the crop debris on the land, combined with narrow rotation schemes is a sure way towards disaster. It is not known whether heavily infested soils can be replanted without risk after 4-8 years. This question may be investigated after developing counting methods or a bioassay for soil inoculum (Drenth *et al.*, 1994; Van der Gaag, in preparation). Such methods may also reveal differences in soil receptivity for inoculum (Oyarzun *et al.*, 1995). Whatever the outcome of such a study, we may safely recommend rotation cycles of at least 3 years and a proper disposal of leaf debris.

Future epidemiological research on *P. porri* should concentrate on the role of leaf debris in the disease cycle. We expect a beneficial effect of phytosanitary measures. Experiments are needed to prove and quantify the effect. Leaf debris may be disposed of by burning or burying (Foister, 1961) or by composting (Bollen *et al.*, 1989). In chapter 3 it was hypothesised that oospores can be killed by high temperatures during composting. This hypothesis can be tested. It is not yet clear, however, if composting of leek refuse is economically feasible.

The adoption of straw mulching will give relief in some, but not in all cases. New mulching techniques limiting *P. porri* and other pests or diseases simultaneously, particularly thrips (Csizinsky *et al.*, 1995), are worth investigating.

It is now possible to breed for resistance to *P. porri*, using the screening techniques described in this thesis, and the selected material from the backcross of a highly resistant landrace with winter leek. In 10-20 years, *P. porri* resistant cultivars may become available which are better adapted to narrow rotation schemes. The basis for this development is described in this thesis.

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Summary

Introduction (Chapter 1)

Leek. Leek (*Allium porrum* L.) is an important vegetable crop in the Netherlands. In 1995 the crop was grown on ca. 4,000 ha and its total production value was ca. Dfl. 100 million (US$ 60 million). About 50% of the Dutch leeks are exported. The Dutch seed industry has the largest share of the world market for leek seed.

Leek breeding is mainly based on mass- and half-sib family selection. Leek cultivars are open-pollinated, ill-defined populations, which may contain a considerable amount of genetic variation. Cultivars may contain ca. 20% selfed, relatively weak plants. No cytoplasmic male sterility is available yet. Therefore, F_1 hybrids are being developed, using vegetatively propagated nuclear male sterile plants as mothers. Little is known yet about the genetics of leek, which is complicated because of autotetraploidy and the localisation of chiasmata, preventing the formation of tetravalents.

Phytophthora porri. In winter, white tip disease (*P. porri*) is the most important leek disease in the Netherlands. Chemical control of *P. porri* is often unsatisfactory. Straw mulching may give some immediate relief. In the long term, host resistance is the most attractive tool for disease control.

P. porri was recorded for the first time in Scotland in 1931. In the Netherlands it was first described in 1959, and it became a serious problem since ca. 1985. *P. porri* is also recorded from other crops in various parts of the world. Some degree of host specificity seems to be present.

P. porri is a homothallic oomycete. The fungus is present in infected tissue as non-septate mycelium. The fungus can grow *in vitro* on natural media. Sporangia can develop *in vitro* and in under field circumstances in wet lesions and may release 10-30 zoospores. Oogonia are produced in abundance in leaves, while the thick-walled oospores are formed as the leaves dry up. The fungus depends on oospores for survival during the crop free period in summer.

Epidemiology

Field inoculation (Chapter 2). Field inoculation of leek with zoospores of *P. porri* resulted in high infection levels within a short time. Inoculation with infected leaf tissue resulted in a more gradual increase of disease incidence. Inoculation with oospores was relatively unsuccessful. Zoospores were produced in Petri-dishes by treating fast-growing, young mycelium with a diluted soil extract for at least two days, followed by a cold treatment in sterile demineralized water.

Temperature effects (Chapter 3). Cardinal temperatures for mycelial growth of *P. porri* on corn-meal agar were <5 (minimum), 15-20 (optimum) and just above 25°C (maximum). The number of infections after zoospore inoculation of young leek plants was relatively low at supra-optimal temperatures, but was not reduced at sub-optimal growth temperatures. Even at 0°C plants were infected. The incubation periods needed for symptom formation were 36-57 d at 0°C, 13-18 d at 5°C, and 4-11 d at >11°C. They were fitted to temperature between 0 and 24°C with a hyperbolical model (1/p= 0.00812*T+ 0.0243).

Oospore germination, reported for the first time for *P. porri*, was strongly reduced after 5 h at 45°C, and totally absent after 5 h at 55°C. However, soil solarization for six weeks during an exceptionally warm period in May-June 1992 in the Netherlands raised the soil temperature at 5 cm depth for 17 h above 45°, but did not reduce the initial level of disease in August significantly.

Rain effect (Chapter 4). On fields infested by soil-borne inoculum (oospores), relatively short periods of explosive disease increase alternated with periods in which apparently no new infections occurred. The analysis of rain data and disease data, using a degree-day model for incubation periods at various temperatures, confirmed the hypothesis that disease increase of *P. porri* is significantly correlated with rain; R^2_{adj} was 0.91, 0.41 and 0.51 in 1992, 1993 and 1994, respectively. Correlations were highest early in the season. Zoosporangia were observed in fields on water-logged light-green lesions. High lesion densities of leaf tips and leaf units at 10-20 cm above the leaf axils indicated that most infections depend on free water, either in puddles or in the water basin formed by the leaves near the leaf axils.

Focus expansion (Chapter 5). The spatial and temporal development of *P. porri* on leeks was studied in artificially infected fields. Disease sources of various shapes and sizes were created in eight adjoining plots of 10 x 10 m in 1991 and 1993. An exponential model was fitted to disease progress curves. The doubling time of disease was ca. 20 days in December. The disease progress curves converged, and uninfected control plots became infested at the end of the season, indicating interplot interference. In spite of severe infestation at the end of the season, epidemics did not enter the saturation phase. Low temperatures in December did not slow down the epidemics.

Disease gradients were analysed with the negative exponential model and the inverse power model. The gradients were initially steep and gradually flattened. The half-distances of the gradients increased from <35 cm in October to >140 cm in December. The displacement velocity of the focal front, which could only be estimated early in the epidemic, was ca 3 $cm.d^{-1}$. Breeders planning field trials to select for resistance under climatic conditions as occur in the Netherlands are recommended to initiate epidemics

on at least 2 % of the plants in spreader rows at most 3 m apart, in the first half of September. Such trials should be laid out on well-drained fields.

Resistance

Initial resistance studies (Chapter 6). Significant differences in partial resistance to *P. porri* were found between winter leek accessions in field tests with zoospore and natural inoculation. These differences were consistent over three years, and indicate that resistance can be improved through mass selection in winter leek cultivars. Some accessions of wild or cultivated relatives of winter leek with higher levels of resistance were found in a glasshouse test with zoospore inoculation. These sources of resistance were also highly resistant in field experiments over a two year period.

New screening method (Chapter 7). A new screening method was developed to evaluate resistance to *P. porri*, based on inoculation by 24 h-immersion of leek plantlets in the 3-6 leaf stage in a suspension of ca. 100 zoospores.ml^{-1}. The immersion test was used for advanced resistance studies.

Advanced resistance studies (Chapter 7). Within winter leek, five resistance classes were defined on the basis of average field scores of 21 plants. Clones from these plants were tested with the immersion technique. The average scores per resistance class in immersion and field tests were significantly correlated. The correlation of single-date field scores with the immersion test scores was better in the second half of the season, after November. A selection experiment yielded a strong response to selection for resistance but no response to selection for susceptibility. This may indicate that resistance is due to 2-4 recessive genes in the winter leek cultivars studied.

Within partially resistant landraces genetic variation for resistance was demonstrated with inbred lines. Crosses between landraces and winter leek were analyzed by means of F_2 (selfed F_1) and BC_1 progenies. This analysis indicated the presence of 2-4 loci with dominant genes for resistance in accession CGN 873243 and additive polygenes in accession PI 368351.

General discussion (Chapter 8)

The ultimate aim of the project, effective control of *P. porri*, can only be achieved if the current cropping method is revised. The common practice of leaving crop debris on the land, combined with narrow rotation schemes, is a sure way to disaster. It is now possible to breed for resistance to *P. porri*, using the screening techniques described in this thesis, and the selected material from the backcross of a highly resistant landrace with winter leek. In 10-20 years, *P. porri* resistant cultivars may become available which are better adapted to narrow rotation schemes.

Samenvatting

Inleiding (Hoofdstuk 1)

Prei. Prei (*Allium porrum* L.) is een belangrijk groentegewas in Nederland. In 1995 was het areaal 4000 ha, en de totale produktiewaarde ca. 100 miljoen gulden. Ca. 50% van de prei wordt geëxporteerd. De Nederlandse zaadbedrijven bedienen gezamenlijk het grootste deel van de wereldmarkt voor preizaad.

Veredeling van prei is hoofdzakelijk gebaseerd op massa- en half-sib-familieselektie. Cultivars zijn vrij-bestoven, moeilijk definieerbare populaties, die een aanzienlijke hoeveelheid genetische variatie kunnen bevatten. Cultivars kunnen voor 20% uit zelfbevruchte, vrij zwakke planten bestaan. Er is nog geen cytoplasmatische mannelijke steriliteit beschikbaar. Wel worden momenteel F_1 hybride rassen ontwikkeld met behulp van vegetatief vermeerderde genisch mannelijk steriele planten. Over de genetica van prei is weinig bekend. De combinatie van autotetraploïdie en chiasma-localisatie, waardoor de vorming van tetravalenten wordt voorkomen, kan voor vreemde complicaties zorgen.

Phytophthora porri. Papiervlekkenziekte (*P. porri*) is 's winters de belangrijkste preiziekte in Nederland. Chemische gewasbescherming geeft in veel gevallen geen bevredigend resultaat. Stro mulch kan op korte termijn de ergste nood lenigen. Op de lange termijn is waardplantresistentie het beste middel voor de onderdrukking van de ziekte.

P. porri werd voor het eerst in 1931 in Schotland beschreven. De eerste beschrijving in Nederland stamt uit 1959. Na ca. 1985 werd de ziekte een ernstig probleem in de preiteelt. *P. porri* is op verschillende gewassen verspreid over de hele wereld gevonden. De schimmel lijkt niet makkelijk van de ene op de andere waardplantsoort over te gaan.

P. porri is een homothallische oomyceet. In geïnfecteerd weefsel groeit de schimmel als een ongesepteerd mycelium. De schimmel kan *in vitro* op een natuurlijk medium worden gekweekt. Sporangiën kunnen *in vitro* worden gekweekt, en worden onder veldcondities gevormd op het oppervlak van natte, geïnfecteerde bladeren. Ze kunnen 10-30 zoösporen voortbrengen. Oögoniën worden in overvloed binnenin het blad gevormd. De dikwandige oösporen verschijnen als bladeren droog worden. De schimmel overleeft de gewasvrije periode in de zomer via oösporen.

Epidemiologie

Veldinoculatie (Hoofdstuk 2). Veldinoculatie van prei met zoösporen van *P. porri* gaf binnen korte tijd veel infectie. Inoculatie met stukjes ziek blad gaf een wat geleidelijker toename van de ziekte. Inoculatie met oösporen ging relatief slecht. Zoösporen werden

gekweekt in Petri-schalen door snelgroeiend, jong mycelium te behandelen met verdund grond-extract gedurende minstens twee dagen. Daarna volgde een koudebehandeling met steriel gedemineraliseerd water.

Temperatuur (Hoofdstuk 3). Kardinale temperaturen voor myceliumgroei van *P. porri* op maismeel-agar waren <5 (minimum), 15-20 (optimum) en net boven 25°C (maximum). Het aantal infecties na zoöspore-inoculatie van jonge preiplanten was relatief laag bij temperaturen boven het optimum voor myceliumgroei, maar bleek niet te dalen bij temperaturen beneden dat optimum. De incubatietijden waren 36-57 d bij 0°C, 13-18 d bij 5°C en 4-11 d bij >11°C, en pasten goed bij een hyperbolisch model (1/p= 0.00812*T+ 0.0243).

Kieming van *P. porri* oösporen, hier voor het eerst beschreven, bleek sterk te verminderen na 5 uur incubatie bij 45°C, en geheel afwezig te zijn na 5 uur incubatie bij 55°C. In een solarizatie-experiment gedurende zes uitzonderlijk warme weken in Nederland, in Mei-Juni 1992, steeg de bodemtemperatuur op 5 cm diepte gedurende 17 uur boven 45°, maar desondanks bleek de eerste aantasting in Augustus niet significant lager te zijn dan in de niet-gesolarizeerde velden.

Regen (Hoofdstuk 4). Op door oösporen in de bodem besmette velden wisselden korte perioden met een explosieve ziektetoename af met langere perioden waarin kennelijk geen nieuwe infecties plaatsvonden. De analyse van regen- en ziekte waarnemingen, met behulp van een graaddagen-model voor incubatieperioden bij verschillende temperaturen, bevestigde de veronderstelling dat ziektetoename gecorreleerd is met regenval; R^2_{adj} was 0.91, 0.41 en 0.51 in 1992, 1993 en 1994, respectievelijk. Vroeg in het seizoen waren de correlaties hoger.

Zoösporangiën werden in het veld waargenomen op waterige lichtgroene lesies. Hoge lesiedichtheden op bladtoppen en 10-20 cm boven de bladoksels betekenen dat infecties waarschijnlijk alleen in vrij water plaatsvinden, in plassen of in het waterbadje bij de bladoksels.

Haarduitbreiding (Hoofdstuk 5). De ontwikkeling in ruimte en tijd van *P. porri* op prei werd bestudeerd in kunstmatig geïnfecteerde velden in 1991 en 1993. Ziektebronnen met verschillende omvang en vorm werden aangelegd in acht aan elkaar grenzende velden van 10 x 10 m. Een exponentieel model werd toegepast op de ziekteontwikkelingscurves. De verdubbelingstijd van de ziekte was ca. 20 d in December. De ziekteontwikkelingscurves convergeerden, en ongeïnfecteerde contrôlevelden raakten tegen het eind van het seizoen besmet, hetgeen wijst op onderlinge beïnvloeding van de velden. Ondanks de zware besmetting tegen het einde van het seizoen kwam de epidemie niet in een verzadigingsfase. Bij lage temperaturen in December vertraagde de ziekteontwikkeling niet.

De ziektegradiënten werden met een negatief-exponentieel en een inverse-machtsmodel geanalyseerd. De gradiënten waren aanvankelijk steil en vlakten geleidelijk af. De halfwaarde-afstanden namen toe van <35 cm in Oktober tot >140 cm in December. De verplaatsingssnelheid van het ziektefront, die alleen vroeg in de epidemie bepaald kon worden, was ca. 3 $cm.d^{-1}$. Wie in het Nederlands klimaat in het veld wil selecteren op resistentie dient begin September minstens 2% van de planten in ten hoogste 3 m uit elkaar staande verspreiderrijen te besmetten. Selectie voor resistentie dient op goed gedraineerde proefvelden te worden uitgevoerd.

Resistentie

Inleidend onderzoek (Hoofstuk 6). Significante verschillen in partiële resistentie voor *P. porri* werden gevonden tussen verschillende nummers van winterprei in veldtoetsen met kunstmatige (zoösporen-) en natuurlijke inoculatie. Deze verschillen waren consistent in drie opeenvolgende jaren, en geven aan dat resistentie kan worden verbeterd door massaselectie binnen winterprei. Landrassen met een hoge mate van resistentie werden gevonden in een kastoets met zoöspore-inoculatie. Deze bronnen van resistentie waren tevens in hoge mate (maar niet absoluut) resistent in een tweejarige veldproef.

Nieuwe toetsmethode (Hoofdstuk 7). Een nieuwe toetsmethode voor *P. porri* resistentie werd ontwikkeld. De methode maakt gebruik van jonge preiplanten met 3-6 bladeren. De plantjes worden geheel ondergedompeld in een suspensie van ca. 100 $zoösporen.ml^{-1}$. De dompeltoets werd gebruikt in het vervolg-onderzoek.

Vervolg-onderzoek (Hoofdstuk 7). Binnen winterprei werden vijf resistentieklassen gedefinieerd op grond van de gemiddelde veldscores van 21 planten. Klonen van deze planten werden met de dompeltoets geëvalueerd. De gemiddelde scores per resistentieklasse in dompel- en veldtoets waren significant aan elkaar gecorreleerd ($P<0.01$). De correlatie van de veldscores van één observatiedatum met de scores in de dompeltoets was relatief hoog in de tweede helft van het seizoen, na November. Selektie voor resistentie was zeer effectief, maar selektie voor vatbaarheid helemaal niet. Dit zou kunnen betekenen dat resistentie in de onderzochte winterprei berust op 2-4 recessieve genen.

Binnen de partieel resistente landrassen bleek genetische variatie voor resistentie aanwezig te zijn. Dit bleek uit beproeving van inteeltlijnen. Kruisingen tussen landrassen en winterprei werden geanalyseerd met behulp van F_2 (zelfbevruchte F_1) en BC_1 nakomelingschappen. De analyse doet vermoeden dat in accessie CGN 873243 de resistentie berust op 2-4 loci met dominante genen, en in PI 368351 op additieve polygenen.

Slotbeschouwing

Het einddoel van dit project, de onderdrukking van *P. porri*, kan slechts bereikt worden via een hervorming van de preiteelt. De gewoonte om bladafval op het land achter telaten, in combinatie met de vaak korte gewasrotatie moet haast wel leiden tot rampzalige gevolgen. Het is nu mogelijk om gericht te werken aan resistente rassen, met behulp van de in dit proefschrift beschreven toetsmethoden, en met de geselekteerde planten afkomstig uit een terugkruising van een resistent landras met winterprei. Over 10-20 jaar zullen er mogelijk *P. porri* resistente cultivars verkrijgbaar zijn die beter zijn aangepast aan korte gewasrotaties.

Curriculum vitae

Willem Diederik Smilde werd geboren op 1 april 1963 te Rotterdam, en groeide op in Bolnes, gemeente Ridderkerk. Na het behalen van het gymnasium-β-diploma aan de Gereformeerde Scholengemeenschap Rotterdam en het propaedeuse-diploma van de Evangelische Hogeschool te Amersfoort begon hij in 1982 met de studie Plantenveredeling aan de Landbouwuniversiteit in Wageningen (LUW). Deze studie werd in 1988 voltooid. Daarna werkte hij achtereenvolgens bij het Instituut voor Plantenziektenkundig Onderzoek (IPO) te Wageningen, het Rijksinstituut voor Rassenonderzoek (RIVRO) te Wageningen, de vakgroep Plantentaxonomie van de LUW, en de International Service for National Agricultural Research (ISNAR) te 's Gravenhage. In december 1990 trad hij in dienst van de Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO), en werd onder verantwoordelijkheid van de Stichting voor de Technische Wetenschappen (STW) gedetacheerd als onderzoeker-in-opleiding (OIO) bij de vakgroep Fytopathologie van de LUW en bij het Centrum voor Plantenveredelings- en Reproduktieonderzoek van de Dienst Landbouwkundig Onderzoek (CPRO-DLO) te Wageningen. Op deze twee plaatsen heeft hij het in dit proefschrift beschreven onderzoek verricht.