On ecology and control of *Mycocentrospora acerina* in caraway (*Carum carvi*)

Albartus Evenhuis

On ecology and control of *Mycocentrospora acerina*
in caraway (*Carum carvi*)

Over ecologie en bestrijding van *Mycocentrospora acerina*
in karwij (*Carum carvi*)

Promotor: dr. J.C. Zadoks
emeritus hoogleraar ecologische fytopathologie

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On ecology and control of *Mycocentrospora acerina* in caraway (*Carum carvi*)

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Bibliographic abstract

Mycocentrospora acerina causes anthracnose, an important disease of caraway (*Carum carvi*) which can cause severe yield loss in wet years. The fungus survives in the soil and inoculum levels are probably maintained by alternate hosts. Although most of the cover crops are susceptible to *M. acerina* their impact on anthracnose development in caraway is small. Seed infection has been demonstrated. Infected seeds contribute to the inoculum, but seed-borne inoculum is probably less important than soil-borne inoculum. *M. acerina* conidia are splash dispersed over short distances (< 9 m). Therefore, inoculum sources from outside caraway fields are of little importance for caraway infection. A damage threshold between 6 and 12 % disease severity of the caraway crop is proposed. Minimizing the occurrence of *M. acerina* should suffice to control anthracnose in caraway. Cultural measures to control anthracnose of caraway include all activities leading to an open crop canopy, among which moderate nitrogen application, intermediate row spacing and moderate sowing rate.

The research described in this thesis was carried out at the Research Station for Arable Farming and Field Production of Vegetables (PAV). It was supported financially by the Ministry of Agriculture, Nature Management and Fisheries within the scope of the Dutch Caraway Research Programme.

Ter herinnering aan mam, voor pap

Bloemen

soms vallen knoppen en bloemen
maar de meesten zetten vrucht
ik zal je ze te eten geven

Berend Verdam

Stellingen

1. Goeie karwij, daar kan een kat overheen en een hond onderdoor lopen (Groningse boerenwijsheid). Dit gewastype beperkt ook het optreden van verbruining in karwij (Dit proefschrift).
2. Het telen van loszadige karwij, terwijl er vastzadige rassen beschikbaar zijn (Rassenlijst, 1998), staat gelijk aan het nemen van een niet te rechtvaardigen risico.
3. Met de introductie van eenjarige karwij (Toxopeus & Lubberts, 1994. Prophyta **1**: 18-19) zal het speculatieve karakter van de karwijteelt minder worden, en deze verliest daarom voor de handelende boer een deel van zijn aantrekkelijkheid.
4. De waardplantenreeks van *Mycocentrospora acerina* kan waarschijnlijk onbegrensd uitgebreid worden, waarbij de schimmel zich op het ene gewas meer als pathogeen gedraagt en op andere gewassen een meer saprofytische leefwijze kent (Dit proefschrift).
5. In tegenstelling tot de bewering van Wall & Lewis, spelen conidiosporen van *Mycocentrospora acerina* op het gewas waarschijnlijk wel een rol bij de problemen met wortelen in de bewaring. Deze veldfase verdient dan ook meer aandacht. (Wall & Lewis, 1980. Transactions of the British Mycological Society **74**: 587-593; Dit proefschrift).
6. Inzet van chemische middelen in de land- en tuinbouw kan minder milieubelastend zijn dan het alternatief, gebruik van niet recycleerbare hulpmiddelen (Roovers *et al.*, 1998, Ekoland **2**: 10-15).
7. Onderzoek op Regionale Onderzoek Centra blijft noodzakelijk omdat streek- en grondgebonden problemen niet centraal opgelost kunnen worden.
8. Door privatisering zijn de leden van het drieluik Onderzoek - Voorlichting - Onderwijs van synergistische tot concurrerende organisaties verworden (Vijverberg, 1996, Spil 137-138/139-140).
9. Flexibiliteit in arbeid vraagt een solide onderbouwd sociaal beleid om van de kansarmen geen kanslozen te maken.
10. Een toename van flexwerk gaat gepaard met een toename van mobiliteits-problematiek.
11. De charme van het vroegere Europa Cup 1 toernooi was het knock-out systeem, met kansen voor de kleine clubs op een stunt. Met de introductie van de "Champions league" is een commercieel gedrocht ontstaan dat de grote clubs bevoordeelt en de ongelijkheid vergroot.
12. Nederland heet een fietsland te zijn, maar de praktijk is dat bijna iedereen als vanzelfsprekend voor elke onbenulligheid de auto pakt. Nederland autoland zou helaas een betere benaming zijn.

Stellingen behorende bij het proefschrift: "On ecology and control of *Mycocentrospora acerina* in caraway (*Carum carvi*)", door A. Evenhuis

Wageningen, 10 juni 1998.

Woord vooraf

De verspreiding van sporen van *Mycocentrospora acerina* is beperkt in aantal sporen en afgelegde afstand. Het gevolg van die enkele verspreide spore kan echter groot zijn. Hopelijk hebben enkele verspreide exemplaren van dit proefschrift, in tegenstelling tot de sporen van de verbruiningsziekte, een positief gevolg voor die paar karwijtelers op de Groningse of Zeeuwse klei en peen telers in Ås (N) of Norwich (GB).

Zoals het ontstaan van de verbruiningsziekte afhankelijk is van bronnen, zo is het ontstaan van een proefschrift dat ook. De oorsprong ligt bij de mogelijkheid en de stimulans die mijn ouders me gegeven hebben om te gaan studeren. Hiermee was, hoewel nog niet zichtbaar, het infectie proces gestart. Als niet het toenmalige PAGV, nu Praktijkonderzoek voor de Akkerbouw en Vollegrondsgroenteteelt (PAV), gelegenheid had geboden om het toegepaste onderzoek te verwerken tot een proefschrift dan was het proces reeds vroegtijdig gestopt. Dankzij de nauwe samenwerking met het Instituut voor Plantenziektekundig Onderzoek (IPO-DLO) kon het proefschrift verder uitgroeien.

Net zoals bij de verbruiningsziekte hangt het al of niet slagen van de infectie nauw samen met de omgeving. Zonder hulp van de mensen van de proefboerderijen van het PAV, ROC-Ebelsheerd, IPO-DLO, Droevendaal (AB-DLO) en de Bouwing (AB-DLO) en de karwijtelers verenigd in de karwijstudieclub zou het onmogelijk geweest zijn het karwei uit te voeren. Langs deze weg wil ik jullie allemaal bedanken voor jullie inzet. De inbreng van de leden van het Karwij Onderzoek Overleg, wat heeft geleid tot gezamenlijke proeven met AB-DLO, CPRO-DLO en het Van Hall Instituut, mag niet onvermeld blijven.

Berend, jij hebt er mede voor gezorgd dat het proces in de exponentiële groeifase terecht kwam. Jouw inzet ging verder dan het praktisch uitvoeren van de proeven. Je dacht mee over de opzet en uitvoering van het onderzoek. Ik heb bewondering voor het feit dat je de koe bij de horens hebt gevat en nu mensen helpt om dat ook te doen.

Tegen het eind van een epidemie zullen nieuwe verspreidingsstructuren gevormd moeten worden die kunnen dienen als bron voor een volgend infectieproces. Zonder iemand tekort te willen doen, Albert, Ben, Johan, Thijs, Willem en Wim, bedankt voor jullie kritische inbreng bij het schrijven van de artikelen.

Het schrijven van een proefschrift is vergelijkbaar met een monocyclische epidemie. Net zoals bij een epidemie moet aan veel voorwaarden worden voldaan om het proces in gang te houden. Aan allen die dit op enigerlei wijze mogelijk hebben gemaakt hiervoor bedankt.

Professor Zadoks, dank voor de 1000 en 1 ideeën die een inspiratiebron zijn geweest bij de uitvoering van het onderzoek. Slechts een fractie hiervan is in dit manuscript terug te vinden.

Anneke, jij was een stimulans om dit karwij (of is het karwei?) te volbrengen, bedankt.

Contents

CHAPTER 1

General introduction

The National Research Programme on caraway

Caraway has been grown as a crop for over three centuries, in the Netherlands. At the beginning of the 20th century caraway was considered the most important umbelliferous crop in the Netherlands, even preceding carrots (Zijlstra, 1915). Caraway cultivation has always been the domain of tradesmen among the farmers. The hectareage of the crop varies from 10 to 10000 hectares annually and is a reflection of the prices paid for caraway seeds in the market in previous years. Traditionally, caraway seed is used as a spice, in the kitchen and in the bakery, nowadays potential new markets for caraway seeds are based upon the chemical structure of carvone, the main component of caraway essential oil. (S)-(+)-carvone[a] is able to inhibit sprouting of potatoes in warehouses and has antifungal properties (Meigh, 1969; Oosterhaven, 1995). (S)-(+)-carvone[a] was successfully used as the chiral starting material in the synthesis of biologically active compounds (Verstegen-Haaksma, 1994).

A national research programme on caraway improvement and marketing potential of its essential oil was started in 1989. The programme was initiated by a group of caraway growers and funded by the Ministry of Agriculture, Nature Management and Fisheries. In the beginning of the 1980's farmers from the province of Groningen asked attention for publications regarding the biological activity of essential oil from caraway. The aim of the programme was to find new applications for caraway essential oil, to improve caraway cultivars by plant breeding and selection, and to elucidate factors involved in seed yield and essential oil production. Simultaneously, a pharmaceutical company and the Agrotechnological Research Institute (ATO-DLO) started research on the application of carvone as a sprout inhibitor of potatoes (Hamster, 1994). The research was successful and a commercial sprout inhibitor of potatoes, based upon carvone, was placed on the market in 1995 (Hartmans *et al.*, 1995).

[a]: In nature two enantiomers of carvone are found. (R)-(-)-carvone is found in spearmint. Caraway and dill contain (S)-(+)-carvone. In this thesis (S)-(+)-carvone is simply referred to as carvone.

Unfortunately, the use of carvone as a sprout inhibitor is rather expensive in comparison with the traditional chemical compounds used (Van der Mheen *et al.*, 1994). To lower the costs of carvone production, an increase in the seed production of caraway was and is necessary. An increase in seed yield gives an increase in essential oil production per hectare as well. Agrobiological studies were performed to elucidate the factors affecting essential oil production (Bouwmeester & Smid, 1995; Bouwmeester *et al.*, 1995). Plant breeders tried to improve the essential oil content of caraway (Toxopeus & Bouwmeester, 1993). Caraway yield fluctuation is very large between years and regions, and varies from 600 to 2500 kg ha^{-1} (Bouwmeester *et al.*, 1995). Apart from weather factors, plant diseases and insects may play a key role in the lack of yield stability of caraway in the Netherlands. The caraway root aphid (*Pemphigus passeki* Börner (*Homoptera: Aphidoidea*)) is considered the most damaging insect in caraway production (Prinsen, 1991). *Mycocentrospora acerina* (Hart.) Deighton and *Sclerotinia sclerotiorum* (Lib.) de Bary are the two most important diseases of caraway (Evenhuis *et al.*, 1995). In Germany, *M. acerina* is considered the most important pathogen of caraway stems (Heeger, 1956).

The present thesis is the third (Verstegen-Haaksma, 1994; Oosterhaven, 1995) and last one arising from the research programme on caraway and deals with *Mycocentrospora acerina* in relation to the production of caraway seed. The ecology of the pathogen was hardly known in 1990. *M. acerina* was found to infect caraway (Westerdijk & Van Luijk, 1924; Heeger, 1956; Erfurth, 1976; Plescher & Herold, 1983; Müller *et al.*, 1989), but data on yield loss were scarce. Measures to control the disease are hardly developed. Some results had been obtained with chemical control but success was not guaranteed (Erfurth, 1976; Müller *et al.*, 1989; Floot, 1991; Evenhuis & Verdam, 1995). The aim of the present study is to investigate the biology of *M. acerina* and to develop control measures to reduce yield loss due to anthracnose.

Caraway botany

Caraway is a biennial species belonging to the *Umbelliferae*. Caraway grows vegetative in the first year, and after winter becomes reproductive. Recently an annual type of caraway has been introduced, referred to as spring caraway. Occasionally caraway is triennial. Caraway originates from Asia and Southern Europe (Van der Meer, 1960). The wild caraway plant can be found throughout Europe, the Middle

East, Tibet and Russia. In Northern America caraway plants found in nature originate from caraway imported for cultivation. Wild caraway was found readily alongside dykes and on haughs of the bigger rivers in the Netherlands at the beginning of this century (Zijlstra, 1915). The species grew also adventively in caraway growing areas (Zijlstra 1915). Nowadays, caraway is found occasionally in nature but it is regarded a rare species in the Netherlands (Brandsma, 1991).

Zijlstra (1915) described caraway thoroughly. Caraway forms a long pen root usually without branches. The diameter of the root collar depends on plant density, but can be up to 2 cm. In the first year only rosette leaves are formed. Leaves are compound and double finned. In the second year caraway bolts. Usually one erect main stem is formed. From the main stem some 8 secondary stems grow under an angle of about 45°. These secondary stems also branche into tertiary and quarternary stems. Each stem ends with an umbel. Thus, umbels of the first, second, third and higher orders are formed. The main umbel is the first to flower, followed by umbels of higher order. An umbel consists of 8 to 16 umbellets. An umbellet consist of approximately 10 flowers. Caraway is andromonoecious and predominantly a protandrous cross-fertilizer. Caraway is insect pollinated (Zijlstra, 1915), but wind pollination does occur (Bouwmeester & Smid, 1995). Caraway produces split fruits (*diachenium*), which are usually referred to as seeds.

Biennial caraway cultivation

Traditionally, biennial caraway or winter caraway is grown on heavy clay soils. The main growing areas are to be found in the provinces of Groningen, in the northeast of the Netherlands, and Zeeland in the southwest. In the Netherlands caraway is usually sown under a cover crop early in spring (March or April). Seed rates vary from 6 - 15 kg ha^{-1}. On heavy clay soils a high sowing rate is usually applied to ensure crop establishment under difficult conditions. When, however, conditions are favourable for germination more plants than necessary will emerge and competition among caraway plants may occur, resulting in thin roots. The development of caraway under a cover crop is slow. When the cover crop is harvested caraway has managed to form a few leaves only. Cover crops used are winter wheat, spring barley, peas, oil seed poppy and spinach for seed production. Since the market for agronomically interesting cover crops, such as peas or spinach, is poor, less attractive cover crops have to be used,

among which winter wheat or spring barley. A cover crop which can be harvested early in the season is preferred because it prolongs the growing period for the caraway plants before winter. Cover crops with open canopies are particularly suitable because light can reach the young caraway plants, such as oil seed poppy and dry peas. Caraway plants must develop a root collar with a diameter of at least 6 mm before winter in order to bolt next year. Plants which have not grown a root of such size will not be able to bolt and flower in the next year but will remain vegetative for one more year. This principle induces farmers sometimes to sow caraway to a high plant density which assures them to be able to harvest caraway twice (Bernelot Moens, 1968).

Biennial caraway usually flowers in May. The seed shattering varieties are windrowed and left on the field in swaths to dry. They are left to dry for some 10 days before the crop is harvested by a combine harvester. Non seed shattering varieties are harvested in July by a combine harvester. The average yield of caraway is approx. 1500 kg seeds per hectare. Occasionally, just over 3000 kg ha^{-1} has been harvested (Van de Mheen *et al.*, 1994). The essential oil content of biennial caraway seeds varies between 2 and 7% (Toxopeus & Bouwmeester, 1993) and is higher than that of annual or spring caraway. The essential oil of caraway consists mainly of two monoterpenes, carvone (50-60%) and limonene (35-45%), the remainder being made up of a mixture of several compounds (Toxopeus & Bouwmeester, 1993). Caraway production is described in detail in a manual for cultivation of caraway (Wander, 1994).

Spring caraway cultivation

In general, growing spring caraway is comparable to growing biennial caraway, although there are some striking differences. The most important difference is the fact that spring caraway is sown and harvested in the same growing season. Obviously, spring caraway is not sown under a cover crop. Spring caraway flowers in July and is usually harvested in September. The yield potential of spring caraway is less than that of biennial caraway. Probably, the highest yield on record is 2370 kg ha^{-1} (Evenhuis & Verdam, 1995). On average spring caraway produces less than 1000 kg ha^{-1}. The essential oil and carvone content of the spring variety Karzo is approximately 30 to 50 % lower than that of the biennial varieties (Toxopeus & Bouwmeester, 1993).

Mycocentrospora acerina (Hart.) Deighton

The fungus *M. acerina* is considered a threat to caraway cultivation. The following list of synonyms of *M. acerina* is based upon Osterwalder (1924), Newhall (1946), Neergaard & Newhall (1951), Viennot-Bourgin (1955) and Sutton & Gibson (1973).

Cercospora acerina Hartig	1880
Sporidesmium acerinum (Hart.) Frank	1896
Cercosporella acerina (Hart.) Arnaud	1918
Cercospora macrospora Osterwalder	1924
Cercospora cari Westerdijk & Van Luijk	1924
Cercospora praegrandis Sprague	1937
Centrospora ohlsenii Neergaard	1942
Centrospora macrospora (Osterw.) Neergaard	1943
Ansatospora macrospora (Osterw.) Newhall	1944
Ansatospora acerina (Hart.) Hansen & Tompkins	1945
Centrospora acerina (Hart.) Newhall	1946
Mycocentrospora acerina (Hart.) Deighton	1972

M. acerina is prevalent in temperate zones of Europe, North America, New Zealand and Australia (Neergaard & Newhall, 1951). The host range of *M. acerina* is large (Tompkins & Hansen, 1950). It includes several weeds (Rintelen & Klewitz, 1976; Hermansen, 1992b). Other economically important umbelliferous crops such as carrot (*Daucus carota* L.; Rader, 1945) and celery (*Apium gravelolens* L.; Newhall, 1944) are susceptible to *M. acerina*. From other plant families, pansy (*Viola tricolor* L.) is mentioned to be highly susceptible to *M. acerina* (Tompkins & Hansen, 1950). Lettuce can suffer severely from the fungus too (Tompkins & Hansen, 1950; Griffin & Simkin, 1977; Mercier *et al.*, 1986).

No sexual phase of the fungus is known (Neergaard & Newhall, 1951). According to Iqbal & Webster (1969) isolates from different crops are morphological identical. However, morphological variation has been mentioned by Constantinescu (1978). Physiological specialization of the fungus was not found (Sutton & Gibson, 1977). The mycelium is superficial and immersed, septate, branched and hyaline (4-8 µm). Chlamydospores are groups of swollen, brown cells with a diameter of 17-30 µm. Chlamydospore chains usually consist of 8 -15 cells. *M. acerina* produces large conidia, 150-200 µm long and 8-15 µm thick in the broadest part, tapering to 1-2 µm apex, 4-5 µm wide at the base. The conidia are septate and sometimes carry an appendage (Sutton & Gibson, 1977).

Outline of this thesis

This thesis describes aspects of the biology of *M. acerina* and its implication for caraway cultivation. The biology of *M. acerina* had not yet been described satisfactory under field conditions. *M. acerina* is more known as the causal agent of liquorice rot, a post harvest disease in carrots (Rader, 1945, Årsvoll, 1969, Wall & Lewis, 1978a,b, Hermansen, 1992a). Various studies in caraway were performed in the period from 1990 until 1995. The aim was to obtain information on *M. acerina* and to generate a set of crop protection methods based on this information, so that crop loss due to anthracnose can be avoided.

Spread of anthracnose from field to field and in the crop is important for the development of an epidemic. Data on spread of the disease may also be important to identify sources of inoculum of this fungus. In chapter 2 dispersal characteristics of *M. acerina* conidia are described.

The soil is considered a major source of inoculum for *M. acerina* in celery (Day *et al.*, 1972). The most important source of inoculum on carrot roots at harvest arises from development of the fungus in the rhizosphere of growing plants and the subsequent formation of chlamydospores (Wall & Lewis, 1980a). In carrots (*Daucus carota* L.) the fungus enters the root mainly through wounds (Davies *et al.*, 1981). The soil may be an important source of inoculum for *M. acerina* in caraway as well. The relation between root injury and root infection was investigated, chapter 3.

Westerdijk and Van Luijk (1924) suggested that seeds of caraway could be infected with *M. acerina*, but according to Neergaard and Newhall (1951) seed transmission was not proven. Chapter 4 describes seed transmission of *M. acerina*. The amount of seed infection by *M. acerina* is also reported. Its effects on crop establishment and yield are described in chapter 5.

The host range of *M. acerina* consist of many dicotyledonous plant species (Tompkins & Hansen, 1950; Rintelen & Klewitz, 1976; Hermansen, 1992b). In the Netherlands caraway is usually sown under a cover crop. These cover crops may be susceptible to *M. acerina* infection and inoculum build up is probable. The effect of susceptible cover crops on anthracnose development in caraway is described in chapter 6.

Caraway yields vary from 600 to 2500 kg ha^{-1}, depending on year, region and field. Part of this variation is ascribed to divergence in photosynthetically active radiation during flowering (Bouwmeester *et al.*, 1995). *M. acerina* may have a profound effect on caraway yield. Crop and disease management, essential to increase the crop's yield stability, demand better knowledge of pathogen/crop interactions. Experiments to study the effect of nitrogen rate, sowing rate and row spacing on anthracnose are described in chapter 7.

The general discussion deals with aspects of the biology of *M. acerina* and the growing of caraway. Possibilities and limitations to control anthracnose in relation to host-pathogen interactions are discussed in chapter 8.

CHAPTER 2

Splash dispersal of conidia of *Mycocentrospora acerina* in the field

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Abstract

The dispersal of conidia of *Mycocentrospora acerina* was studied in caraway field trials. A Burkard spore trap, rotorods, inverted Petri dishes containing sucrose agar and rain gauges were used to trap conidia of *M. acerina*. Sporulation was stimulated by rainfall (≥ 2 mm) and moderate temperatures (around 15°C). Solar radiation had a negative effect on sporulation. Hardly any conidia were found in the spore traps on rainless days. Short distance (≤ 9 m) spread of *M. acerina* is mainly caused by splash dispersal of its conidia. Trap plants at 0, 0.1, 1 and 4 m from the inoculum source were readily infected under moist conditions. Beyond 9 m from an inoculum source no infection of caraway trap plants was found. Trap plants at 9 m from an inoculum source were infected in one out of three seasons only. Long distance (> 9 m) spread could not be demonstrated by the techniques used in this study. The results suggest that, usually, a caraway field is infected by inoculum sources within that field.

Introduction

In the Netherlands, biennial caraway (*Carum carvi* L.) is an arable crop grown mainly on heavy clay soils. In the 1980's anthracnose caused by *Mycocentrospora acerina* (Hart.) Deighton was responsible for severe yield losses. The host range of *M. acerina* is broad, including several economically important crops (Tompkins & Hansen, 1950).

Dispersal of conidia may attribute to the progress of an anthracnose epidemic in caraway, but the literature provides no data. Therefore, spore dispersal of *M. acerina* on caraway and environmental conditions favouring spore dispersal were studied. Since little information was available on the suitability of spore traps for catching *M. acerina* conidia several types of spore traps were used. Horizontal and vertical dispersal of conidia were investigated in the field. Horizontal dispersal was studied to obtain information on dispersal distances and gradients of *M. acerina* conidia. Vertical dispersal was investigated to examine the possibility that *M. acerina* moves upwards in a crop to infect umbels.

Materials and methods

Experimental fields

Biennial caraway was sown together with a cover crop. The experiments were situated at Lelystad, Wageningen, Lienden and Randwijk (table 1). The cover crop, spring barley, was harvested in July of the first year. Spore sources were created by inoculation of field plots, using a knapsack sprayer. Inoculum was prepared according to Evenhuis *et al.* (1995). Spore traps were placed in inoculated caraway plots (6 x 6 m) at Lelystad and in a naturally infested caraway field at Wageningen (table 1).

Table 1. Locations and fields planted with winter caraway where spore trapping was conducted using various spore traps at given heights. Entries are numbers of days a Burkard spore trap, rotorods, rain gauges and Petri dishes were exposed as spore traps. The last column represents the number of exposures (NE) involving trap plants.

				Spore traps						
	Soil	Field		Burkard	Rotorod	rain gauge		Petri dish		Trap
Location	type	code	Season	30/50[a]	15[a]	30[a]	120[a]	30[a]	70[a]	plants (NE)
Lelystad	clay	2496	1991/92	18	17	9	9	-[b]	-	3
Lelystad	clay	3071	1992/93	-	22	5	-	30	30	8
Lelystad	clay	3434	1993/94	-	13	-	-	25	25	8
Wageningen	sand	Dr941	1993/94	28	-	-	-	48	6	-
Randwijk	clay	Bo941	1993/94	-	-	-	-	-	-	1
Lienden	clay	Ln941	1993/94	-	-	-	-	-	-	1

a: Height of mounting in cm

b: No observations.

Spore trapping

Spore catches were made discontinuously over the period from harvest of the cover crop until harvest of the caraway crop in the following year, from August until July. A Burkard spore trap was used to assess periodicity in spore catches. Rotorods were used to establish wind dispersal of conidia of *M. acerina*. Petri dishes containing sucrose agar were used to assess splash dispersal of *M. acerina*. Rain gauges placed above crop height were used to determine splash dispersal of conidia. Rain gauges beneath crop height were used to determine drip-off as described by Savary & Janeau (1986) and rain splash. Trap plants were placed in the field to estimate horizontal dispersal distances of conidia.

Burkard spore trap

A Burkard spore trap (Burkard Manufacturing Company Ltd, Rickmansworth, UK) was placed in the field with the orifice 50 cm above the ground at Lelystad and 30 cm at

Wageningen. The suction rate of the Burkard trap was adjusted to 10 L min^{-1} air. Tape greased with vaseline was wrapped around the revolving drum of the trap. The tape was replaced weekly. Conidia of *M. acerina* deposited on the tape, measuring 100-200 µm x 10-30 µm (Neergaard, 1952) and easily recognizable, were counted under a light microscope (100x).

Power could not be supplied continuously to the Burkard spore trap at Lelystad (1992). It ran from 9.00 to 16.15 hours and from 16.30 to approximately 05.00 hours the next morning. The actual operation time was determined by means of a timer. At Wageningen (1993) around the clock observations were made.

Rotorods

Three rotorods (Rotorod Sampler, Ted Brown Associates, Los Altos Hills, USA) were placed in an inoculated caraway plot. The lower end of the rods was at 10 cm above soil level. The rotation velocity, measured at the end of the experiment, was slightly less than 2400 r.p.m. In the spring of 1992 the rotorods had a run time of 20 minutes. Starting in autumn 1992 they had a run time of 24 hours from 9.00 a.m. to 9.00 a.m. The rods were examined for conidia under a light microscope (40x).

Petri dishes

Three inverted Petri dishes (diameter 9 cm) containing SuA+ (sucrose 6g l^{-1}, agar 15g l^{-1} and 0.02% streptomycin, after Day *et al.*, 1972) were placed at each of two heights, 30 and 70 cm above ground level, and exposed for 24 hours. After exposure the plates were incubated in a closed plastic bag at 12°C in the dark. After two weeks the plates were examined and the colonies of *M. acerina* counted. Such colonies are easily recognizable but, in case of doubt, colonies were examined for the presence of the typical conidia of *M. acerina*. If no conidia were found, sporulation of the colonies was stimulated by UV-irradiation. Two days after UV-treatment sporulation was observed.

Rain gauges

In 1992, three rain gauges were placed in the crop with their rims at 20 cm above the ground and three others just above average crop height (*c.* 1 m). In 1993, only the three 20 cm rain gauges were used. The water from the rain gauges was poured into a plastic jar and taken to the laboratory. A drop of soap was added to diminish the surface tension

of the water in order to prevent the conidia from floating. The suspension was thoroughly shaken and centrifuged at 3000 r.p.m. for 20 minutes. The supernatant was carefully removed. The sediment was resuspended and the conidia were counted under a light microscope (40x).

Caraway trap plants

Seven to nine weeks old caraway plants, raised in a greenhouse, were placed at different distances from an inoculated caraway plot. The experiment was carried out 21 times in a period from 1992 until 1994 (table 1).

Trap plants were placed in the centre of the inoculated plot, and at 0.1, 1, 4, 9, 16 and 25 metres from the plot's edge in an easterly direction, i.e. downwind relative to the prevailing wind direction. Ten plants were placed at every distance. The pots containing the trap plants were dug into the ground, so that the rim of the pot was level with the soil surface. Every time an experiment was conducted, ten trap plants were placed outside but far from the field, as a control, to check for eventual seed infection.

After a forthnight's exposure the oldest leaves of the trap plants, with or without symptoms were collected. Leaf fragments of about 2 cm^2 were incubated on moist filter paper in Petri dishes for two days. Preferably leaf fragments with symptoms were incubated, if not available older leaf fragments were chosen randomly. Sporulation of *M. acerina* on leaves was checked under a microscope (40x). If sporulation was found the trap plant was considered to be infected. For each distance, disease incidence was determined as the percentage of trap plants infected.

Weather data during spore trapping (Petri dishes & rotorods)

Rainfall (mm) was measured using rain gauges. Weather data (temperature (°C), wind velocity (m/s), relative humidity (%), solar radiation (J/cm^2) rain duration (h) and precipitation (mm) during a 2-days period before each spore catch) were obtained from nearby (± 2 km) standard weather stations at Lelystad and Wageningen, respectively. At Wageningen rain intensity (mm/h) was estimated by the quotient of the hourly precipitation (mm) and rain duration (h). At Lelystad, where only hours with rain were recorded, average rain duration per hour was taken to be 0.5 after careful comparison of Lelystad and Wageningen rain data. Rain intensity was estimated using the corrected rain

duration. The estimates thus obtained, though inaccurate, were adequate for the present purpose.

Weather data during dispersal gradient experiments (trap plants)

Total rainfall, mean temperature, mean relative humidity, mean solar radiation, mean wind velocity, and mean wind direction were measured during the exposure period of the trap plants in the field. Various wind directions contribute differently to spore deposition on the trap plants. To accommodate for such differences a cosine transformation was applied to the angle (α) between the daily average of the wind direction and the direction in which the trap plants were placed in the field ($\cos\alpha$). The result is a figure between -1 (wind from the east) and 1 (wind from the west). A wind vector (Wv) was calculated as the sum of the positive values of the products of daily average wind direction (α) and daily average wind speed (v); equation 1:

$$Wv = \sum_{t=1}^{T} (\cos\alpha \times v) \qquad (1)$$

with t = exposure day and T = last exposure day

Statistical analysis

Data were analyzed using Genstat 5, release 3.1.

Rotorods and Petri dishes

Gompertz curves were fitted to the numbers of conidia and the daily amounts of precipitation. Linear regression of the ln-transformed number of conidia (ln(n+1)) on the ln-transformed daily amount of rainfall (ln(rf+1)) was also calculated.

Trap plants

Dispersal gradients were estimated by regressing disease incidence on distance from the inoculum source, using a negative exponential curve (equation 2). Parameter B is an estimate of disease incidence at distance 0 m and R is a shape parameter. The shape parameter describes the rate of decrease of the disease incidence (y) with distance (x) from the inoculum source.

$$Y_x = B \times R^x \mid 0.1 \leq x \leq 9 \text{ m} \qquad (2)$$

Data from distances 0 and 25 metres were excluded from the analyses. At distance 0, disease incidence of trap plants was high, but the trap plants were placed in a caraway crop, whereas at the other distances the trap plants were placed in grass. At the distances 16 and 25 m no infection of the trap plants was found, so that observations at 25 m were considered redundant. The area under the dispersal gradient curve (AUDGC) was calculated for distances from 0.1 to 16 metres from the inoculum source using the parameters B and R generated by the regression equation.

Weather data

The effects of weather factors on spore catches of *M. acerina*, disease incidence of trap plants and AUDGC were studied by multiple regression. The number of conidia trapped with rotorods and Petri dishes was ln-transformed. Multiple regression models were selected with high coefficients of determination (r^2) and with explanatory variables having significant ($P \leq 0.05$) partial regression coefficients.

Results

Comparison of spore traps

No conidia of *M. acerina* were found on the tapes of the Burkard spore trap, during a total of nearly 1000 run hours. Conidia of *M. acerina* were trapped by rotorods and by inverted Petri dishes. The mean daily catches of conidia of *M. acerina* by rotorods, Petri dishes and rain gauges did not exceed 400, 80 and 57, respectively.

The correlation matrix for spore catches in two seasons is given in table 2. In each of two seasons a significant linear correlation was found ($P<0.001$) between the number of conidia trapped per day by rotorods at 15 cm height and by Petri dishes at 30 cm height. The same holds when the two seasons were taken together ($r^2=0.79$, $n=33$, $P<0.001$).

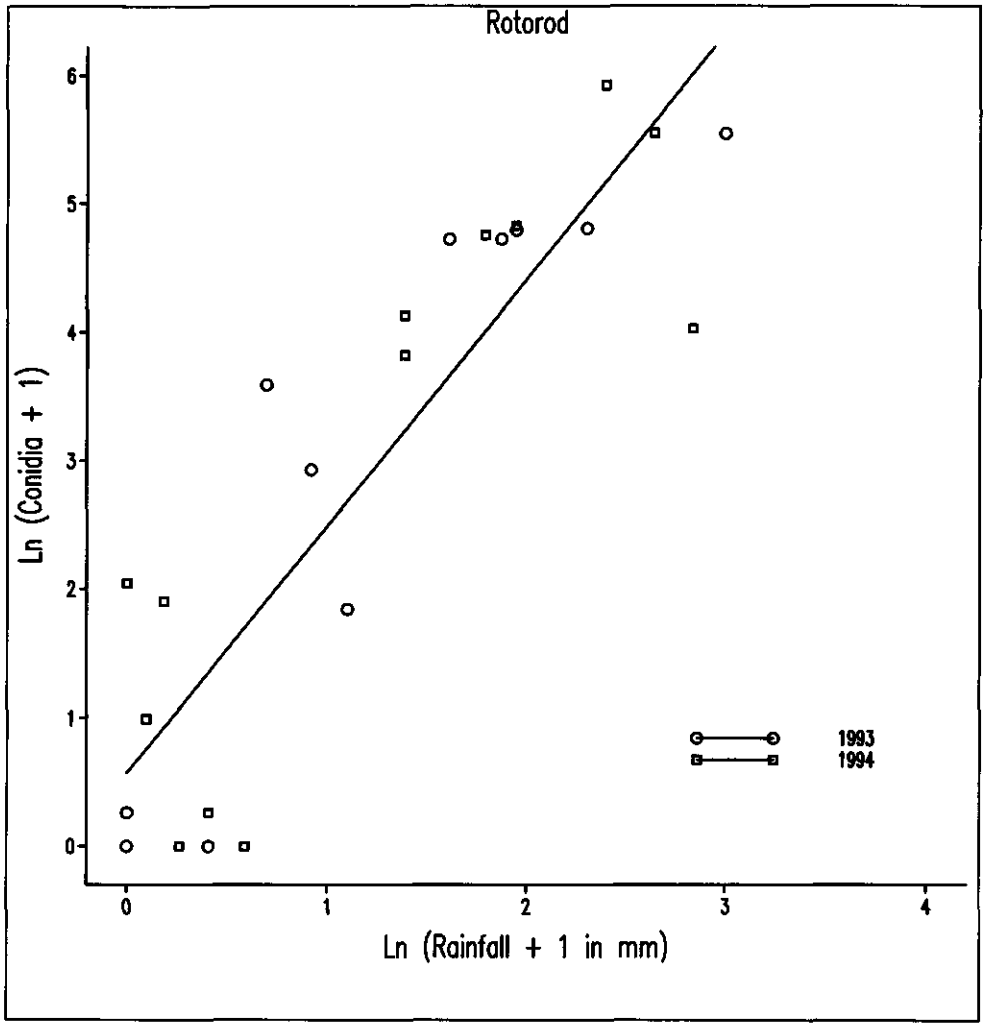

Figure 1. Ln-transformed daily catches of *M. acerina* (ln(n+1)) conidia trapped by rotorods in autumn 1992 / winter1993 and autumn 1993 / winter1994 plotted against ln-transformed daily rainfall (ln(rf+1)). $y = 0.6 + 1.9\,x$, $r^2=0.78$, n=24, $P<0.001$.

Table 2. Correlation matrix (r) for the mean daily number of conidia of *M. acerina* trapped with different spore traps near Lelystad in 1992/3 (n=21) and 1993/4 (n=12).

Season	Field	Sampling method	Petri dish 30 cm[a]	Petri dish 70 cm[a]
1992/93	3070	Petri dish 70 cm	0.84 ***	
1992/93	3070	Rotorod	0.87 ***	0.55 **
1993/94	3434	Petri dish 70 cm	0.81 ***	
1993/94	3434	Rotorod	0.95 ***	0.90 ***

a: height of mounting, *: significant at $P \leq 0.05$; **: significant at $P \leq 0.01$; ***: significant at $P \leq 0.001$

Rotorods.

In autumn and winter, a positive correlation was found between the average daily total number of conidia trapped by rotorods (N, dependent variable) and daily rainfall (rf, independent variable). Linear regression of N on rf gave a coefficient of determination $r^2=0.57$, with n=25 and $P<0.001$. A Gompertz regression of N on rf yielded $r^2=0.68$. A linear regression (figure 1) of ln(N+1) on ln(rf+1) gave $r^2=0.78$ ($P<0.001$).

In spring, the maximum number of conidia trapped per day by rotorods was 16, with a daily mean of 7. Notwithstanding the low numbers, a positive correlation was found between daily rainfall and the daily number of conidia trapped in spring. A linear regression of ln(N+1) on ln(rf+1) gave $r^2=0.49$ ($P<0.001$).

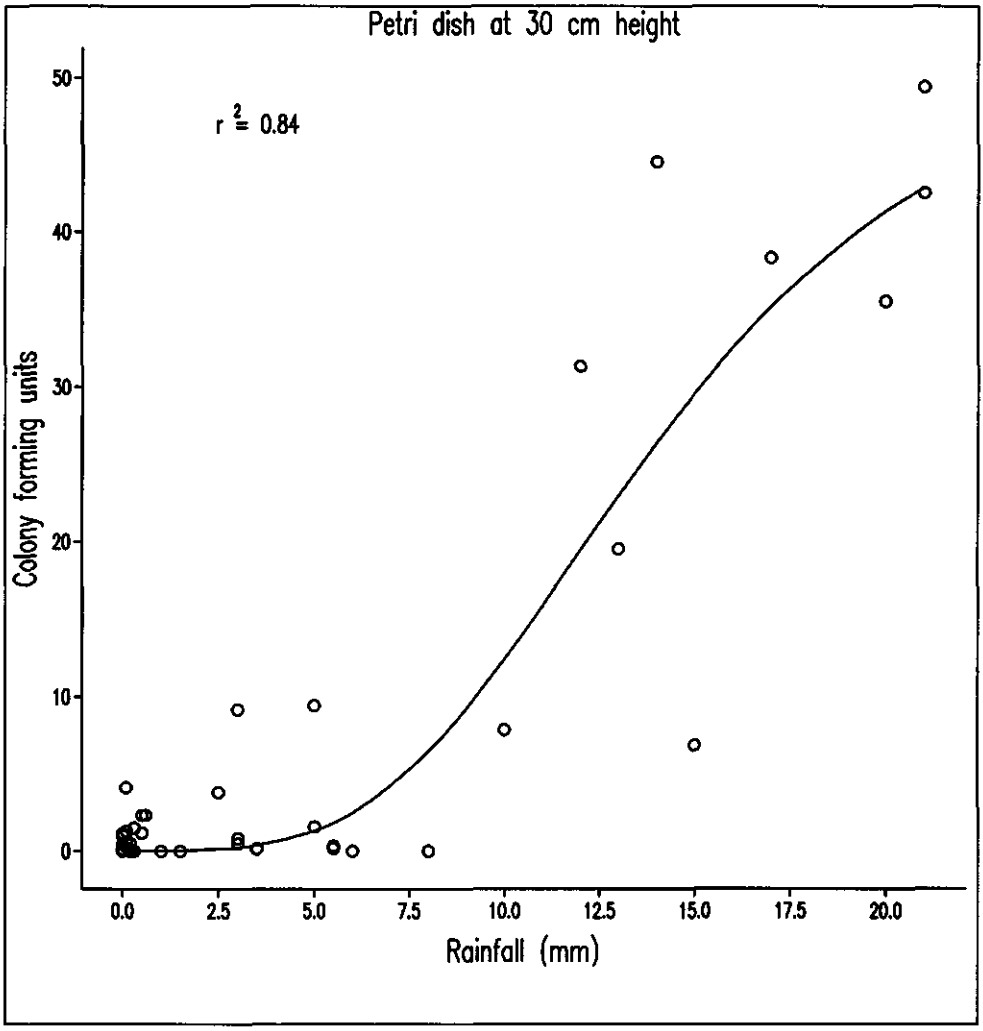

Figure 2 Effect of daily rainfall on colony forming units of *M. acerina* trapped by inverted Petri dishes after exposure in caraway field Dr941 (1993/4) at 30 cm height, with an exposure time of 24 hours.

$y = 51 \times EXP(-1 \times EXP(-1 \times 0.2(x-11.8)))$, $r^2=0.83$, n=48.

Rain gauges.

Conidia of *M. acerina* were caught using rain gauges. Data were available for 14 observation days. On one day only, no conidia were trapped. The following figures represent numbers of spores per rain gauge per day. Averages were calculated over three

rain gauges in similar positions. The highest number was 80, the highest average 57. Numbers varied considerably between rain gauges and days, so that no regressions could be calculated. In spring, an average over 9 days of 30.2 was found in rain gauges with the rim beneath crop height (30 cm), and of 0.8 conidia in rain gauges above crop height (1.20 m). In autumn, an average over five days of 13.6 conidia was found in rain gauges placed at 30 cm height, at that time above crop level.

Petri dishes.

In autumn and winter up to 80 conidia forming colonies were found at 30 cm height. After a period of frost in 1992 numbers dropped, whereas in 1993 numbers increased after frost. Conidia were still trapped when the daily mean temperature dropped to 2°C. In spring the number of conidia trapped did not exceed 20 per day.

Spore catches with Petri dishes at 30 cm height were approximately 8 times higher than at 70 cm. Throughout the years the number of conidia trapped per day and the daily rainfall were positively correlated. Significant year effects were found. The numbers of colony forming units on agar plates exposed at 30 cm were regressed to rainfall using the Gompertz transformation, for field 3070 (1992/93, $r^2 = 0.45$, n=30), field 3434 (1993/94, $r^2 = 0.56$, n=25) and field Dr941 (1993/94, $r^2 = 0.83$, n=48, figure 2). Linear regression was performed on the ln-transformed number of conidia (ln(n+1)) and the ln-transformed amount of rainfall in mm (ln(rf+1)). Ln-transformations gave better fits than Gompertz transformations for fields 3070 (1992/93, r^2=0.72, n=30, P<0.001, figure 3) and 3434 (1993/94, r^2=0.64, n=25, P<0.001), but not for field Dr941 (1993/94, r^2=0.56, n=48, P<0.001).

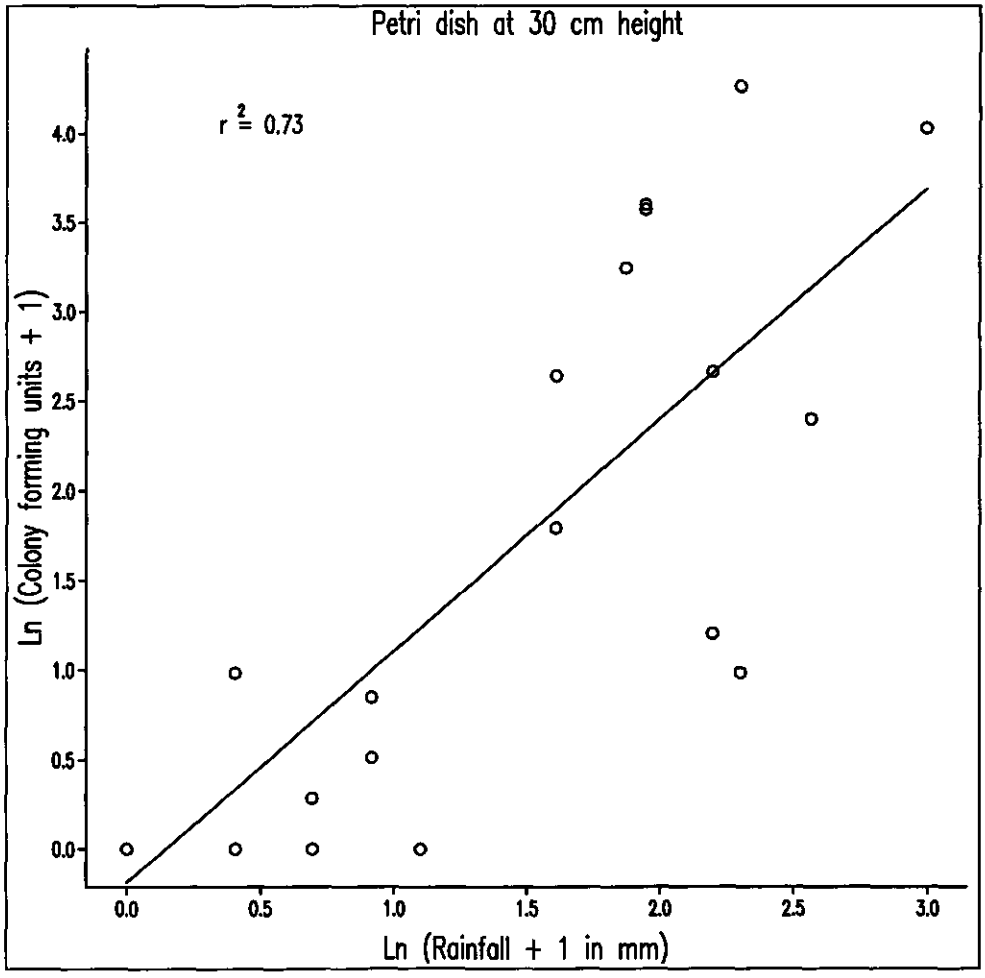

Figure 3 Effect of daily rainfall in mm (ln(rf+1)) on daily catches of *M. acerina* conidia (ln(n+1)) by Petri dishes exposed in caraway fields, field 3070 (1992/3): $y = -0.2 + 1.3x$, r^2=0.72, n=30, P<.001. Data for field 3434 (1993/4): $y = 0.6 + 1.2x$, r^2=0.64, n=25, P<.001 and field Dr941 (1993/4): $y = 0.1 + 0.88x$, r^2=0.56, n=48, P<.001 are not shown.

Table 3. The effect of weather variables on *M. acerina* conidia trapped by rotorods at 15 cm, and by Petri dishes at 30 cm and 70 cm height[a].

$Y_{rotorod} = 0.21 + 0.930 \times ri - 0.41 \times Tm + 0.45 \times Tmax - 0.0018 \times SR;$	n=52	r^2=0.58	(3)
$Y_{Pd30} = 0.22 + 0.193 \times rf - 0.16 \times Tm + 0.17 \times Tmax - 0.0006 \times SR;$	n=103	r^2=0.59	(4)
$Y_{Pd70} = -0.20 + 0.063 \times rf + 0.11 \times Ws;$	n=61	r^2=0.28	(5)

a: $Y_{Rotorod}$: ln-transformed daily mean number of conidia trapped with rotorods; Y_{Pd30}, Y_{Pd70}: ln-transformed daily mean number of conidia trapped by Petri dishes at 30 and 70 cm height, respectively; rf: daily rainfall (mm); ri: rain intensity (mm/h); Tm: daily mean temperature (°C); Tmax: daily maximum temperature (°C); Ws: mean daily wind speed (m/s); SR: solar radiation per day (J/m^2).

Spore catches under dry conditions.

On 12 of 14 rainless observation days no conidia were trapped by rotorods. On the two other rainless days mean spore catches per day by rotorods were less than 1 conidium. On eight of 25 rainless days some conidia were trapped by Petri dishes at 30 cm height. On these days the mean number of conidia forming colonies per day never exceeded five, and usually was less than one.

Other effects of weather.

The best multiple regression equations for the effects of weather factors on *M. acerina* spore catches using rotorods and Petri dishes are given in table 3. Temperature, rainfall and solar radiation had an effect on spore catches at 15 cm (rotorod, equation 3) and 30 cm (Petri dish, equation 4) above the ground. Relative humidity, wind speed and precipitation during a 2-day period before spore catching days had no significant effect on spore catches with rotorods and Petri dishes at 30 cm (table 4). However, wind speed and rainfall had a significant positive effect on spore catches at 70 cm height (Petri dishes, equation 5). The results suggest that rainfall in combination with strong winds is necessary to catch spores at a height of 70 cm. Table 5 gives the linear correlation coefficients of the weather variables during days when Petri dishes at 30 cm were placed in the field.

Table 4. Linear correlation coefficients of the log number of *M. acerina* conidia (ln(y+1)), caught by rotorods or Petri dishes, and weather variables[a] during spore catch experiments.

	Rotorod 15 cm n = 52	Petri dish 30 cm n = 103	Petri dish 70 cm n = 61
rf	0.54***	0.76***	0.47***
Tm	ns	ns	ns
Ws	ns	0.22*	0.39**
RH	0.28*	0.20*	ns
ri	0.60***	0.55***	0.26*
rf48	ns	0.23*	ns
SR	-0.36**	-0.23*	ns

a: rf: rainfall; Tm: mean temperature; Ws: wind speed; RH: relative humidity; ri: rain intensity; rf48: rainfall over a two day period previous to the observation day; SR: solar radiation. Values marked by ***, **, and * were significant at P≤0.001, P≤0.01, and P≤0.05, respectively. Non-significant values are indicated with ns.

Table 5. Linear correlation coefficients of the weather variables[a] (n=103) during days with spore catches by Petri dishes at 30 cm height.

	rf	Tm	Ws	RH	ri	rf48
Tm	ns					
Ws	0.28**	ns				
RH	0.20*	ns	-0.21*			
ri	0.66***	0.28**	ns	ns		
rf48	ns	ns	0.19*	ns	ns	
SR	ns	0.34***	-0.31**	-0.61***	ns	ns

a: rf: rainfall; Tm: mean temperature; Ws: wind speed; RH: relative humidity; ri: rain intensity; rf48: rainfall over a two day period previous to the observation day; SR: solar radiation. Values marked by ***, **, and * were significant at P≤0.001, P≤0.01, and P≤0.05, respectively. Non-significant values were replaced by ns.

Horizontal movement of conidia estimated by trap plants.

When, during an experiment, less than 50 % of the plants in the centre of the field were infected, the data of that experiment were discarded as being non-informative (10 discarded out of 21). In such an experiment conditions were unfavourable for sporulation so that no useful information on dispersal of conidia would be obtained.

In contrast, a study of the effect of weather conditions on infection of the trap plants requires inclusion of experiments with unfavourable weather conditions. Thus, data from all experiments (n=21) were included in calculations to estimate effects of weather conditions on infection of trap plants.

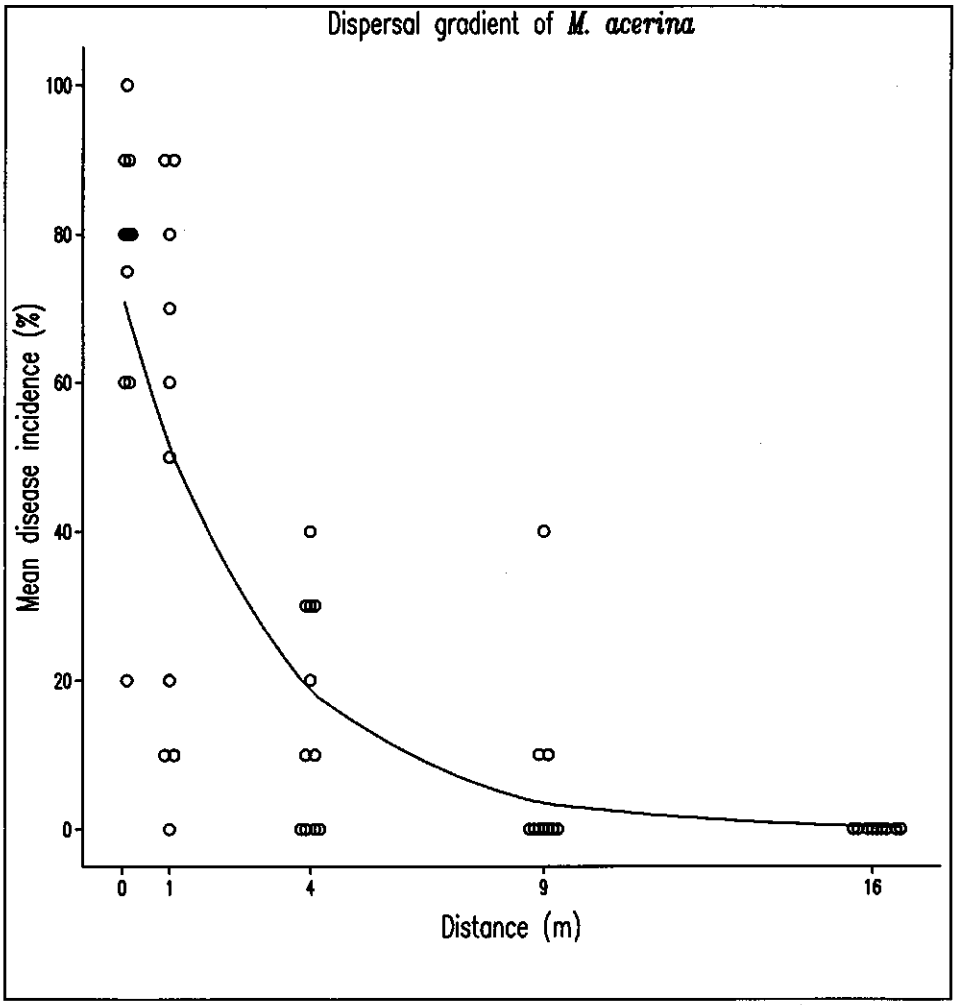

Figure 4 The mean percentage of caraway trap plants infected by *M. acerina* at different distances from an inoculum source in caraway plots at Lelystad, in 1992/3 and 1993/4, at Randwijk, 1993/4, and at Lienden, 1993/4. Data were fitted to a negative exponential curve. $Y = 75.8(6.2) \times 0.67(0.06)^{x}$, $r^2=0.67$, n=55, $p<0.001$.

Dispersal gradients

Plants at 16 and 25 m were never found to be infected by *M. acerina*. At a distance of 9 m seven plants out of 110 were infected by the pathogen. Dispersal gradients were calculated as negative exponential curves with the asymptote approaching zero (equation

2). Figure 4 shows the mean percentage of diseased caraway trap plants (11 experiments) at different distances from the inoculum source.

Effect of weather conditions on infection of trap plants

To assess the effect of weather conditions on infection of the trap plants all 21 experiments were used in the analyses. The best multiple regression equations for the effects of weather factors on infection of trap plants at each distance are given in table 6. Infection of trap plants in the inoculum source and at 0.1 m was related to rain intensity, the number of days the plants were left in the field, temperature and wind direction (equations 6 and 7). Infection of trap plants at 1, 4 and 9 m from the inoculum source depended on temperature, solar radiation and wind speed (equations 8 to 10). The effects of weather conditions on AUDGC are described in equation 11 (table 6).

Table 6. The effect of weather variables on *M. acerina* infection of trap plants at various distances from an inoculum source (n=21).

$Y_0^a = -359 + 25 \times Tm + 4.5 \times d + 21 \times ri + 3.5 \times RH + 52 \times Wd - 18 \times Tmax;$	$r^2=0.68$	(6)
$Y_{.1} = -312 + 23 \times Tm + 4.6 \times d + 17 \times ri + 3.0 \times RH + 53 \times Wd - 17 \times Tmax;$	$r^2=0.71$	(7)
$Y_1 = 6 + 4.4 \times Tmin + 9.2 \times Ws - 0.043 \times SR;$	$r^2=0.36$	(8)
$Y_4 = -8 + 1.7 \times Tm + 5.2 \times Ws - 0.019 \times SR;$	$r^2=0.55$	(9)
$Y_9 = -9 + 1.3 \times Tmax + 2.4 \times Ws - 0.014 \times SR;$	$r^2=0.23$	(10)
$AUDGC = 3 + 2.2 \times Tm + 3.7 \times Wv - 0.024 \times SR;$	$r^2=0.43$	(11)

a: Y_x: disease incidence of trap plant at distance x (m) from an inoculum source; AUDGC: Area Under Dispersal Gradient Curve; Tm: daily mean temperature; Tmax: daily maximum temperature; Tmin: daily minimum temperature; d: number of days the trap plants were exposed; ri: rain intensity; RH: mean daily relative humidity; Wd: mean daily wind direction; Ws: mean daily wind speed; Wv: mean daily wind vector; SR: daily solar radiation.

Discussion

Burkard spore trap

The Burkard spore trap is said to be more efficient than traps based on passive deposition, especially at low aerial spore densities (Nakamura, 1971). However, in our experiments no conidia of *M. acerina* were trapped. One explanation might be that the orifices of the Burkard spore traps were placed too high (50 cm at Lelystad, 30 cm at Wageningen), but this point is refuted by two observations. In our experiments conidia of *M. acerina* were trapped at heights of 30 and 70 cm using inverted Petri dishes. Hermansen (1992a), working with carrot, generally trapped one or two conidia of *M. acerina* per day using a Burkard trap at 40 cm height. He suggested that a Burkard spore trap was less suitable to trap splash dispersed conidia at this height. Another explanation might be that the rain droplets carrying *M. acerina* conidia were too large to be trapped by a Burkard spore trap. Darby (1977) found that *M. acerina* conidia were mainly dispersed in water droplets larger than 900 µm which could, in fact, contain several conidia of 100-200 µm x 10-30 µm. Such large droplets might not easily be sucked in through the Burkard's orifice of 2000 µm width.

Rain gauges

Conidia were regularly found in rain gauges. Numbers varied from 0 to 80 conidia per rain gauge per day. A large variation in numbers was found between rain gauges and days. Thus, interpretation of the data is awkward. In spring more conidia were found in rain gauges at 30 cm than in rain gauges at 120 cm, suggesting a combination of drip-off and rain splash. Since in autumn the rim of the rain gauges was above crop level the catches indicate upward splash dispersal of *M. acerina* conidia, probably in combination with turbulent dispersal of spore-loaded droplets. Counting conidia in rain gauge catches was laborious and the rain gauge method was, in the present case, unsuitable to quantify splash dispersal.

Petri dishes

The method of the inverted Petri dish is less time consuming. Only viable conidia, forming colonies, will be counted. Other possible colony forming units, like chlamydo-

spores or mycelium fragments, are probably not trapped by inverted Petri dishes at 30 and 70 cm height. Thus the method gives an estimation of viable *M. acerina* conidia in the air. The medium used is selective. This selectivity was enhanced by a low incubation temperature which gives *M. acerina* an advantage over many other micro-organisms. Unfortunately, Petri dish traps are sensitive to particle size, wind speed, and aerodynamic effects, and they sample only a small volume of air (Gregory, 1973). The large size of the *M. acerina* conidia overcomes these disadvantages in part, since large spores more easily impacted on leaves than small spores (Gregory, 1973). The same will hold for impaction on Petri dishes. Calculations on the absolute spore density in the air cannot be made using Petri dish traps, but comparative studies can be performed (Savary & Janeau, 1986).

Rotorods

Rotorods could be examined adequately since the conidia of *M. acerina* are large and easily recognizable. Because of the large sized conidia collection efficiency must be approaching 100% (Edmonds, 1972). Rotorods are assumed to trap dry spores only (Savary & Janeau, 1986) and are thought less suitable to trap splash dispersed spores, especially those in large droplets. However, *M. acerina* conidia were found on the rods, which suggests that water droplets, containing conidia, fall apart when impacted onto the rods, leaving some conidia behind. Evaporation of spore carrying droplets is unlikely, because the water droplets carrying conidia are so big (Darby, 1977). Since only low densities of air-borne conidia of *M. acerina* were present, rotorods had to run day and night, which made them sensitive to breakdown. In autumn and winter the rotorods were not overloaded with dust. In spring, the rotorods sometimes collected too much material, especially on sunny and windy days. Thus spore catches in spring may have been underestimated.

Spore catches by rotorods (15 cm) and Petri dishes (30 and 70 cm) were highly correlated (table 2). Apparently the two methods respond to the same dispersal characteristics of *M. acerina*, i.e. splash dispersal. The methods are about equally satisfactory to quantify sporulation of *M. acerina*.

Liberation of conidia

Few conidia were trapped on rainless days, indicating involvement of rain in conidium liberation. Wind speed had no effect on spore catches using rotorods, and only small effects on spore catches using Petri dishes (table 4). The importance of wind seems to increase with height of the trap. Therefore we conclude that wind does not play a role in the liberation of conidia, but may contribute to the impaction of spore carrying droplets on spore traps. Spore catches increased with increasing rainfall (figures 1, 2 & 3). Therefore, we assume that water is necessary for the formation and/or liberation of conidia of *M. acerina*. Inverted Petri dishes readily trapped *M. acerina* conidia. On rotorods, *M. acerina* conidia were usually found in clumps, suggesting dispersal by droplets. All observations are compatible with the hypothesis that most, if not all, conidia are splash dispersed. We surmise that rain splash is the most important take-off mechanism of *M. acerina* conidia. Spore carrying droplets will be impacted onto plant and trap surfaces, and small droplets may become air-borne.

Aerial spore density

Using rotorods the maximum spore density was estimated as 2.33 conidia per m^3 air (i.e. 373 conidia per 2 rods in one day). Calculations were made according to the description of the manufacturers of the rotorods. In pansies, 25 - 75 conidia of *M. acerina* per m^3 air were found at 15 cm height using a Hirst spore trap (Rintelen & Klewitz, 1976). In carrot, the maximum spore density found was 47 conidia per m^3 at a height of 40 cm using a Burkard spore trap. (Hermansen, 1992a; 1-h sampling). Since in our experiments the rotorods were left in the field for 24 h, estimation of the maximum aerial spore density is difficult. It is unlikely that spore liberation took place during the whole day. If it is assumed that spore liberation only took place during rain showers (i.e. 5 h on the day with maximum spore catch) the maximum spore density would become 11 conidia per m^3. This figure is of the same order of magnitude as those quoted by Rintelen & Klewitz (1976) and Hermansen (1992a). Spore catches with rotorods were five times higher than catches with Petri dishes, the difference being ascribed to the difference in catching efficiency between volumetric and non-volumetric spore traps (Gregory, 1973).

In general a low spore production is associated with high efficiency of spore dispersal and with large sized spores (Ingold, 1953). This association seems to hold for *M. acerina* infecting caraway. In aquatic fungi, spore production often is quite low (Read *et al*,

1991). *M. acerina* was frequently found as a component of the aquatic hyphomycetes community in rivers (Shearer & Webster, 1991). Aquatic isolates of *M. acerina* were pathogenic to carrot and parsnip, and produced similar symptoms as isolates from carrot (Iqbal & Webster, 1969). The evidence indicates *M. acerina* isolates, whether from aquatic origin or isolated from plants, have comparable characteristics, including low spore production and high efficiency of spore dispersal.

Effect of temperature on sporulation and germination

Conidia were trapped over a range of temperatures from 2°C to 18°C. Gündel (1976) found sporulation of *M. acerina* between temperatures from 5 to 25°C. Our results indicate that sporulation in the field is possible at even lower temperatures than expected from the literature, although the numbers of conidia trapped were small. Trap plants were readily infected in autumn and winter. Probably the availability of the substratum plays a role. During long periods of frost leaves decay completely, but after a short period of frost decayed leaves still allow *M. acerina* to sporulate.

Germination of *M. acerina* conidia *in vitro* was found in a temperature range from below 4°C to 29°C (Gündel, 1976). In pansy, infection took place even at temperatures of 0-2°C after inoculation with conidia of *M. acerina* (Rintelen & Klewitz, 1976). The related species *Mycocentrospora filiformis* was shown to have a germination rate of 95% at 15°C, and this germination rate was not significantly reduced at low temperatures (Read *et al.*, 1991). Such data indicate that *M. acerina* conidia are able to germinate and infect caraway in autumn and winter.

Effect of weather factors on infection of trap plants

Near the inoculum source, rainfall, relative humidity and temperature were the major factors determining disease incidence (table 6). Rainfall and temperature were shown to be important for spore liberation and dispersal in the spore catch experiments (table 3). A high relative humidity and/or leaf wetness is needed for sporulation and subsequent infection of the plants. In caraway, an increase of disease severity coincided with longer leaf wetness duration (Evenhuis *et al.*, 1955). In carrots, *M. acerina* produced many conidia when the temperature was between 15-20°C and the relative humidity close to 100% (Årsvoll, 1969). With increasing distance from the inoculum source wind speed became more important. Solar radiation was negatively correlated with infection (table

6). Apparently, effective dispersal of *M. acerina* conidia over medium distances (i.e. a few metres) of open area, covered by short grass only, occurs on rainy days with high wind speed and turbulence and low solar radiation. In pansy, the number of conidia trapped at 15 cm increased 5 to 20 times during heavy showers, (Rintelen & Klewitz), a result emphasizing splash dispersal.

Vertical movement of conidia

Both at 30 and 70 cm height *M. acerina* conidia were trapped by inverted Petri dishes, showing vertical movement of *M. acerina*. These conidia must have originated from the caraway crop, which at the time was in the rosette stage, with a maximum height of 10 cm. Apparently *M. acerina* conidia became air-borne by splash dispersal, since rotorod catches were small compared to Petri dish catches, considering the air volume sampled. Neergaard & Newhall (1951) indicated that conidia were not easily detached from the conidiophores, which suggests that wind does not play a major role in the liberation of conidia. Gregory *et al.* (1959) showed splash dispersal to be a mechanism by which spores can be thrown up into the air. The positive correlation between wind speed and spore catches at 70 cm suggests that turbulent deposition plays a role in the catches of *M. acerina* conidia on Petri dishes.

Probably, turbulence contributes to the transportation of spores, at least up to 9 m, and the impaction of *M. acerina* conidia on stems and umbels of caraway in spring. Attachment of *M. acerina* conidia to flowers and stems of caraway after drip splash and splash dispersal is probably quick and strong. Conidia of *M. filiformis* in streams readily and firmly attach to branches in the river. Read *et al.* (1991) showed that after a few minutes *M. filiformis* conidia could no longer be washed off their substratum, branches in the river.

Horizontal movement of conidia

Trap plants placed in the middle of a diseased caraway field were usually infected by *M. acerina* if weather conditions were favourable, showing an adequate source strength. *M. acerina* was not found on control plants placed outside the greenhouse, far from potential inoculum sources, indicating that trap plants were healthy at the start of the experiments. Other caraway plots, which might have served as inoculum sources, were only lightly infected and further away from the trap plants than the inoculated plot. Thus, the

probability of trap plants being infected by inoculum from outside the inoculated plots is vanishingly small.

Disease incidence of the trap plants decreased steeply with distance from the inoculum source. Various gradient models (Zadoks & Schein, 1979; Campbell & Madden, 1990) were tested. Equation 2, resembling the gradient model used by Gregory & Read (1949), gave the best fit. Conidia of *M. acerina* are carried in droplets larger than 480 µm in diameter, and mainly larger than 900 µm (Darby, 1977). Such large droplets only travel short distances. Even when conidia are not in droplets their large size may further sedimentation or impaction (Sreeramulu & Ramalingam, 1961; Ingold, 1965). Although the odd spore may travel over a distance beyond 9 m, our results imply in practical terms that a caraway crop is usually infected by an inoculum source within the field, i.e. infested soil, other host plants and/or infected seeds (Evenhuis & Verdam, 1995). Host plants outside the field including other caraway crops probably play no substantial part in the onset of an epidemic. This conclusion is in accordance with data from Norway, where Hermansen (1992a) found a few trapped spores just outside carrot plots. He concluded that conidia were not important in the spread of liquorice rot from one plot to another.

Inoculum build-up due to sporulation

Observations in carrots suggested that *M. acerina* conidia produced on young leaves in spring were washed into the soil (Wall & Lewis, 1978a). Conidia of *M. acerina* could survive the summer in the soil, because they formed thick-walled chlamydosporic conidia (Wall & Lewis, 1978b). These conidia infected senescent leaves in autumn, before lifting the carrots (Wall & Lewis, 1978a; Wall & Lewis, 1980a). The observations suggest that (chlamydosporic) conidia play a role in the inoculum build-up and subsequent infection of the carrot crop (Wall & Lewis, 1978a; 1978b). Caraway trap plants were readily infected throughout autumn and winter, if weather conditions were favourable. Conidia of *M. acerina* are able to survive for at least six months (Wall & Lewis, 1978b) and probably initiate another phase of infection on senescent leaves, stems and umbels of caraway in spring. Thus, in caraway, sporulation of *M. acerina* during autumn and winter may increase the inoculum potential and supplement the other inoculum source, chlamydospores surviving in soil (Evenhuis & Verdam, 1995).

Indications for control measures to prevent inoculum build-up

In Norway, carrots are treated four times with iprodione in autumn before harvest (Netland, 1993). In winter caraway, a single application of iprodione in autumn had no effect on inoculum build-up as disease severity in spring was not reduced (Evenhuis & Verdam, 1995). Repeated application of fungicides in caraway is economically and environmentally undesirable. Biological control might reduce inoculum build-up. *Trichoderma harzianum* (Tronsmo, 1989) and *Pythium oligandrum* (Lutchmeah & Cooke, 1984) were found to control *M. acerina* to some extent.

Conclusions

As a support to crop management recommendations, spore dispersal can be quantified satisfactorily by using inverted Petri dishes or trap plants. Spore dispersal was stimulated by rainfall and moderate (15-20°C) temperatures, whereas solar radiation had a negative effect on spore catches. Dispersal gradients of *M. acerina* are so steep that the primary inoculum sources must be present within the caraway fields themselves. Considering the dispersal characteristics of *M. acerina* conidia, verges and nearby diseased crops hardly play a role as an inoculum source. Because of the restricted dispersal of conidia, we surmise that inoculum from neighbouring caraway crops or from host plants in verges does not substantially affect a caraway crop.

CHAPTER 3

Effect of root injury on lesion development of caraway roots infected by *Mycocentrospora acerina*

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Abstract

The effect of injury on disease incidence, incubation period and lesion development rate on caraway roots by *Mycocentrospora acerina* was studied in three laboratory experiments. After inoculation with *M. acerina*, disease incidence of injured roots was significantly ($P < 0.001$) higher than of non-injured roots. The incubation period of *M. acerina* was significantly ($P < 0.001$) shorter on injured roots than on non-injured roots. The incubation period shortened with increasing root injury level. Younger injured roots tended to be more resistant to *M. acerina* infection than older injured roots, expressed by longer incubation periods. The lesion development rate was, on average, higher on heavily injured roots than on non- or slightly injured roots. The lesion development rate remained fairly constant after the first emergence of the symptoms on the caraway root, until the whole root was colonized. Caraway roots carefully dug up in autumn frequently showed injuries enabling *M. acerina* to penetrate the roots. However, the correlation between root injury and root rot after cold storage was weak. Injury of roots had a stimulating effect on infection and development of *M. acerina*, but roots without wounds could be infected too. Some relevant field observations are discussed.

Introduction

Biennial caraway (*Carum carvi* L.) is an arable crop which, in the Netherlands, is grown mainly on clay soils. In the 1980's anthracnose (Westerdijk & Van Luijk, 1924) caused by *Mycocentrospora acerina* (Hart.) Deighton was responsible for severe yield losses. The soil is probably a major inoculum source (Truscott, 1944; Newhall, 1944; Rader, 1945). In carrots (*Daucus carota* L.) the fungus enters the root mainly through wounds (Davies *et al.*, 1981) and causes liquorice rot (Rader, 1945). Caraway roots frequently suffer mechanical injury by farm equipment and gnawing insects.

In stored carrots a positive correlation was found between the degree of root injury at harvest and the occurrence of liquorice rot after two months in storage (Davies *et al.*, 1981). In agricultural practice, injury on caraway roots might be inflicted by mechanically weeding. Wounds can be caused by insects, mice and organisms of the soil fauna. Especially the caraway root aphid (*Pemphigus passeki* Börner) may injure caraway roots (Prinsen, 1991), and this insect possibly provides points of entry to *M. acerina*.

The aim of this study was to find out whether the development of lesions on caraway roots by *M. acerina* was enhanced by mechanical injury. The phenomenon was studied under controlled conditions in a climate chamber. Root injury under field conditions was considered. Root injury in autumn may be indicative for the risk of anthracnose development, and might be used to decide upon whether action is needed.

Materials & methods

Treatments

In experiments 1 and 2, caraway was sown early, in March 1991 and March 1992, respectively. Spring barley (*Hordeum vulgare* L.) was used as a cover crop. In experiment 3 no cover crop was used, therefore caraway was sown late, in June 1994. Caraway roots were collected from the field in spring 1992, autumn 1992 and autumn 1994. The ages of the caraway roots at lifting for use in the experiments 1, 2 and 3 were 373, 227 and about 100 days, respectively. The roots were carefully lifted, though the tap root inevitably broke off, thus creating a distal wound. The roots were gently

washed to remove adhering soil. The leaves were removed by cutting, leaving the petiole bases. Roots with diameters between 8 and 11 mm and a length of approx. 15 cm were used in the experiments. The roots used had no apparent injury or infection, the distal wound excepted. The roots were artificially injured with a punching apparatus, creating one wound per root. A cylinder of tissue (diameter 5 mm) was removed to different depths, in order to create different levels of injury (Davies *et al.*, 1981). Four levels of injury were applied.

0: no injury

1: only the epidermal layer removed

2: cortex cells were removed but the central cylinder was not injured

3: the central cylinder was injured

The roots thus prepared were placed on moist filter paper and incubated in covered plastic boxes.

Inoculation

An isolate of *M. acerina* originally obtained from a caraway root was used in all experiments. Chlamydospores were obtained by growing colonies for three weeks on sucrose agar (6 g sucrose, 15 g agar and 200 mg streptomycin per litre medium, after Day *et al.*, 1972) at 12°C. Inoculum was prepared by blending agar discs with *M. acerina* colonies in tap water. The suspension was sieved through double cheese cloth. The inoculum density was adjusted to 5 x 10^4 chlamydospore chains of *M. acerina* per ml suspension. With a pipette a drop of 0.1 ml suspension was placed in the wound created on the root. Non-injured roots were inoculated too. As a control treatment some roots of each injury level were treated with tap water instead of a chlamydospore suspension. Three experiments were carried out. In experiment 1 four levels of root injury were distributed in 8 boxes in a completely randomized design with two replicates. Each box contained 4 inoculated roots and one root treated with water. Experiments 2 and 3 were carried out in the same manner but had 4 replicates, whereas each box contained 2 roots inoculated with *M. acerina* and two treated with water.

Incubation and disease observations

During the experiments the temperature was kept at 5°C. This temperature was chosen to simulate conditions in the field during winter. A high humidity was maintained by placing plastic covers over the boxes. A high humidity (RH > 90%) is necessary for the development of *M. acerina*, as shown by Newhall (1944) who worked with stored celery. Experiments 1, 2 and 3 continued for eight, 13 and 32 weeks, respectively. The experiments were carried out in the dark. Lesion development was assessed weekly by measuring the length (mm) of the lesions caused by *M. acerina*. Isolations from the roots were made at the end of the experiments to confirm that *M. acerina* had caused the lesions.

Field observations

Caraway root injury in autumn was assessed in nine field experiments and in one commercial crop between 1992 and 1994. In another commercial crop, root injury was assessed in spring. The commercial crop was grown in the province of Groningen (Gr). The field experiments were situated in the provinces of Groningen (Gr), Flevoland (Fld) and Gelderland (Gld). Caraway was sown on clay soils, except for two field experiments in the vicinity of Wageningen (Gld) which had a sandy soil. Per field between 16 and 64 samples were taken. Each sample consisted of 10 to 20 caraway roots. The roots were carefully lifted in December when most leaves had already decayed. The root samples were washed and injury assessments were made. The degree of root injury was rated as follows:

0: no root injury

1: 1 - 10% of the root length covered with injuries

2: 11 - 30% " " " " " " "

3: 31 - 70% " " " " " " "

4: 71 - 100% " " " " " " "

A root injury index was calculated per sample by multiplying class ratings by class frequencies, adding the products, and dividing the sum by the number of roots per sample.

After injury rating the roots were placed in cold storage as described above for the root injury experiments. After six months in cold storage the roots were examined for the presence of *M. acerina*. The percentage of the roots rotted (disease incidence) was determined. The roots were rated according to the percentage decayed tissue, using the above mentioned root injury scale. A root rot index was calculated in the same way as the root injury index. The root rot ratings were based upon the classes used by Hermansen (1992a) to classify liquorice rot (*M. acerina*) on the mid-root of carrots, but where Hermansen used lesion number and size, we used percentages of root surface rotted.

Statistics

Results of injury experiments were analyzed by linear regression and analysis of variance (ANOVA). Linear regression was conducted on the development of the lesion length (mm) with time, starting from lesion initiation. For proper comparison of the three experiments only data of the first eight weeks were used. The slope of the regression line represents the lesion development rate (mm $week^{-1}$). The incubation period (weeks) was estimated as the intercept of the regression line with the time-axis. The incubation period and the log-transformed lesion development rates were subjected to analysis of variance. Least significant differences (LSD) at the $P = 0.05$ level are used to indicate differences between injury levels.

The effect of root injury in the field, assessed in autumn, on root rot after cold storage was studied by linear regression analysis with Genstat 5, release 3.1.

Results

Injury experiments

Disease incidence

Observations on disease incidence were made eight weeks after inoculation. Non-inoculated roots showed no anthracnose. Roots treated with water were omitted from further analysis, since no *M. acerina* infection occurred. With inoculation, disease incidence of injured roots was significantly ($P < 0.001$) higher than disease incidence of non-injured roots. Inoculated roots with injury had 100% disease incidence in all three experiments. Inoculated roots without injury had disease incidences of 50, 68 and 50% in the experiments 1, 2 and 3, respectively. On inoculated caraway roots lesions expanded with time. The lesions were caused by *M. acerina*, as was confirmed by re-isolation of the pathogen. On non-injured roots lesions were restricted, with hardly any expansion after eight weeks (2.8 mm), due to a longer incubation period (table 1) and a tendency for a lower lesion development rate (table 1) than found on injured roots (20.4 mm). On severely injured roots (classes 2 and 3) lesions became progressive, and finally covered whole roots.

Incubation period

The incubation period of *M. acerina* on non-injured roots was significantly ($P < 0.001$) longer than on injured roots. The incubation period shortened significantly with the level of root injury in experiments 1 and 3, but not in experiment 2 (table 1). The mean incubation period of *M. acerina* on injured roots in experiment 3 (2.9 days) was significantly ($P < 0.001$) longer than in experiments 1 (1.8 days) and 2 (1.9 days). The roots used in experiment 1 and 2 were considerably older (> 200 days) at lifting than the roots used in experiment 3 (100 days). The result suggests that the incubation period decreased with increasing age of the roots at the time of inoculation, but the relationship may have been caused by other differences between the experiments.

Table 1. Effect of root injury levels (four classes) on incubation periods and lesion development rates of M. acerina on caraway roots in cold storage after inoculation. The experiments 1, 2, and 3 began in March and October 1992 and September 1994, respectively. Non-inoculated controls showed no infections.

Class	Root injury level	Incubation period (weeks)				Lesion development rate (mm week^{-1})			
		Exp. 1	Exp. 2	Exp. 3	Mean	Exp. 1	Exp. 2	Exp. 3	Mean
0	Non-injured control	6.6 a [b]	5.4 a	6.7 a	6.3 a	2.1	1.0 a	3.3	2.1 a
1	Epidermal layer	2.7 b	2.1 b	4.5 b	3.1 b	4.2	1.6 a	2.8	2.8 ab
2	Cortex parenchyma	1.4 c	1.6 b	2.4 c	1.8 c	4.7	3.1 b	2.9	3.6 bc
3	Central cylinder	1.3 c	1.8 b	1.8 c	1.6 c	3.8	4.5 b	4.4	4.2 c
LSD[a]		1.0	1.8	1.1	0.7	n.s.[c]	[d]	n.s.[c]	[d]

a: Least significant differences for treatments 1-3 at P = 0.05.

b: Different letters indicate significant differences between injury treatments within columns, based upon LSD values for incubation periods.

c: No significant differences at P = 0.05, but significantly different at P = 0.10.

d: Different letters indicate significant differences between injury treatments within columns of log-transformed lesion development rates.

Lesion development

A tendency ($0.05 \leq P \leq 0.10$) was found that the lesion development rate of *M. acerina* increased with the root injury level (table 1). No significant differences between treatments were found in lesion development rate on roots in experiment 1 and 3. In experiment 2 the lesion development rate increased significantly with increasing root injury. The mean lesion development rate of the roots in experiment 2 (2.6 mm week^{-1}) was significantly lower (P = 0.005) than in experiments 1 and 3 (3.4 and 3.4 mm week^{-1}), but the difference could not be ascribed to root age. Experiments 2 and 3 continued for 13 and 32 weeks, respectively. During the experiments the lesion development rate remained fairly constant from first emergence of the symptoms on the caraway roots until the roots were colonized completely.

Field observations

Injury

The incidence of injury on caraway roots under field conditions in autumn was usually found to be less than 25% (table 2). Injury was found on 85% of the roots in a commercial crop, where caraway was grown for a second seed harvest. In another commercial crop, which was also to be harvested for the second time, root injury in spring was 61%. The root injury index of a caraway crop to be harvested for the second time was approximately five times higher than of first year's caraway crops.

Disease incidence

In cold storage pathogenic micro-organisms colonized the roots. After six months in cold storage approx. 50% of the roots had rotten. Root rot was caused by *M. acerina*, *Fusarium* spp., bacteria and other unidentified micro-organisms.

Wounding and root rot

When all fields were taken together the rotting of the roots was not significantly correlated with the level of injury assessed in autumn. However, in six out of ten fields a positive correlation between root injury index per sample and root rot index per sample was found (table 2).

Table 2. Effect of root injury in the field (9 trials and one commercial crop (G93a)) on root rot after cold storage. Linear regressions of the root injury index in autumn on the root rot index after cold storage were positive, r^2-values and P-values are given.

Experiment	Year of sowing/ harvest	Number of samples examined [a]	Root injury percentage	Root injury index	Percentage infected roots	Root rot index	Coefficient[b] of determination (r^2)	P-value
		(#)	(%)	(-)	(%)	(-)	(-)	(-)
G93a	1991/93	50	85	1.16	57	1.85	-[c]	0.73
EH659	1992/93	50	19	0.27	52	1.43	0.13	0.006
EH699	1992/93	50	17	0.22	35	0.87	0.14	0.006
EH705	1993/94	48	19	0.21	55	1.07	-	0.25
EH707	1993/94	16	10	0.12	50	1.02	-	0.43
PAGV3433	1992/93	24	25	0.34	49	0.79	0.18	0.022
PAGV3737	1993/94	36	8	0.08	51	1.53	0.16	0.010
DR93B	1992/93	24	5	0.06	47	1.41	-	0.078
DR94B	1993/94	24	4	0.06	44	0.70	-	0.37
LN94B	1993/94	64	5	0.05	50	1.87	0.14	0.003

a: Assessments were made on 10 to 20 roots per sample.

b: Coefficient of determination for linear correlation of root injury index in autumn with root rot index after cold storage.

c: Residual variance exceeds the variance of the Y variate.

Discussion

Inoculum source

In celery, chlamydospores in the soil were shown to be the main inoculum source of *M. acerina* (Day *et al.*, 1972). In the present experiments, chlamydospores were chosen as the inoculum, since caraway root infection in soil is probably due to chlamydospores too. It is unlikely that a natural inoculum source interfered with the results, since no infection was found on control roots treated with water.

Injury experiments

Cold storage

Roots of caraway remain in the field during autumn and winter. Circumstances in the field in winter are to some extent comparable to cold storage of carrots, although circumstances in the field are much more variable than in storage. The development of carrot plants slows down and growth almost ceases as the temperature falls during autumn (Lewis & Garrod, 1983), as will happen to caraway. But where carrots are lifted, caraway stays in the soil to flower next spring. The physiological activity of both carrot and caraway roots in winter is low. In such a period the defence of the roots probably weakens, so that *M. acerina* might penetrate and colonize the roots. If the physiological activity of caraway roots decreases relatively more than that of *M. acerina*, the fungus gains an advantage over the host in winter time.

Wounding and disease incidence

Davies *et al.* (1981) stated that liquorice rot was more often found in stored carrots severely injured at harvest than in carrots only slightly injured. They also found that liquorice rot was almost always connected with root injury, be it severe damages or ones invisible to the naked eye. They concluded that *M. acerina* was predominantly a wound pathogen. In parsnips (*Pastinaca sativa* L.), however, lesion development was not increased by wounding the roots before inoculation (Channon, 1965). Artificial wounding had no influence on disease incidence of celery roots in storage (Gündel, 1976). In contrast, severe damage to the petiole of celery (*Apium graveolens* L.) plants considerably increased the rate of *M. acerina* infection (Day *et al.*, 1972).

Wounding of caraway roots

M. acerina does not need visible wounds to infect caraway roots under the conditions provided in the study. Though care was taken not to injure the roots at lifting, some minute wounds might have been inflicted, especially on or near small lateral roots and root hairs. These minute injuries might have been the points of entry for *M. acerina* in the inoculated non-injured roots (class zero treatment). Both disease incidence and disease severity increased when roots were artificially injured. Minute wounds of caraway roots can easily occur during the formation of lateral roots. Organisms of the soil fauna can cause minute to medium sized wounds. Some agricultural practices cause injuries, such as weeding. Under cool conditions wound healing will be slow, as indicated by the fact that a period of high temperatures before cold storage accelerated wound repair of harvested carrot roots and diminished infection by *M. acerina* (Lewis *et al.*, 1981). Especially in winter, conditions are relatively favourable for *M. acerina* development since the fungus is able to grow at low temperatures (Newhall, 1944; Neergaard & Newhall, 1951; Channon, 1965; Gündel, 1976). Any injury during such a period might lead to successful penetration and colonization of the caraway root. Roots of caraway to be harvested for a second year in succession tend to be more injured than roots of a first year caraway crop. This finding indicates that a second harvest caraway crop runs a higher risk to become seriously infected by *M. acerina* than a first harvest crop.

Incubation period and lesion development rate

The incubation period of *M. acerina* in stored carrots was up to four months when the roots were not injured (Lewis & Garrod, 1983). In carrots, progressive lesions developed rapidly in wounds which reached into the xylem parenchyma (Lewis *et al.*, 1981). The incubation period of *M. acerina* on stored celery heads was seven weeks (Truscott, 1944). In celery plants, infection did occur at an early stage when the petioles were severely damaged (Day *et al.*, 1972). In our experiments the incubation period was longer than six weeks when the caraway roots were not injured, which is in accordance with the observations in carrots. By damaging the caraway roots the incubation period for *M. acerina* decreased drastically, as also found in carrots. On celery heads in storage *M. acerina* seems to behave similarly. No data on lesion development rates were found in the literature.

Incubation period and root age

The longer incubation period in experiment 3 as compared to experiments 1 and 2 may be caused by the age of the caraway roots at lifting. In carrot, wound infection increased with age of roots at harvest. Injured carrot roots harvested 127 days after sowing were more resistant to *M. acerina* infection than carrot roots harvested 162 to 211 days after sowing (Davies & Lewis, 1980). The same was found for colonization of caraway roots. In experiment 3 caraway roots were lifted about 100 days after sowing, and in experiments 1 and 2 after 373 and 227 days, respectively. The incubation period in experiment 3 was much longer than in experiments 1 and 2. The effect of root age on susceptibility of caraway to root colonization has an indicative value only since the results were obtained from three experiments not designed for this purpose. Nevertheless, the results suggest that serious caraway root infection might be expected at the end of winter rather than during the autumn. Furthermore, a caraway crop which will be harvested for the second time might run more risk of root infection, as observations in a commercial crop seem to confirm. In Germany, root infection of biennial caraway, probably caused by *Fusarium* spp. increased 2.5 times between September and June the following year (Plescher & Herold, 1983).

Lesion types

Davies *et al.* (1981) described two lesion types on carrot roots. The first type is a restricted lesion, which expands very slowly. The second type is a progressive lesion which expands rapidly. The first stage is often followed by the second when the carrot roots have been in storage for some months. Le Cam *et al.* (1993) studied lesion development on carrots under various environmental conditions. On caraway roots these two lesion types were found too. Restricted lesions were prevalent on non-injured and slightly injured roots, whereas progressive lesions were prevalent on roots with medium and severe injuries. Caraway roots were relatively thin. As lesions rapidly ringed the roots, lesion development was adequately assessed by measuring lesion length.

Carrot roots have some degree of resistance to *M. acerina* but this resistance is easily overcome by mechanical injury. In carrot root tissues, a decrease in the concentration of falcarindiol was found from the epidermic layer to the central cylinder. Falcarindiol is believed to be the resistance factor since the concentration measured in the outer

tissues is toxic to *M. acerina* (Davies & Lewis 1981b; Garrod & Lewis 1982). Such a chemical component may exist in caraway roots too. A decrease in the concentration of such a component in the inner tissues would explain why injury of caraway roots leads to the formation of progressive lesions, as in carrots. For breeding purposes, identification and measurement of the chemical involved could be interesting. An alternative explanation might be that the mechanical resistance to penetration is much higher in the epidermal layer than in tissues beneath.

Caraway root aphid

The caraway root aphid injures the roots of caraway while feeding on phloem cells. The insect migrates to caraway from mid-June to mid-August and prevails during dry periods. Most insects leave the crop before winter, and only a few are able to overwinter on caraway (Prinsen, 1991). During the period when the majority of the caraway root aphids are present on caraway, conditions are unfavourable to *M. acerina* but favourable for wound healing. Considering the life cycles of the organisms involved, it is unlikely that the caraway root aphid causes root injury through which the fungus could penetrate the roots. In a small survey among some fields nor in field experiments any relation was observed between the presence of caraway root aphids and the incidence of anthracnose.

Field observations

Root injury and root rot

Caraway roots in commercial crops were frequently injured at the end of autumn. Root injury was especially marked in caraway crops to be harvested a second time. These injuries increase the probability of roots becoming infected by *M. acerina*, as found in the root injury experiments. Because disease assessments were hard to make on roots dug up in autumn, the roots were placed in cold storage in order to allow the pathogenic micro-organisms present to colonize the roots. In carrots, *M. acerina* does not spread from root to root in storage (Davies *et al.*, 1981) and it is unlikely the fungus will do so in caraway in the field. Approximately 50 percent of the caraway roots from commercial crops were rotten after cold storage. *M. acerina* infection was

found occasionally but anthracnose was hard to distinguish from infections by other micro-organisms.

In several fields positive relationships were found between root injury and root rot, though correlations were poor (table 2). Taking the fields together, linear regression showed no significant relation between mean root injury index in autumn and mean root rot index after six months of cold storage. Possibly the correlation was masked because the mean root injury levels did not vary much per field. Another explanation might be that *M. acerina* is not strictly a wound pathogen in caraway, so that even roots not injured at the time of lifting could have been infected by *M. acerina*. Because of the long duration of storage all the infected roots could become colonized entirely, despite a lower lesion development rate of *M. acerina* on non-injured roots. A third explanation might be that injuries not visible to the naked eye played a part in the infection of caraway roots by *M. acerina*. A fourth explanation might be that the occurrence of other pathogenic and saprophytic micro-organisms might have caused root rot irrespective of the injury level of the caraway roots at autumn, thus obscuring any correlation between root injury and root infection by *M. acerina*.

It is not known whether a positive relation between root injury and *M. acerina* infection under field conditions exists. From the results presented in the laboratory experiments such a relationship is plausible, but observation of root rot after cold storage could not confirm the relationship. The coefficients of determination were low (0.13-0.18), but the number of samples per field were high (24-64), so that (r from 0.36-0.43) the significance of the regression was high ($P \leq 0.01$). Apparently root injury can play a role in the loss of caraway plants through root rot, but that role is not very prominent. Root injury assessment in autumn cannot be used as an indication for anthracnose development on roots. Many micro-organisms were found in infected roots. It is not known whether these are secondary pathogens following *M. acerina* infection or whether they are primary pathogens to caraway roots.

Further research

The relative importance of the different pathogens involved in root infection must be investigated further. The importance of root infection on caraway growth and yield, relative to the effects of stem infection, has to be established. Data gathered so far

indicate that infection of the stems is more important than infection of the roots in respect to yield loss (Evenhuis & Verdam, 1995).

Conclusions

This study shows that injury on roots increases the risk of root infection by *M. acerina*. An indication was found that a caraway crop which is to be harvested a second time runs a higher risk of becoming seriously infected by *M. acerina* than a first harvest crop.

CHAPTER 4

Seed infection of caraway (*Carum carvi*) by *Mycocentrospora acerina*

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B. Verdam

Submitted to Seed Science and Technology

Abstract

Methods to quantify *M. acerina* seed infection in caraway are presented and discussed. Seed infection levels were usually less than 3%, but infection levels up to 50% were found. Seed transmission of *Mycocentrospora acerina* in caraway (*Carum carvi* L.) is demonstrated. Seed transmission efficiency varied between 27 and 62%. *M. acerina* remained viable in caraway seeds for at least three years. Results suggest that infected caraway seed can be an inoculum source for the development of anthracnose in caraway crops. The importance of seed infection for long distance dissemination and the onset of an epidemic is discussed.

Introduction

In the Netherlands, anthracnose of caraway (*Carum carvi* L.), caused by *Mycocentrospora acerina* (Hart.) Deighton, is a major constraint in caraway production (Evenhuis & Verdam, 1995). Caraway is an arable crop grown for its seeds, which are used for consumption and for extraction of its essential oil for industrial purposes. A little over 50% of the oil consists of carvone (Toxopeus & Bouwmeester, 1993). Recently a sprouting inhibitor of potatoes in storage based upon carvone, has been marketed (Hartmans *et al.*, 1995). Caraway is a biennial species belonging to the family of the *Umbelliferae*. Recently, spring caraway, an annual form, was introduced in the Netherlands (Toxopeus & Lubberts, 1994). Botanically the term seed (*semen*) is incorrect since the harvestable product consists of split fruits (*diachenium*). However, the term seed is generally used, and will be used here too.

The establishment of primary infection by *M. acerina* leading to anthracnose in a caraway crop has not been described satisfactorily. Westerdijk and Van Luijk (1924) suggested that seeds of caraway could be infected with *M. acerina*. However, according to Neergaard and Newhall (1951), seed transmission was not proven. Gill (1971) demonstrated seed transmission of *M. acerina* in pansy (*Viola tricolor* L.). The host range of *M. acerina* is broad (Rader, 1945; Newhall, 1944; Tompkins & Hansen, 1950; Rintelen & Klewitz, 1976; Hermansen, 1992b). There are no further reports on seed infection of plant species belonging to the host range of *M. acerina*. Repeated attempts to isolate *M. acerina* from celery (*Apium graveolens* L.) seeds failed (Truscott, 1944).

The aim of this study is to demonstrate seed transmission of *M. acerina* in caraway. Methods to determine seed infection levels were investigated for application in a seed certification scheme.

Materials and methods

Seed lots

Caraway seeds harvested in the years 1990 until 1994 were used in the experiments (table 1). In 1990, 1991, and 1992 seed lots were obtained from farmers fields in the provinces of Groningen (Gr) and Zeeland (Zld). Seed lots were also collected from field experiments conducted at Nieuw Beerta, Lelystad, Lienden, Randwijk and Wageningen, from 1991 until 1994. Some plots in these experiments had been inoculated with *M. acerina*, using a knapsack sprayer (Evenhuis *et al.*, 1995). These inoculations were carried out approx. half a year (biennial caraway) and 2 months (spring caraway) before flowering of the caraway crop. Special mention is made of seeds collected from a heavily diseased part of a caraway crop at Wageningen in 1992. This sample was coded WdG92-1. The sample taken randomly from the mixed seed yield of the whole field was coded WdG92-2. Usually, the seeds had been in storage for more than half a year before disease assessments were made. Seed lots were stored in a dark room with a constant temperature of 8°C and a relative humidity of 40%. Once, trash originating from a seed lot was checked for the presence of *M. acerina*. Three methods were tested to determine seed infection by *M. acerina*.

Greenhouse method (G)

Seeds were sown to a depth of 0.5 - 1 cm, in plastic containers measuring 45 * 30 * 7 cm, filled with 5 cm river sand. The distances between rows and between seeds within a row were both 4 cm. High relative humidity was maintained by placing plastic covers over the containers. The temperature in the greenhouse was kept as close as possible to 20°C. During a period of sunny weather the temperature could rise to almost 25°C. When necessary, water was added directly onto the soil. Eight samples were tested in 1990 (table 2). Per sample three replicates of 50 seeds each were sown. In 1991, thirteen samples consisting of two replicates of 104 seeds each, were tested.

Seedlings and diseased seedlings were counted weekly up to eight weeks after sowing. Plants showing damping-off were removed and examined under a light microscope (40x) for the presence of the typical conidia of *M. acerina*. If the fungus could not be recognized immediately, the plants were incubated for two days on one layer of moist

filter paper (T 10 D, diameter 13.5 cm, Papierfabriek Schut BV, Heelsum, The Netherlands) in closed Petri dishes in darkness at 18°C, and examined again. The emergence percentage is defined as the percentage of the seeds developing a seedling. The infection level is defined as the percentage of the total number of seeds giving a *M. acerina* infected seedling, and was not adjusted for emergence percentage.

Table 1. Caraway seedlots tested for *M. acerina* infection.

Year	Field code	Crop type	Location	Soil type	Origin
1990	Gr[a]	biennial	Prov. Groningen	clay	8 farmers fields
1991	Gr[b]	biennial	Prov. Groningen	clay	7 farmers fields
1991	Zld91a	biennial	Prov. Zeeland	clay	1 farmers field
1991	PAGV2316	biennial	Lelystad	clay	experiment
1991	Wag	biennial	Wageningen	sand	experiment
1992	Gr[c]	biennial	Oldambt	clay	7 farmers fields
1992	Zld[d]	biennial	Prov. Zeeland	clay	2 farmers fields
1992	WdG92-1	biennial	Wageningen	sand	experiment
1992	WdG92-2	biennial	Wageningen	sand	experiment
1992	PAGV2496	biennial	Lelystad	clay	experiment
1994	Bo943	biennial	Randwijk	clay	experiment
1994	Dr941	biennial	Wageningen	sand	experiment
1994	Ln941	biennial	Lienden	clay	experiment
1993	PAGV3069	spring	Lelystad	clay	experiment
1993	EH690	spring	Nieuw Beerta	clay	experiment
1991	trash[e]	biennial	Lelystad	clay	experiment

a: Consist of 8 samples from farmers' fields coded Gr90a-Gr90h.

b: 7 samples coded Gr91a-Gr91g.

c: 7 samples coded Gr92a-Gr92g.

d: 2 samples from farmers' fields coded Zld92a and Zld92b.

e: Samples do not consist of seeds but of fragments of umbels.

Blotter (B) and freezing-blotter method (F)

To determine seed infection levels, seeds were placed on one layer of moist filter paper (T 10 D) either by hand or with the aid of a metal slide containing 50 holes (diameter 0.4 mm, at least 1 cm apart) attached to a vacuum cleaner. The Petri dishes (14 cm in diameter) with 50 seeds each were incubated at 18°C in a climate chamber in the dark. In the blotter method incubation was continued for 12-14 days without a cold treatment. In the freezing-blotter method incubation was interrupted, after two days, by deep freezing at -20°C for a period varying from one to three days. After deep freezing the filter paper was wetted with tap water until saturation and the seeds were incubated for approximately twelve more days before they were examined for sporulation of *M. acerina* under a light microscope (40x). Seeds showing sporulation were counted. Mean infection levels and 95% confidence intervals were calculated per field or experiment. A yearly average for seed infection levels was calculated for the farmers' fields in Groningen and Zeeland. The fungus was isolated from the seed and grown on Sucrose Agar (sucrose 6g/l, agar 15g/l and 0.02% streptomycin, modified after Day *et al.*, 1972). Spore suspensions were made from two week old cultures. Four week old caraway seedlings were inoculated to confirm that the conidia belong to *M. acerina*, infectious to caraway. Caraway was grown on sterile river sand in plastic containers, as in the greenhouse tests. After inoculation the humidity was kept high by placing plastic covers over the containers. The seedlings were checked regularly for symptom development.

Comparison of the blotter and freezing-blotter method

To compare the blotter and freezing-blotter methods, seed lots with different levels of seed infection were prepared. Sample WdG92-2 was used as the diseased seed lot. The seed infection level was 50%, as assessed directly after harvest in 1992, based upon 200 seeds tested with both methods. This seed lot was mixed in various proportions with seeds from a non-contaminated field at Lelystad (PAGV2496). Seed lots with *M. acerina* infection levels of 0, 1, 6, 13, 29, and 50% were created. For each seed infection level, 50 seeds were incubated in each of six replicate Petri dishes according to either method. Three experiments were carried out, in 1993, 1994 and 1995. In the 1993 experiment 0, 29 and 50% levels were tested with the blotter method, and all six levels were tested with the freezing-blotter method. In the 1994 experiment seed infection levels of 0, 1, 6, and 13 percent were tested with both methods. In the 1995 experiment all six infection levels

were examined for the presence of *M. acerina*, with both methods but with two replicates only. Observed seed infection levels were compared to the expected seed infection levels by linear regression. The slope of the regression, a measure for the efficiency of the method to determine seed infection rates, should be approximately one.

Transmission efficiency

The transmission efficiency is defined as the percentage of infected seeds yielding a diseased seedling (Schilder & Bergstrom 1995), see appendix 1. To estimate transmission efficiency, four replicates of 84 seeds each, from a seed lot with a calculated infection level of 29% were sown on river sand, as in the greenhouse tests. The experiment was conducted twice. The emergence percentage, the infection level of the seedlings and transmission efficiency were determined.

Statistics

Data were analyzed using Genstat 5.0, release 3.1 (Genstat 5 Committee, 1993). Seed infection levels observed with the greenhouse, blotter and freezing-blotter methods had a binomial distribution. Therefore seed infection levels were logit transformed to calculate the 95% confidence interval. The confidence limit values were back-transformed to percentage values. The blotter and the freezing-blotter methods were compared by linear regression.

Results

Conidia of *M. acerina* have a characteristic and distinctive appearance. Examined under a light microscope (40x) they cannot easily be mistaken for spores of other fungi. *M. acerina* was demonstrated in or on seeds of caraway by means of the greenhouse, the blotter, and the freezing-blotter method. Isolates taken from infected seeds and seedlings proved to belong to *M. acerina*. These isolates were tested successfully for pathogenicity on caraway, thus fulfilling Koch's postulates.

Storage effects

Seeds were stored for more than half a year before seed infection levels were determined. There is no evidence that *M. acerina* lost viability while the seeds were in storage. Over

a three year period (1992 - 1995) seed infection levels of sample WdG92-2 did not decrease significantly as established by the blotter and freezing-blotter methods.

Greenhouse method

In the greenhouse experiments, biennial caraway germinated and emerged within two weeks. Infection of the hypocotyl of the emerging plant could first be found two to three weeks after sowing, within a few days after emergence of the seedlings. The hypocotyl was colonized by the fungus and turned dark brown to black. Sometimes a grey cover, consisting of mycelium and conidia, was seen by the naked eye. When seedlings were infected in an early growth stage they usually died. Damping-off of seedlings was restricted to the first eight weeks after sowing. Mostly, the typical conidia of *M. acerina* were observed under the light microscope, but sometimes a positive identification could be made after incubation only. Occasionally, *Alternaria* spp. and *Fusarium* spp. were found on seedlings. Seed infection levels assessed with the greenhouse method in 1990 and 1991 were low (table 2).

Table 2. Percentage of caraway seedlings infected by *M. acerina* assessed in the greenhouse test, with back-transformed 95% confidence intervals of the logit-transformed data. The seed samples were collected in farmers' fields and in field experiments, including an inoculated field experiment. The number of samples and seeds per object are given, including the number of replicates per sample between brackets.

Year	Province of origin	Field code	Samples	Total seeds	Emergence percentage	Infected seeds		Confidence interval
			(#)	(#)	(%)	(#)	(%)	(%)
1990	Groningen	Gr90a-h	8 (3)	1200	84	1	0.1	0.01 - 0.7
1991	Groningen	Gr91a-g	7 (2)	1456	83	15	1.0	0.6 - 1.8
1991	Zeeland	Zld91a	1 (2)	208	80	0	0.0	-[b]
1991	Flevoland	PAGV2316[a]	3 (2)	607	88	7	1.2	0.5 - 2.5
1991	Gelderland	Wag	2 (2)	416	82	7	1.7	0.8 - 3.7

a: Plots were inoculated with *M. acerina*.

b: The 95% confidence interval could not be calculated because seed infection levels were zero.

Blotter and freezing-blotter methods

The blotter and freezing-blotter methods were compared by incubating seed lots with different levels of infection by *M. acerina*. In either method sporulation by *M. acerina* on caraway seeds was found, and in the blotter method also on germlings (figure 1). Linear regression of observed on expected seed infection levels shows that the slope with the blotter method is significantly lower than 1, whereas with the freezing-blotter method no significant difference from 1 was found. In general, seed infection levels found with the freezing-blotter method were higher and closer to the expected seed infection levels than with the blotter method. However, the variance of the freezing-blotter data was larger than of the blotter data. Another way to estimate the suitability of either method was a linear regression of the seed infection levels on the fraction of sample WdG92-2 in the mixture. The slope of the regression line represents the fraction of the seeds infected by *M. acerina* in sample WdG92-2. This figure was multiplied by 100 to estimate the seed infection level in percentages. In the three experiments, the estimates of the seed infection level of sample WdG92-2 with their standard errors, as predicted by linear regression, were 35±3%, 4±2% and 41±4% for the blotter method and 62±4%, 38±5% and 46±4% for the freezing-blotter method. The expected seed infection level of sample WdG92-2 was 50%. With both methods, the predictions of the seed infection level in the second experiment were rather low, possibly because seedlots with the two highest seed infection levels were not included in that experiment.

Comparison of healthy and infected seeds

Observation under the light microscope often revealed chains of chlamydospores of *M. acerina* in the pericarp of infected caraway seeds, where the oil streams are situated. Infected seeds were usually darker in colour than healthy seeds. The surface of infected seeds appeared to be less smooth than that of healthy seeds. The infected seeds were not always completely filled and often they were smaller than disease-free seeds. For example, the mean kernel weight of the highly infected seed lot (WdG92-2; sandy soil) was 2.0 mg whereas that of a healthy seed lot (PAGV2496; clay soil) was 2.9 mg. The difference in mean kernel weights attributed to the difference in soils did not exceed 0.1 mg (Evenhuis & Verdam, 1995) (2.94 mg for sandy soil, 2 experiments; and 2.90 mg clay soil, 11 experiments).

Provenance of seed infection

Samples of caraway seeds from farmers' fields (table 3) and from experiments were screened for seed infection (table 4) using the blotter and the freezing-blotter methods. Seed infection levels varied from 0 to 50%, but were usually below 3%. The seed infection levels from farmers' field were less than one percent in 1990 (greenhouse method) and 1992 (freezing-blotter method). In 1991, seed infection levels from several farmers' fields were approx. 2% (blotter method), or 1% (greenhouse method). Using Neergaard's (1977) terminology, seed infection of seedlots was frequent, but inoculum potential was low. The low inoculum potential might be due to the weather conditions during flowering which were not favourable for *M. acerina* development in 1990 and 1992. High seed infection levels were found in biennial caraway at Wageningen, 1992, and in spring caraway at Lelystad (PAGV3069) and at Nieuw Beerta (EH690) in 1993 (table 4). These fields had in common that they had severely lodged and, in addition, precipitation in September 1993 was high (table 4). Inoculated plots generally had higher seed infection levels than non-inoculated plots.

Transmission efficiency

In the two greenhouse experiments, the emergence percentages of the infected caraway seed lots were 53% and 55%, respectively. Of the resulting seedlings 33% and 15%, respectively, were infected by *M. acerina*. In both experiments the infection level of the seed lot was 29%, according to the freezing-blotter method. The transmission efficiencies of *M. acerina* from seed to seedling were 62% and 27%, respectively, though the seed lots had the same origin.

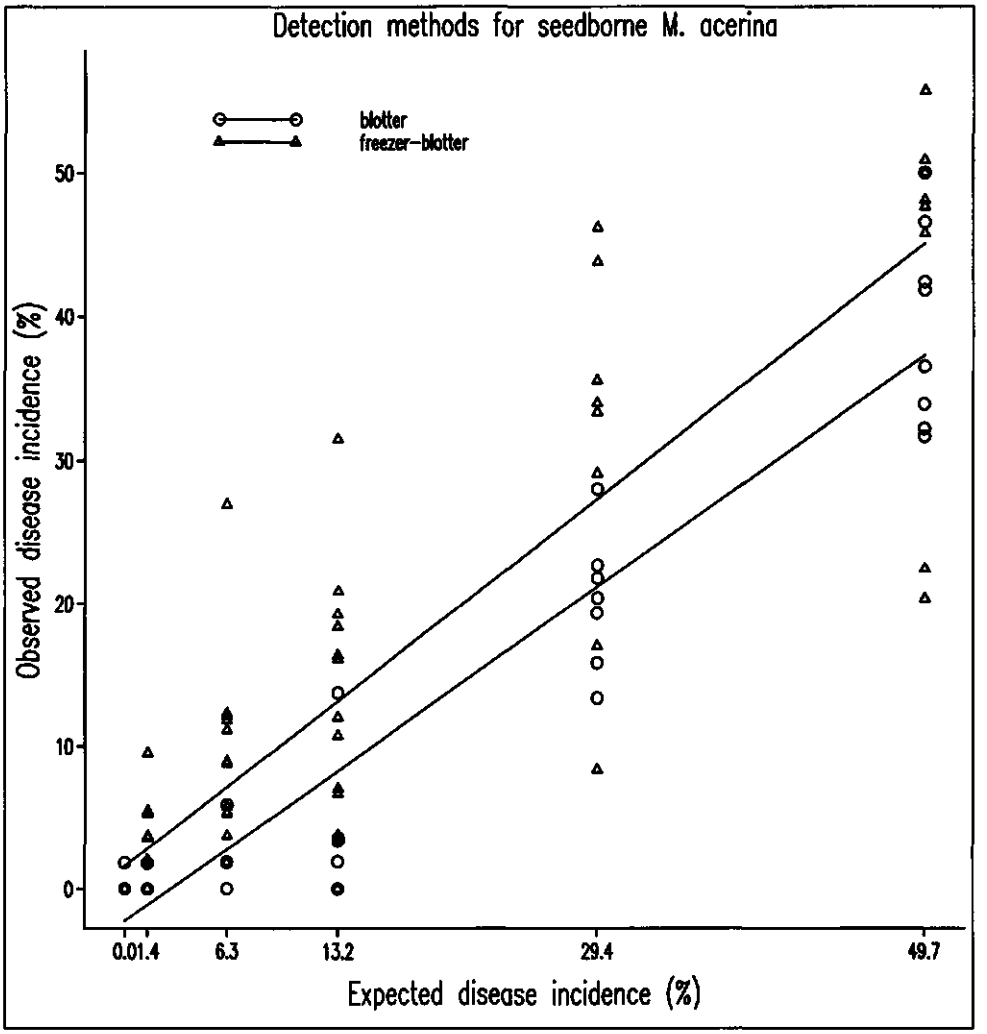

Figure 1. The reliability of two detection methods for seed infection at various levels of caraway seed infection. Data from three experiments were pooled and analyzed together. Linear regression equations for the blotter method (n=52; r^2=0.91; P<0.001) and the freezing-blotter method (n=71; r^2=0.89; P<0.001) are:

$Y_{blotter}$ = -2.25 (0.80) + 0.80 (0.04) X;

$Y_{freezing\text{-}blotter}$ = 1.56 (0.82) + 0.99 (0.04) X;

Y is the observed seed infection level and X the expected seed infection level. Standard errors are given between brackets.

Table 3. The percentage of diseased seed in biennial caraway determined by means of the blotter (B) and the freezing-blotter (F) methods, with the back-transformed 95% confidence intervals of the logit-transformed data. Seeds used in the experiments were harvested from farmers' fields in the provinces of Groningen (Gr) and Zeeland (Zld).

Year	Field code	Method	Replicates	Total seeds	Infected seeds		Confidence interval
				(#)	(#)	(%)	(%)
1991	Gr91a	B	2	47	1	2.1	0.2 - 21[a]
1991	Gr91b	B	2	37	1	2.7	0.2 - 26
1991	Gr91c	B	2	50	0	0.0	-[b]
1991	Gr91d	B	2	42	0	0.0	-
1991	Gr91e	B	2	78	0	0.0	-
1991	Gr91f	B	2	38	1	2.6	0.2 - 25
1991	Gr91g	B	2	39	1	2.6	0.2 - 25
1991	Zld91a	B	2	42	1	2.4	0.2 - 23
1992	Gr92a	F	6	315	0	0.0	-
1992	Gr92b	F	6	330	1	0.3	0.1 - 1.2
1992	Gr92c	F	6	317	0	0.0	-
1992	Gr92d	F	6	301	1	0.3	0.1 - 1.3
1992	Gr92e	F	6	321	0	0.0	-
1992	Gr92f	F	6	330	1	0.3	0.1 - 1.2
1992	Gr92g	F	6	312	2	0.6	0.2 - 1.6
1992	Zld92a	F	6	326	1	0.3	0.1 - 1.2
1992	Zld92b	F	6	322	1	0.3	0.1 - 1.2

a: Confidence intervals were large because the total number of seeds tested was small, the conclusion is that seed infection levels were larger than 0.

b: The 95% confidence interval could not be calculated, because seed infection levels were zero.

Table 4. The percentage of diseased seeds in biennial (B) and spring (S) caraway determined by means of the blotter (B) or freezing-blotter methods, with the back-transformed 95% confidence intervals of the logit-transformed data. Seeds used in the experiments were harvested from farmers' fields (Gr and Zld), in experimental plots, and in lodged plots at Wageningen (WdG92). The numbers of samples are given, with the number of replicates per sample between brackets. Rainfall was measured in the month caraway flowered, and lodging was observed shortly before harvest.

Year	Field code	Crop	Method	Inoculation	Samples	Total seeds	Infected seeds		Confidence interval	Lodging autumn	Rain
					(#)	(#)	(#)	(%)	(%)	(%)	(mm)
1991	Gr[a]	B	B	-	7 (2)	331	4	1.2	0.4 - 3.9	-[d]	60
1991	Zld[a]	B	B	-	1 (2)	42	1	2.4	0.2 - 21	-	39
1991	PAGV2316	B	B	-	3 (2)	157	3	1.9	0.5 - 7.2	0	38
1991	Wag	B	B	-	1 (2)	43	2	4.7	0.9 - 21	-	37
1992	Gr[a]	B	F	-	7 (6)	2226	5	0.2	0.1 - 0.4	-	69
1992	Zld[a]	B	F	-	2 (6)	648	2	0.3	0.1 - 0.7	-	58
1992	WdG92-1	B	B	-	1 (1)	43	38	88	71 - 96	95	61
1992	WdG92-2	B	B	-	1 (2)	200	97	48.5	40 - 57	75	61
1992	WdG92-2	B	F	-	1 (2)	200	103	51.5	43 - 60	75	61
1992	PAGV2496	B	F	-	1 (1)	100	0	0.0	-	0	60
1994	Bo943	B	F	+	12 (1)	1124	6	0.6	0.2 - 1.3	33	87
1994	Bo943	B	F	-	12 (1)	1089	1	0.1	0.01 - 0.8	24	87
1994	Dr941	B	F	-	24 (1)	1984	21	1.1	0.7 - 1.6	50	87
1994	Ln941	B	F	-	9 (1)	739	5	0.7	0.2 - 2.2	0	87
1994	Ln941	B	F	+	9 (1)	751	15	2.0	1.0 - 3.9	0	87
1994	Ln941	B	F	s[b]	3 (1)	251	7	2.8	1.0 - 7.4	0	87
1993	PAGV3069	S	F	+	12 (1)	1251	68	5.4	4.1 - 7.2	49	162
1993	PAGV3069	S	F	-	12 (1)	1058	19	1.8	1.0 - 3.2	51	162
1993	EH690	S	F	+	24 (1)	962	186	19.3	16 - 23	36	137
1993	EH690	S	F	-	24 (1)	898	146	16.2	13 - 20	33	137
1991	trash[c]	B	F	-	1 (1)	185	3	1.6	-	-	-

a: Mean seed infection rates of several farmers' fields sampled.

b: The plots were inoculated with infested caraway straw.

c: samples do not consist of seed but of fragments of umbles.

d: no data available.

Discussion

Storage effects

Preservation of viability over a three year period (1992 - 1995) may be ascribed to *M. acerina* being mainly present in the form of thick walled chlamydospores in the pericarp. Chlamydospores of *M. acerina* were shown to survive for at least two years in soil (Wall & Lewis, 1980c).

Greenhouse tests

Infection of seedlings by *M. acerina* was found in the greenhouse tests (table 2). As the substrate was river sand, the soil is not likely to be an inoculum source. It is unlikely that conidia were blown in from outside, since plastic covers were placed over the containers. The covers were only removed during disease assessments. Dispersal of the fungus from one diseased seedling to another within an experimental unit, i.e. a container, is improbable as conidia of *M. acerina* are mainly splash dispersed. During the experiment no water was added over the top of the plants, thus minimizing the risk of infection among neighbouring plants. Transport of conidia by water running over the soil is not impossible, but infection of seedlings neighbouring a diseased seedling was not found. It is concluded that infected seeds must have been the source of inoculum.

Comparison of methods for the determination of seed infection levels

Infection of caraway seeds by *M. acerina* was found using the greenhouse, the blotter and the freezing-blotter methods.

The greenhouse method, which requires much space and time, provides an estimate of the number of plants that might serve as inoculum sources in the field. Under field conditions the emergence percentage of infected seedlings is probably smaller than in the greenhouse. The greenhouse method probably underestimates the percentage of seeds infected by *M. acerina* since emergence percentages of highly infected seed lots are generally lower than of non-infected seed lots. Moreover the environmental conditions (i.e. light, temperature, relative humidity) in a greenhouse cannot be controlled fully as evidenced by the difference in transmission efficiency among two experiments. The higher transmission efficiency in the first experiment might also be due to sowing the seeds a little shallower than in the second experiment.

In both the blotter and the freezing-blotter method, a small probability exists that healthy seeds are infected by neighbouring infected seeds, leading to an overestimation of the seed infection level. *M. acerina* infection levels in the freezing-blotter method were not significantly different from the expected seed infection levels (figure 1). The blotter method yielded seed infection levels which were significantly lower than expected (figure 1), especially in experiment two.

With the blotter method, mites and nematodes were often observed in the Petri dishes, possibly disturbing the mycoflora and masking the presence of *M. acerina*. The microfauna was killed when seeds were exposed to -20°C (freezing-blotter method). Killing of the microfauna might explain the significantly higher infection percentages found in the freezing-blotter method. Another explanation (Limonard, 1966) might be that the active resistance of the living seed in the blotter method suppresses sporulation whereas in the freezing method the seeds were dead. The regression coefficient found in the freezing-blotter method is not significantly different from 1, whereas the regression coefficient found in the blotter method is significantly smaller than 1. The variance with the freezing-blotter method is larger than with the blotter method. Thus, the freezing-blotter method estimates seed infection levels of *M. acerina* more precisely than the blotter method but, to overcome the larger variance, more replicates are needed.

Seed infection level

The seed infection levels of caraway samples harvested from 1991 to 1994 were usually between 0% and 3%. The sampled crops had in common that they had not or barely lodged, and had not received abundant rainfall during flowering. In some cases higher infection levels were found. The high seed infection level (50%) found in field WdG-92 was probably due to severe lodging of the crop even before flowering, which led to circumstances conducive to development of seed infection by *M. acerina*. In experiments discussed elsewhere (Evenhuis *et al.*, 1995), an increase of the leaf wetness periods due to lodging coincided with an increased disease severity. Lodging combined with heavy rainfall led to high seed infection levels in spring caraway at Lelystad (PAGV3069) and Nieuw Beerta (EH690), in 1993 (table 4). Seed infection levels appeared to increase when the crop had lodged and/or when precipitation during or after flowering was high. Trash in seed lots was sometimes infected by *M. acerina*, but infected trash does not

seem to increase the level of the inoculum in the seed lot substantially (Evenhuis & Verdam, 1995).

Seed transmission of M. acerina

In pansy, *M. acerina* grew from 7 (2.3%) of the 300 seeds tested for the presence of the fungus (Gill, 1971). Infected seeds produced heavily infected seedlings. In another experiment only 0.8% of 520 seeds tested were infected (Rintelen & Klewitz, 1976). In contrast to Gill (1971), Rintelen and Klewitz (1976) concluded that infected seed could not be responsible for heavy outbursts of the disease in pansy, at least not in Germany. The results on seed transmission of *M. acerina* in caraway are in accordance with those on transmission of the fungus through pansy seed. Infected caraway seeds gave infected seedlings. Thus, long distance dissemination of *M. acerina* must be possible through caraway seed transmission. Farmers should be aware that sowing infected seed leads to infected caraway plants. These diseased plants might be the origin of an anthracnose epidemic.

Conclusions

The experiments demonstrate that *M. acerina* is seedborne on caraway, as suggested by Westerdijk and Van Luijk (1924). For at least three years, *M. acerina* remains viable on caraway seeds in storage. To overcome part of the problems in caraway due to anthracnose, and to establish a viable crop (sufficient plant density and root diameter), seeds should be free from *M. acerina*. Seed lots should be routinely tested for the presence of the fungus.

For rapid screening of seedborne *M. acerina* the freezing-blotter method is suggested as the most reliable and suitable method. Formal introduction of this technique makes it possible to set an upper limit to the percentage of caraway seed infected by *M. acerina* in certified seed lots.

When a seed test cannot be performed it is recommended to use seed with minimum exposure to *M. acerina*, e.g. seed from fields with an open stand and without lodging, and/or seeds from years with low rainfall during bolting, flowering and ripening.

Appendix 1 Transmission efficiency equations.

Schilder and Bergstrom (1995) introduced the term transmission efficiency, but used it in a qualitative manner only. Here, we propose a quantitative approach. We define transmission efficiency (T) as the fraction of the diseased seeds yielding a diseased seedling. Once the transmission efficiency is established the farmer is able to calculate the number of diseased seedlings he might expect when he is sowing a seed lot with a known seed infection level (I) and a known germination rate (G).

The total amount of seeds can be divided into four fractions:

HG: The fraction of seeds which are healthy and germinate.

HN: The fraction of seeds which are healthy but do not germinate.

IG: The fraction of seeds which are infected and germinate.

IN: The fraction of seeds which are infected and do not germinate.

Thus HG + HN + IG + IN = 1.

To estimate the transmission efficiency we need to define the following parameters:

I: The fraction of the seeds infected by *M. acerina*, i.e. seed infection level.

G: The fraction of the seeds which germinate and yield a seedling, i.e. the germination rate.

D: The fraction of diseased seedlings compared to the total number of seedlings.

These parameters can be rewritten as:

$$I = IG + IN$$

$$G = HG + IG$$

$$D = IG * (IG + HG)^{-1}$$

From our definition of the transmission efficiency (T) it follows that

$$T = IG * (IG + IN)^{-1},$$

which can be rewritten as

$$T = G * D * I^{-1},$$

and

$$D = T * I * G^{-1}$$

When G, I and T are known D can be calculated. Usually the value for G is provided on the certificate attached to the seed lot. The value for I can be estimated by the freezing-blotter method. The value for T is established under greenhouse conditions. Thus, the farmer could be informed on the fraction of diseased seedlings to expect, or, in other words, the initial inoculum imported into his field by the seed.

CHAPTER 5

Effects of seed infection (*Mycocentrospora acerina*) on caraway (*Carum carvi*) crop establishment and yield

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Abstract

The effect of seed infection of caraway (*Carum carvi*) by *Mycocentrospora acerina* on crop establishment and yield was studied in two field experiments. High seed infection levels hampered crop establishment of caraway and limited the number of plants producing a root diameter large enough to permit flowering in the next year.

Introduction

In the Netherlands, anthracnose of caraway (*Carum carvi* L.), caused by *Mycocentrospora acerina* (Hart.) Deighton, limits caraway production. Westerdijk and Van Luijk (1924) suggested that seeds of caraway could be infected with *M. acerina*, a suggestion proven to be correct (Evenhuis & Verdam, submitted.).

Caraway is usually sown under a cover crop (peas, seed spinach, spring barley), where environmental conditions are suboptimal for establishment. Caraway plants grown under a cover crop may become vulnerable to pathogens due to etiolation of plants and high humidity. Seedlings are easily attacked by soil-borne *M. acerina*, causing damping off (Evenhuis & Verdam, 1995). Infection of seed by *M. acerina* might also cause damping off and reduce crop establishment. The effect of different seed infection levels on crop establishment and development of anthracnose in caraway crops was studied in field experiments.

Materials and methods

Treatments

Two field experiments were conducted to examine the effect of seed infection on disease development in biennial caraway. Caraway was sown (8 kg ha^{-1}) on a heavy clay soil at Lelystad at April 14th, 1993 and April 19th 1994. Spring barley, sown on the same day as caraway, was used as a cover crop. Row distances were 25 cm. In 1993 the *M. acerina* infection percentages of the seed lots used were 0, 1, 6, 13, 29 and 50%. These seed lots were achieved by mixing two seed lots, one with no detectable seed infection and one with a seed infection of 50% (WdG92-2). Seed infection levels were established using the freezing-blotter method (Evenhuis & Verdam, submitted.). In 1994 seed lots were sown with 0, 6, and 29% *M. acerina* infection. Both experiments had a randomized block design with four replicates (blocks). The plots measured 6 * 6 m, separated by a margin of bare soil of 3 m in 1993/94 and 1 m in 1994/95.

Plant density

Plant density was determined 1, 3, 4, 5, and 7 weeks after sowing by counting the number of seedlings in one meter row length at two randomly chosen positions in

every plot (0.5 m² per plot). To estimate emergence rate and total emergence, Gompertz curves (Campbell & Madden, 1990) were fitted to data points representing plant density plotted against time from sowing. Emergence rate is defined as the highest value of the dirivitive of the curve (i.e. parameter R). Total emergence is the plant density represented by the asymptote of the fitted Gompertz curve (i.e. parameter C). Plant density per plot was determined again in autumn. Final assessments were made after harvest by counting caraway stem bases in units of two adjacent rows of one meter at four positions per plot (2 m² per plot).

Root diameter

At the end of autumn, 10 - 20 caraway plants in the rosette stage were dug up per plot. The leaves were removed and the roots were washed. Root diameter was measured just under the head using a calliper gauge (Digit Cal SI, TESA SA, Rennes, Switzerland) with electronic display and output.

Leaf senescence

The percentage of senescent leaf area of caraway plants per plot was estimated in autumn, on 21 September and 18 October, 1993, and 3 November, 1994, respectively. Leaf senescence was estimated as the percentage of brown leaf area in comparison with the total leaf area.

Disease assessment

In the spring of the second year of each crop disease incidence and disease severity were assessed weekly from bolting (April) until harvest (July). Disease incidence was estimated as the percentage of caraway plants infected by *M. acerina* on 25 plants per plot. Disease severity was determined by estimating the percentage of the main stem surface covered with lesions caused by *M. acerina*, sampling 25 plants per plot. Detailed disease assessments were made about four weeks before harvest, sampling 25 plants at 5 randomly chosen positions per plot (125 plants per plot). Ripening of the crop made it increasingly difficult to assess disease incidence and disease severity, but observations continued as long as possible. The progress of *M. acerina* development in caraway was estimated by regressing disease incidence on time, using a Gompertz transformation. The area under the disease progress curve (AUDPC; Campbell &

Madden, 1990) was calculated from the beginning of May, shortly after bolting, until one week before harvest (13/7/94 and 20/7/95).

Yield

Caraway was harvested in July using a combine harvester (Wintersteiger, Nurserymaster Elite, Austria) designed especially for field experiments. Seeds were dried to a moisture content of approx. 12% and weighed. Seed weight per plot was expressed as seed yield in kg per hectare.

Statistics

Data were analyzed using Genstat 5.0, release 3.1 (Genstat 5 Committee, 1993). Analyses of variance (ANOVA) were applied to data from field observations. Least significant differences (LSD) at the P = 0.05 level were calculated from the standard errors of difference generated in the analysis. Gompertz curves (equation 1) were calculated to describe the change of plant density (crop establishment) and disease development with time.

$$Y = A + C * EXP (EXP (-B * (X-M))) \quad (1)$$

Y: dependent variable, i.e. plant density (m^{-2}) or disease incidence (%).

X: time (weeks).

A: estimate of Y at X = 0; i.e. 0.

C: estimate of the asymptote, with A = 0.

B: shape parameter of the curve.

M: estimate of X at which the value of $Y = e^{-1} * C$, with A = 0 and e = 2.72, the Euler constant.

For plant density and disease assessments, parameter A was set to zero, while the variables (Y) were supposed to be zero at the start of the experiment (X=0). The

highest value of the dirivitive of the curve, representing the emergence rate (R), was calculated by equation 2.

$$R = C * B / e \quad (2)$$

The AUDPC was calculated using the AREA directive provided by Genstat. Linear regression was conducted on AUDPC, with seed infection levels.

Table 1. Plant densities of caraway established after sowing at Lelystad in spring 1993 and 1994, respectively, were fitted to Gompertz curves (Y = A + C * EXP (EXP (-B*(X-M))); equation 1). Crop establishment was assessed in plots sown with seed infection levels of 0, 1, 6, 13, 29 and 50% (Y_0 - Y_{50}). Parameter A was set to zero. The parameter C differed significantly between infection levels. The LSD-value was 31.

1993	$Y_0 = 116 * EXP(-EXP(-0.90 * (X-2.83)))$
	$Y_1 = 112 * EXP(-EXP(-7.00 * (X-2.80)))$
	$Y_6 = 115 * EXP(-EXP(-1.25 * (X-2.57)))$
	$Y_{13} = 108 * EXP(-EXP(-1.45 * (X-2.88)))$
	$Y_{29} = 101 * EXP(-EXP(-2.64 * (X-2.82)))$
	$Y_{50} = 80 * EXP(-EXP(-1.29 * (X-2.47)))$
1994	$Y_0 = 106 * EXP(-EXP(-1.53 * (X-3.04)))$
	$Y_6 = 100 * EXP(-EXP(-1.67 * (X-2.91)))$
	$Y_{29} = 96 * EXP(-EXP(-1.62 * (X-2.92)))$

Relative impacts were calculated to compare the effects of seed infection levels (p) on crop establishment, disease intensity and yield. Equation 3 was used to calculate the relative impact of seed infection on total emergence, plant density at autumn and harvest, and yield, since these are influenced negatively by seed infection. Equation 4 was used to calculate the relative impact of seed infection on disease parameters, since disease intensity increases with seed infection levels.

$$\text{Relative impact}_1 = (X_{\max obs} - X_p) / X_{\max obs} \quad (3)$$

where $X_{\max obs}$ is the highest value found for the various parameters and X_p is the value found at seed infection level p.

$$\text{Relative impact}_2 = 1 - ((X_{\max exp} - (X_p - X_{\min obs}))) / X_{\max exp}) \quad (4)$$

where $X_{\max exp}$ is the highest expected value (100, except for AUDPC 10 x 100), $X_{\min obs}$ is the lowest observed value and X_p is the value found at seed infection level p.

Table 2. The effect of seed infection (1993) by *M. acerina* on leaf senescence, plant density and root diameter in 1993, and on disease parameters, plant density anthracnose and yield of biennial caraway in 1994.

Seed infection	Total emergence (C)	Leaf senes-cence	Plant density	Root diameter	Disease incidence	Disease severity	AUDPC	Plant[a] density	Seed yield
	spring	autumn	autumn	autumn	spring	spring	spring	harvest	harvest
	1993	1993	1993	1993	1994	1994	1994	1994	1994
(%)	(m^{-2})	(%)	(m^{-2})	(mm)	(%)	(%)	(-)	(m^{-2})	(kg/ha)
0	116	3	145	9.7	89	3.5	294	105	1778
1	112	10	142	9.0	85	2.5	316	107	1805
6	115	13	130	10.0	84	2.6	298	98	1762
13	108	29	132	9.4	87	3.2	309	88	1781
29	101	30	115	8.5	90	4.6	364	80	1702
50	80	38	88	9.4	94	5.0	392	74	1458
LSD (.05)	31	13	24	1.4	7	1.4	32	13	84

a: Plant density of generative plants were assessed only.

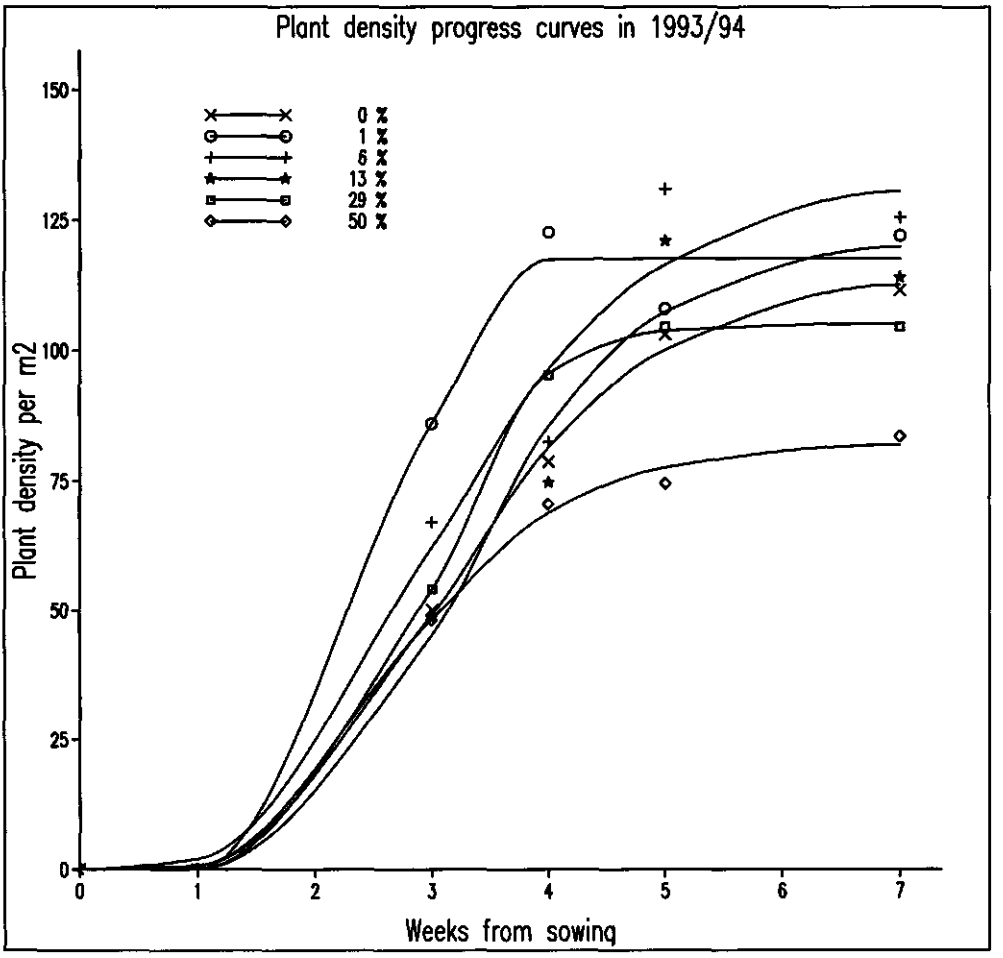

Figure 1 Plant densities of caraway established after sowing at Lelystad in the season 1993/94. Seed lots were sown with seed infection levels of 0, 1, 6, 13, 29 and 50%. The Gompertz fits are given in table 1.

Results

Crop establishment

Total emergence of seedlings decreased significantly with increasing seed infection levels (figure 1; tables 1 & 2). In 1993, plant density in autumn was higher than plant density assessed after seven weeks, possibly because of a second wave of emergence. In the 1994/95 crop, plant density in autumn was lower than plant density seven weeks after sowing. Emergence rate (R; equation 2) was not affected by seed infection levels. In the 1993/94 crop, no effect on root diameter was found. In the 1994/95 crop, in contrast, root diameter tended to decrease with increasing seed infection levels. Both effects led to a decrease in the number of roots with a diameter greater than 6 mm in autumn, needed for bolting in the next spring (Bernelot Moens *et al.*, 1973), with increasing seed infection levels (tables 2 and 3). Subsequently, plant density at harvest, implicitly also plant density at flowering, were significantly lower at higher seed infection levels in both years.

Table 3. The effect of seed infection (1994) by *M. acerina* on leaf senescence, plant density, and root diameter in 1994, and disease parameters, plant density and yield of biennial caraway in 1995.

Seed infection	Total emergence (C)	Leaf senes-cence	Plant density	Root diameter	Disease incidence	Disease severity	AUDPC	Plant[a] density	Seed yield
	spring	autumn	autumn	autumn	spring	spring	spring	harvest	harvest
	1994	1994	1994	1994	1995	1995	1995	1995	1995
(%)	(m^{-2})	(%)	(m^{-2})	(mm)	(%)	(%)	(-)	(m^{-2})	(kg/ha)
0	107	8	95	9.4	46	0.8	165	83	1980
6	101	68	97	8.0	42	0.7	164	77	1870
29	96	83	83	8.5	42	0.7	187	68	1830
LSD (.05)	10	8	37	1.4	7	0.2	11	13	92

a: Plant density of generative plants were assessed only.

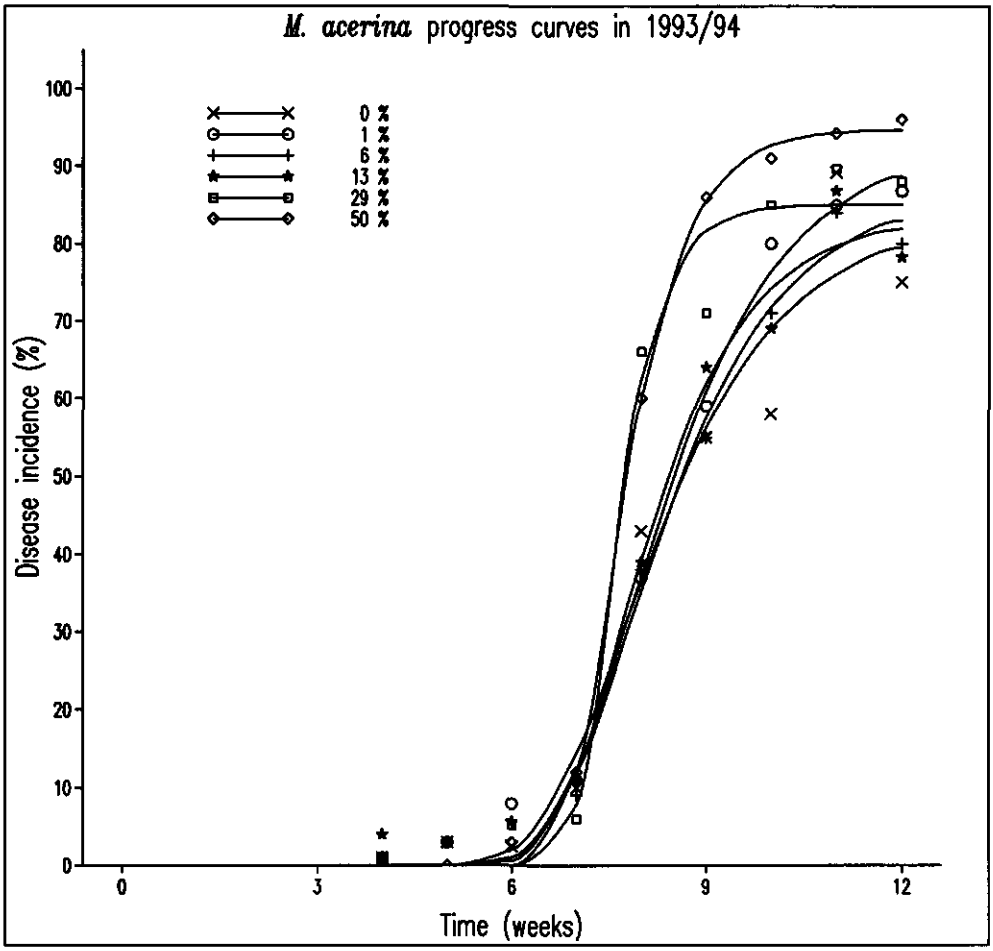

Figure 2 Disease incidence progress curves for *M. acerina* in caraway at Lelystad experiment 1993/94, spring 1994. At X = 0 bolting just started. Seed lots were sown with seed infection levels of 0, 1, 6, 13, 29 and 50%. The Gompertz fits are given in table 4.

Disease development

Higher infection levels of the seed lot resulted in higher percentages of senescent leaves in autumn. In the first experiment (table 2), higher infection levels of the seed resulted in higher disease severity in the following spring, whereas in the second experiment no effect on disease severity was found (table 3). The increase of the disease incidence from bolting until harvest was described by Gompertz curves (figure

2; table 4). In both experiments, the AUDPC increased significantly when seed with higher seed infection levels (29 and 50%) was sown (tables 2 & 3). Linear regression of the AUDPC with seed infection levels (p) for experiment 1 (n=24; $P < 0.01$; $r^2=0.65$) and 2 (n=12, $P = 0.082$; $r^2=0.20$) are described in equations 5 and 6, respectively, with standard errors given between brackets:

$$AUDPC_{1993/94} = 297\ (7.2) + 1.9\ (0.3) * p \quad (5)$$

$$AUDPC_{1994/95} = 164\ (6.9) + 0.8\ (0.4) * p \quad (6)$$

Figure 3 shows the relative impact of seed infection on agronomical and disease parameters in experiment 1. In experiment 2, similar effects were found though less pronounced because insufficient data were available. Effects of seed infection on root diameter were not rendered because effects were too variable.

Table 4. Disease incidence of caraway in spring 1994 and 1995, respectively, were fitted to Gompertz curves (Y = A + C * EXP (EXP (-B*(X-M))); equation 1). Disease incidence was assessed in plots sown with seed infection levels of 0, 1, 6, 13, 29 and 50% (Y_0 - Y_{50}). Parameter A was set to zero. Least significant differences for the parameter B and M were 0.69 and 0.35, respectively.

1994	$Y_0 = 83 * EXP(-EXP(-0.75 * (X-7.7)))$
	$Y_1 = 92 * EXP(-EXP(-0.79 * (X-7.9)))$
	$Y_6 = 86 * EXP(-EXP(-0.80 * (X-7.9)))$
	$Y_{13} = 83 * EXP(-EXP(-0.94 * (X-7.7)))$
	$Y_{29} = 85 * EXP(-EXP(-2.04 * (X-7.4)))$
	$Y_{50} = 95 * EXP(-EXP(-1.50 * (X-7.5)))$
1995	$Y_0 = 66 * EXP(-EXP(-0.66 * (X-10.0)))$
	$Y_6 = 65 * EXP(-EXP(-0.48 * (X- 9.8)))$
	$Y_{29} = 71 * EXP(-EXP(-0.40 * (X- 9.6)))$

Discussion

Effect of seed infection level on crop establishment

In carrot (*Daucus carota* L.), soil-borne *M. acerina* delayed emergence and caused damping-off and death of emerged carrot seedlings, substantially reducing the population density (Wall & Lewis, 1980a). Similar effects were found in caraway. High seed infection levels reduced the total emergence of caraway plants in both years (tables 2 & 3; figure 1). In 1993/94 plant density increased from seven weeks after sowing until harvest of the cover crop, whereas a small decrease was observed in 1994/95. In the first experiment some seeds may have germinated late and emerged later than seven weeks after sowing. In the second experiment some plants were lost, possibly due to low light intensity for the caraway seedlings under the cover crop. High seed infection levels reduced total emergence and subsequent density of the flowering plants and seed yield (tables 2 & 3). Weglarz (1983) showed a positive correlation between root diameter of caraway on the one hand and percentage of flowering plants and seed yield on the other hand. For bolting and flowering of biennial caraway the root diameter of the plants in the autumn of the first year, must be at least 6 mm (Bernelot Moens *et al.*, 1973). The growth of the roots depends on plant density (Hornok & Csaki, 1982), and on the length of the period between harvest of the cover crop in the summer and the moment of leaf senescence in the autumn.

In both experiments, at higher seed infection levels the percentage of senescent leaves was higher at any given time, and leaf senescence occurred earlier in autumn, thus restricting the growth of the caraway roots. A similar effect was found in carrot where plants surviving an early *M. acerina* attack usually senesced earlier than healthy plants (Wall & Lewis, 1980a).

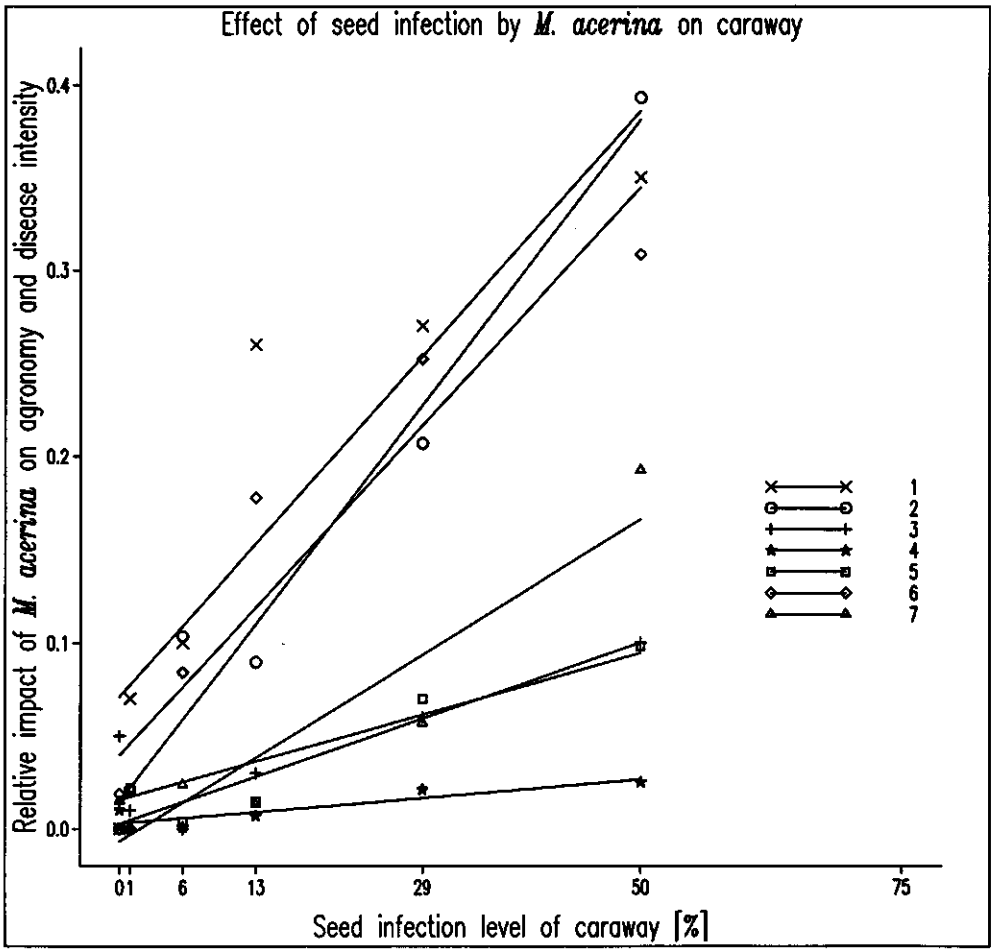

Figure 3 The relative effect of seed infection on leaf senescence (1) plant density in autumn (2), disease incidence (3), disease severity (4), AUDPC (5), plant density at harvest (6) and yield (7), in experiment 1.

In 1993/94, circumstances for germination and emergence apparently were not favourable and plant density in the autumn at higher seed infection levels was significantly reduced (table 2). As a consequence the remaining plants grew relatively thick roots, even at higher seed infection levels (table 2). In 1994/95, plant densities in autumn did not differ significantly between treatments (table 3). Higher leaf senescence levels at higher seed infection rates limited root growth and led to thinner

roots (table 3). In both seasons, 1993/94 and 1994/95, crop establishment was hampered at higher seed infection levels, either by fewer plants or by thinner roots.

Role of infected seeds in an epidemic

In the field experiment of 1993/94, disease severity levels were remarkably high, even when seed was used with no apparent seed infection (table 2). A soil-borne inoculum source might have been present in the soil which infected caraway seedlings. Spread of the disease between plots was unlikely because of the isolation of the plots, and the limited dispersal of *M. acerina* conidia (Evenhuis *et al.*, 1997). Disease severity levels, assessed at the end of spring 1994, were higher when a seed lot with 50% infection was sown than in plots sown with healthy seeds (table 2). In the 1994/95 experiment no significant effect was found on disease severity assessed in spring 1995 (table 3).

Weather conditions in the springs of 1994 and 1995 were not favourable for disease development. The crops did not lodge, so that the microclimatic conditions within the crops were not conducive to disease development.

To elucidate effects of seed infection level on anthracnose development over time, Gompertz curves were fitted to disease incidence from the onset of bolting until harvest. Differences between treatment (% seed infection) come out clearest by taking the integral of disease incidence over time, the AUDPC, (Campbell & Madden, 1990). The effect of seed infection levels on AUDPC was significant in both years, indicating that high seed infection levels promote anthracnose in caraway. Given suitable conditions for *M. acerina*, the effect of seed-borne inoculum might become far more pronounced and the loss might well exceed the 20% level of the 1993/94 experiment.

Theoretically a seed infection level of 1% could lead to 1.4 diseased seedlings per m^2. Assumed is a sowing rate of 10 kg ha^{-1}, a mean kernel weight of 3.0 mg, an emergence of 90% and a transmission efficiency of 43%, as found on average in the greenhouse (Evenhuis & Verdam, submitted.). These infected seedlings might act as inoculum sources, especially for neighbouring seedlings, and initiate an epidemic. When the soil is already contaminated by *M. acerina* low levels of seed-transmitted inoculum would probably not add much to the soil-borne inoculum. The importance of seed-borne inoculum does not depend on infection levels and transmissibility alone, but also on other means of transmission and economic loss (Neergaard, 1977).

Yield loss

High seed infection levels potentially initiate an anthracnose epidemic in caraway. The followthrough depends largely on the weather during flowering of the crop. Yield loss due to a decreased number of generative plants was found to be up to 20%. Accordingly, these investigations indicate that high seed infection levels makes it more difficult to establish a caraway crop with sufficient plants to flower the next year. Since crop establishment is already a bottleneck in the growing of caraway under a cover crop, injury by *M. acerina* should be avoided by sowing healthy seed. In the greenhouse and under adverse conditions in the field, seed dressing of caraway seeds improved total emergence and crop establishment (Evenhuis & Verdam, 1995).

Relative impact

The relative impact of seed infection on agronomical and disease parameters is shown in figure 3. Leaf senescence and plant density in autumn (lines 1 & 2) respond to seed infection indicating that seed infection has an impact on the first year's events. Yield (line 7), disease incidence and severity (lines 3 & 4), and AUDPC (line 5) observed in the second year respond far less to seed infection. The relatively small effect of seed infection on the second year's events may be caused by the effect on plant density in the first year, which levels off some of the effects of seed infection.

Aspects of seed infection which require further attention

Is seedling infection from infected seed systemic? Is only the pericarp infected or other parts of the seed too? Both points have implications for the feasibility of seed dressing to eliminate *M. acerina* from the seed. What factors influence seed infection by *M. acerina*? Can we minimize the use of infected seeds to be sown? What is the importance of seed infection in the dispersal of the fungus from one field to another in relation to other inoculum sources? Answers to these questions are relevant to disease management.

Conclusion

Seed infection hampers caraway crop establishment and promotes anthracnose development in caraway. Seeds should be free from *M. acerina*. Seed lots should be routinely tested for the presence of the fungus.

CHAPTER 6

Effects of cover crops, weeds and plant debris on development of *Mycocentrospora acerina* in caraway

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Abstract

This paper reports on the search for inoculum sources of *Mycocentrospora acerina* on caraway (*Carum carvi* L.). Obvious suspects are cover crops of biennial caraway and preceding crops of annual caraway. Other suspects are weeds in or alongside the field. Finally, survival structures of the fungus, chlamydospore chains, packed in plant debris or naked, are suspected. *M. acerina* is able to infect many plant species, including cover crops of caraway such as spinach for seed production and peas. However, the agronomical suitability of a crop to serve as a cover crop of biennial caraway proved to be a more important factor in determining caraway yield than the susceptibility of the cover crop to *M. acerina*. This finding was corroborated by the fact that spinach and peas as preceding crops had no significant effects on *M. acerina* development in spring caraway sown the next year. Dill, barley and four weed species were found as new hosts of *M. acerina*. The role of weed hosts, susceptible crops and plant debris in the survival of the fungus in years without caraway is discussed. Caraway sown on soil containing infested caraway straw, infested debris of other plant species or chlamydospores grown in pure culture, became infected by *M. acerina*. Only high inoculum densities of chlamydospores in the soil caused severe damping-off of caraway seedlings. The opportunity for disease management by agronomical means is quite limited.

Introduction

Caraway (*Carum carvi* L.) is an arable crop grown for seed and essential oil. Because of new possibilities for application of the carvone component of the essential oil, the interest in growing caraway is increasing. Dill (*Anethum graveolens* L.), also containing carvone, might become an alternative source of carvone because the seed yield is higher than that of biennial caraway. Dill is not yet grown for seed production in the Netherlands (Van der Mheen *et al.*, 1994). Both species belong to the *Umbelliferae*. Anthracnose of caraway is caused by the fungus *Mycocentrospora acerina* (Hartig) Deighton (Westerdijk & Van Luijk, 1924). Infection of stems and umbels can lead to considerable yield losses (Evenhuis & Verdam, 1995). The sources of inoculum have not yet been identified beyond doubt. Possible sources are previous caraway crops, non-caraway crops, weeds, plant debris and chlamydospores.

The host range of *M. acerina* consists of at least 90 plant species, belonging to several dicotyledonous plant families (Tompkins & Hansen, 1950; Rintelen & Klewitz, 1976; Hermansen, 1992b). Among these host plants are frequently grown crops and common weeds. The fungus causes liquorice rot of carrot (*Daucus carota* L.; Rader, 1945) and black crown rot of celery (*Apium graveolens* L.; Newhall, 1944; Day *et al.*, 1972). Common cover crops of biennial caraway such as spinach and peas (Bernelot Moens, 1968) were reported infectible by *M. acerina* (Tompkins & Hansen, 1950). These alternative hosts may play a role in the survival of the fungus in the field when no caraway is grown. An abundance of chlamydospores of *M. acerina* is produced in senescent celery and carrot tissue in autumn (Day *et al.*, 1972; Wall & Lewis, 1978b). Also, a previous caraway crop or another susceptible host might have been infested with mycelium and chlamydospores of *M. acerina*. Infested plant debris ploughed into the soil might add to the inoculum potential of *M. acerina* in the soil. After decay of the plants, the chlamydospores remain in the soil, where they are viable for at least two years (Wall & Lewis, 1980c). An epidemic of anthracnose in a caraway crop might be initiated by infested debris or by surviving chlamydospores in the soil.

This study investigated whether other crops influenced the disease intensity in the following caraway crop. Non-caraway crops were tested in two treatments, as cover crops (experiments 1 & 2) and as preceding crops (experiments 3 & 4). Other plant species growing in and around the field were examined for the presence of *M. acerina*

(experiment 5). The effect of infested caraway stems brought into the soil under field (experiment 6) or greenhouse conditions (experiments 7 & 8) on the onset of anthracnose in caraway was studied. The effect of plant debris of some major cover crops on *M. acerina* infection of caraway was studied under greenhouse conditions (experiments 7 & 8).

Materials and methods

The effect of cover crops and preceding crops on anthracnose development in caraway.

Cover crop experiments

The experiments 1 and 2 (table 1), tried to asses the effect of a cover crop on anthracnose development in biennial caraway. These experiments took two seasons. Treatments (tables 2 & 3) were seed spinach (20 kg ha^{-1}), peas (190 kg ha^{-1}), spring barley (110 kg ha^{-1}) and a control (no-cover-crop). Biennial caraway was sown together with the cover crops. The experimental design was a randomized block design, consisting of three blocks with treatments randomized within blocks.

Preceding crop experiments

Experiments 3 and 4 (table 1), tried to assess the effect of a preceding crop on anthracnose development in a following crop. For practical purposes, we used spring caraway. These experiments again, took two seasons. Treatments (table 4) and experimental design were as in experiments 1 and 2. In this case the control was fallow.

Inoculation

Chlamydospores were obtained by growing *M. acerina* colonies for three weeks on sucrose agar (6 g sucrose, 15 g agar and 200 mg streptomycin per litre medium, after Day *et al.*, 1972) at 12°C. Inoculum was prepared by blending agar discs with *M. acerina* colonies in tap water. The suspension was sieved through double cheese cloth. The inoculum density was adjusted to 10^4 chlamydospore chains per ml. All plots were inoculated in June, 1992 (experiments 1 & 3) and 1993 (experiments 2 & 4), about two months after sowing. In the centre of each plot an area source was established by

spraying 1.2 l inoculum onto the crop with a knapsack sprayer. The inoculated area was 1 m^2 in 1992 (experiments 1 & 3) and 4 m^2 in 1993 (experiments 2 & 4). Inoculation took place during rainy weather.

Table 1. General information on field experiments. Sowing dates of biennial caraway are a year before harvest.

Experiment	1	2	3	4	6
Harvest year	1993	1994	1993	1994	1994
Location[a]	Ld	Ld	Ld	Ld	Ln
Soil type	clay	clay	clay	clay	clay
Caraway crop[b]	B	B	S	S	B
Cover crop	t[c]	t[c]	-[d]	-	SB[e]
Sowing date cover crops	14/4/92	14/4/93	-	-	15/4/93
Sowing date preceding crops	-	-	14/4/92	14/4/93	-
Sowing date caraway	14/4/92	14/4/93	5/4/93	3/5/94	15/4/93
Sowing rate caraway (kg ha^{-1})	7.5	7.5	4	4	8
Row spacing (cm)	25	25	25	25	25
Inoculation date	3/6/92	16/6/93	3/6/92	16/6/93	15/4/93
Disease assessment	28/6/93	27/6/94	18/8/93	13/9/94	29/6/94
Harvest date caraway	13/7/93	13/7/94	-	-	20/7/94
Plot size (m)	3 x 6	6 x 6	3 x 6	6 x 6	6 x 6
Area source (m)	1 x 1	2 x 2	1 x 1	2 x 2	-

a: Field experiments were located at Lelystad (Ld) and Lienden (Ln).

b: S and B are spring caraway and biennial caraway, respectively.

c: See treatments (tables 2, 3, 4 and 6).

d: - not relevant.

e: SB spring barley.

Leaf senescence

Leaf senescence of biennial caraway was estimated per plot as the percentage of yellow and brown leaf area relative to the total leaf area, on 17 September, 1992 (experiment 1), and 7 October, 1993 (experiment 2), respectively.

Lodging

Lodging of biennial caraway was assessed on 7 June, 1993 (experiment 1), shortly after flowering, and on 24 May, 1994 (experiment 2), during flowering. The percentage of lodged crop area was estimated per plot. Plants were considered to be lodged when the stem's angle of inclination deviated more than 45 degrees from the upright position.

Disease assessments

Plants of the cover crops were sampled during the season (table 4). Samples from leaves with lesions and senescent leaves were placed on moist filter paper in Petri dishes in closed plastic bags, and incubated for two days at 18°C (filter paper method). The leaf samples were checked for sporulation of *M. acerina* under a light microscope. Spores of *M. acerina* are easily recognizable because of their size (100-200 mm x 10-30 mm; Neergaard, 1952) and typical shape. Disease assessments on caraway were made about three weeks before harvest (table 1). Disease incidence of caraway (experiments 1 to 4 and 6) was estimated as the percentage of infected plants of 100 plants, 25 plants from each of four randomly chosen locations per plot. The percentage of the main stem surface colonized by *M. acerina* of 25 plants per location, at four locations per plot, was estimated to establish disease severity. Disease incidence and severity were calculated as the means of the four locations per plot.

Plant density

Plant density was assessed as the mean number of stems per meter drill length, averaged over two randomly chosen locations per plot. Intermediate assessments were made in biennial caraway in November 1992 (experiment 1) and March 1994 (experiment 2), respectively. Final assessments were made shortly after the harvest of biennial caraway in July 1992 and 1993 (experiments 1 & 2, respectively) and of cutting the spring caraway in September 1992 and 1993 (experiment 3 & 4,

respectively), averaged over four locations per plot (2 m^2 per plot). Plant density was expressed in numbers of plants per m^2.

Yield

Biennial caraway was combine harvested in July 1993 and 1994 (experiments1 & 2). Seeds were dried to a moisture content of 12% and weighed. Yields were expressed in kg per hectare. Spring caraway was cut and no yield was determined because seedset was poor.

Host plants

Plant species collected from the field

In experiment 5A, plants of different weed species were collected in caraway fields over the years 1991-1994 (table 5). These fields were situated in the Province of Groningen (heavy marine clay), in the vicinity of Lelystad (light marine clay) and of Wageningen (sand). Plants were also collected from field verges e.g. *Anthriscus sylvestris* (L.) Hoffm. At Lelystad, wild caraway (*Carum carvi* L.) was sown as a crop for breeding purposes by DLO-Centre for Plant Breeding and Research. Collected plants were checked for infection by *M. acerina* using the filter paper method. Parts of plants showing symptoms, especially senescent leaves, were placed on moist filter paper in Petri dishes. In the wild caraway plots, disease incidence was determined as described above.

Plant species tested in the greenhouse

In experiment 5B, sets of various plant species, representing weeds and arable crops, were tested for their susceptibility to *M. acerina* in the greenhouse (table 5). Clay and potting soil were pasteurized twice for 24 hours at 70°C, with one day in between. Plastic containers measuring 45 x 30 x 7 cm were filled, each with 4 kg clay mixed with 1 kg of potting soil. Seeds of various plant species including caraway were sown. The number of seeds varied between 10 and 35 per container. Caraway was included in each set. Plastic covers maintained a high relative humidity in the containers. The temperature varied around 16°C at night and 20°C during daytime. On sunny days the

temperature occasionally reached 25°C, as no cooling device was available. The containers were randomly distributed in the greenhouse.

Inoculation

In experiment 5B, the plants (table 5) were inoculated two weeks after emergence, with either chlamydospore chains or conidia. Chlamydospore suspensions were prepared as described above. Conidia were washed from the agar discs. The inoculum density, estimated with a haemocytometer, was adjusted to 10^4 conidia or chlamydospore chains per ml inoculum. Suspensions of either chlamydospore chains or conidia, were sprayed on the seedlings until drip off.

Disease assessment

During the experiments (5B), which usually took eight weeks, the plants were frequently inspected for symptoms. Whenever lesions were found they were examined for the presence of conidia of *M. acerina* by means of the filter paper method. To test the pathogenicity of the *M. acerina* isolates to caraway, disease assessments were made after reisolation and inoculation of fresh caraway seedlings (Koch's postulates).

The effect of plant debris and chlamydospores on anthracnose development

Treatments

In experiment 6 (table 1), the effect of chlamydospores and infested caraway straw brought into the soil on disease intensity of caraway was studied at Lienden. Infested caraway straw was obtained from a field heavily infected with *M. acerina*, disease incidence being close to 100% and disease severity well over 25%. Straw was cut to 10 cm pieces and this inoculum was loosely mixed through the top soil layer (10 cm; table 6). Chlamydospore suspensions were sprayed over the soil surface before sowing. Chlamydospore density per ml in the top soil layer (2.5 cm) is specified in table 6. Biennial caraway was sown (8 kg ha^{-1}) together with spring barley (110 kg ha^{-1}) as a cover crop. The two crops were sown in alternating rows, 12.5 cm apart, so that single-crop row distances were 25 cm.

Plant density was assessed as above, immediately after harvest in July. Disease incidence and disease severity were assessed in June, three weeks before harvest, as above. Caraway was combine harvested.

The experimental design was a randomized block design, consisting of three blocks with treatments randomized within blocks.

The effect of infested plant debris on anthracnose of seedlings under greenhouse conditions

Treatments

In experiments 7 and 8, plant debris and chlamydospores were added to the soil, and an uninoculated control was included. The experiments (7 & 8) began in November of 1992 and 1993, respectively. In both experiments 21 containers measuring 45 x 30 x 7 cm were filled each with 4 kg of pasteurized clay mixed with 1 kg of potting soil. Debris of caraway infested with *M. acerina* was collected at Lelystad and Wageningen in 1992 and in 1993, respectively. Infested plant debris of spinach (*Spinacia oleracea* L.), spring barley (*Hordeum vulgare* L.) and peas (*Pisum sativum* L.) were also collected. Plant debris was mashed in a blender and approximately 50 g dry weight was mixed through the soil of each container, an amount roughly equivalent to the amount of debris ploughed into the soil in standard agricultural practice. Soil was inoculated with *M. acerina* to a density of 100 and 1000 chlamydospore chains per ml soil, as described above. The inoculum was thoroughly mixed through the soil. The experimental design was a randomized block design, consisting of three blocks with treatments randomized within blocks.

Incubation

Seeds of spring caraway were sown to a depth of 1 cm. In each container, 96 seeds were sown. Distances of seeds between and within rows were 4 cm. Plastic covers were placed on the containers to maintain a high relative humidity (>90%). The covers were removed during disease assessment only. The temperature in the greenhouse was about 15°C at night and 20°C during daytime. Light was supplemented for 14 hours per day, by lamps (Son T+; Philips; 100 W/m^2) hanging approximately 1.80 m above the seedlings.

Seedling emergence and dry weight

During the first three weeks the numbers of emerged seedlings were counted weekly. Seedling emergence (%) was assessed by dividing the number of emerged plants by the number of seeds sown, and multiplying the quotient by 100. At the end of the experiment the caraway plants were carefully dug up and washed. The dry weight per container of surviving plants (shoots & roots) was determined after drying for 48 hours in an oven at 70°C.

Disease assessments

Damping-off and leaf infection were assessed weekly. The filter paper method was used to confirm infection of seedlings and leaves by *M. acerina*. The number of infested plants was assessed as the total number of plants per container showing damping-off and/or leaf infection. Disease incidence was calculated by dividing the number of infected plants by the number of emerged seedlings.

Statistics

Data were analysed using Genstat 5.0, release 3.1 (Genstat 5 Committee, 1993). Analyses of variance (ANOVA) was applied to data from field observations. Arithmetic means are presented with Least Significant Differences (LSD) calculated with Student's t-distribution at the 95 % confidence level. To stabilize variance, percentages were analysed after angular transformation (Mead & Curnow, 1983). Angular means and corresponding LSD's on transformed scale are presented between brackets. Disease severity was always in the range 0-30%. In that case a square root transformation can be used to stabilize variance (Gomez & Gomez, 1984). However, we used a logarithmic transformation because it gave a better stabilization of variance. Logarithmic means and corresponding LSD's are also presented between brackets. In experiment 6, at Lienden, mean disease severity and mean plant density per treatment were calculated. Linear regression was conducted to examine the effect of mean plant density on mean disease severity.

Results

The effect of the cover crop (experiments 1 & 2)

Biennial caraway

M. acerina was frequently found on decaying leaves throughout the season. Caraway without cover crop produced more leaves and had a higher percentage of senescent leaves in autumn than caraway grown under cover (tables 2 & 3), thus providing more opportunity for *M. acerina* development.

Table 2. Experiment 1. Effect of cover crops on leaf senescence, lodging, anthracnose (*M. acerina*), plant density and yield of biennial caraway at Lelystad in 1992/93. Angular or logarithmic means and corresponding LSD's on transformed scale are given between brackets (df=6).

Cover crop	Leaf senescence (%)	Lodging[a] (%)	Disease incidence (%)	Disease severity (%)	Plant density[b] autumn (m^{-2})	Plant density[b] harvest (m^{-2})	Seed yield (kg/ha)
None	87 (69)	67 (55)	76 (61)	9.0 (2.06)	83	90	1820
Peas	40 (39)	47 (43)	75 (62)	8.8 (1.82)	75	77	2140
Spinach	60 (51)	20 (22)	58 (50)	4.1 (0.96)	108	97	2134
Spring barley	33 (35)	0 (-)	28 (31)	1.3 (-0.48)	53	60	1456
LSD (0.05)	(10)	(35)	(4)	(0.88)	30	18	400

a: Residual degrees of freedom was equal to 4 for lodging, only.

b: In autumn total plant densities were assessed, at harvest plant density of reproductive plants were assessed only.

Table 3. Experiment 2. Effect of cover crops on leaf senescence, lodging, anthracnose (*M. acerina*), plant density and yield of biennial caraway at Lelystad in 1993/94. Angular or logarithmic means and corresponding LSD's on transformed scale are given between brackets (df=6).

Cover crop	Leaf senescence (%)	Lodging (%)	Disease incidence (%)	Disease severity (%)	Plant density spring (m^{-2})	Plant density harvest (m^{-2})	Seed yield (kg/ha)
None	90 (72)	10 (18)	95 (76)	3.8 (1.30)	119	98	1812
Peas	20 (22)	10 (18)	88 (70)	3.4 (1.21)	70	64	1882
Spinach	27 (26)	0	81 (64)	2.1 (0.75)	94	63	1704
Spring barley	37 (36)	0	67 (55)	1.2 (0.13)	82	46	919
LSD (0.05)	(40)	(0)	(6)	(0.49)	28	14	368

The cover crops had a distinctive effect on plant density of biennial caraway. Plant density under spinach was higher than under peas. Barley gave the lowest plant density, thus being the poorest cover crop. Yield was influenced by both plant density and disease severity. Low plant densities resulted in little anthracnose but also in low yields. High plant densities resulted in high yields, despite the increased disease severity. The effect on yield is shown by linear regression analysis. Disease severity was positively correlated with lodging of the caraway crop. The correlation (n=24, r^2=0.83, P<0.001) between disease severity (S) and lodging (L), in experiments 1 and 2 together, is given in equation 1, with standard errors in parentheses. No significant year effects were found.

$$S = 2.04\ (0.33) + 0.11\ (0.01) \times L \qquad (1)$$

The effect of the preceding crop (experiments 3 & 4)

Spring caraway

As the spring caraway did not lodge, lodging did not interfere with disease development. No correlation (P = 0.169, experiment 3; P = 0.395, experiment 4) between the disease incidence of the preceding crop and the disease severity of spring caraway was found (table 4). With preceding fallow, disease severity was significantly lower than with spinach preceding spring caraway. Peas and spring barley before spring caraway resulted in intermediate levels of disease severity in spring caraway. In both experiments seed set was so poor that caraway was not harvested.

Host range

Caraway

In the greenhouse, biennial and spring caraway were readily infected by *M. acerina*, whether by spray-inoculating the plants (table 5) or by adding inoculum to the soil (table 7; Evenhuis & Verdam, 1995). Apparently the inoculum used was infectious and the inoculation methods effective.

New hosts

Several plant species, including weeds, were found to be susceptible to *M. acerina* (table 5). Creeping thistle (*Cirsium arvense* (L.) Scop.), greater plantain (*Plantago major* L.), curled dock (*Rumex crispus* L.) and long-stalked crane's-bill (*Geranium columbinum* L.) were not previously recorded as hosts of *M. acerina*.

Other hosts

The susceptibility of several other species (table 5) was shown by other authors. On most species the fungus was mainly present on decaying leaves. On pansy (*Viola arvensis* Murray), *M. acerina* was frequently found on green leaves.

Table 4 Experiments 3 and 4. The percentage of leaves infected by *M. acerina* in caraway and three preceding crops (peas, spring barley and spinach). Observations were made before (B) and after (A) the harvest of the preceding crop, the latter being made on debris. Entries were based on 13 to 55 inspected leaves per treatment. Effects of crops grown in rotation with spring caraway on anthracnose (*M. acerina*) and plant density of spring caraway at Lelystad are shown also. Angular or logarithmic means and corresponding LSD's on transformed scale are given between brackets (df=6).

Treatment	Preceding crops				Spring caraway					
	Disease incidence before (B) and after (A) harvest of the preceding crops (%)				Disease incidence (%)		Disease severity (%)		Plant density at harvest (m^{-2})	
	Exp 3 1992 B	Exp3 1992 A	Exp 4 1993 B	Exp 4 1993 A[c]	Exp 3 1993	Exp 4 1994	Exp 3 1993	Exp 4 1994	Exp 3 1993	Exp 4 1994
None	90[a]	85[a]	70[a]	62[a]	77 (62)	91 (72)	6.6 (1.85)	14.2 (2.60)	82	114
Peas	5	0	0	0	85 (69)	98 (82)	8.5 (2.09)	18.1 (2.88)	80	81
Spinach	64	0	13	10	96 (81)	93 (75)	12.0 (2.46)	20.4 (3.01)	104	105
Spring barley	53	0	0	0	87 (72)	89 (71)	7.7 (1.99)	17.1 (2.84)	96	93
LSD (.05)	-[b]	-	-	-	(14)	(6)	(0.50)	(0.60)	22	27

a: Where no preceding crop was grown, biennial caraway plants were sampled in an adjacent plot.

b: No statistical analysis conducted.

c: Data in this column are averages of assessments in November and December.

Infectibility not shown

Neither dill (*A. graveolens* L.; 25 field observations) nor wild chervil (*A. sylvestris*; 99) were found to be naturally infected under field conditions. Common chickweed (*Stellaria media* L.) and cleavers (*Galium aparine* L.) were not infected by *M. acerina* in our experiments, but were successfully inoculated by Viennot-Bourgin (1955) and Hermansen (1992b).

Wild caraway

In the filter paper method, on 20 % of the wild caraway (*Carum carvi* L.) samples *M. acerina* was found in May. The disease incidence of the wild-type caraway, sown as a crop, was 83% at the end of June 1994. Infection originated from natural inoculum.

Other crops

In experiment 3, *M. acerina* was found on spring barley (*Hordeum vulgare* L.), 8 days after inoculation, in the form of chlamydospore chains in decaying leaves. Though the filter paper method did not confirm the presence of *M. acerina*, inoculation of isolates from these chlamydospore chains on caraway confirmed the identity of *M. acerina*. Later, no *M. acerina* was found on barley (table 4). In experiment 4 no early infection was found on spring barley. In experiment 3, *M. acerina* was occasionally found on peas (*Pisum sativum* L.), shortly after inoculation, but not in experiment 4. The fungus was not found on plant debris of peas in autumn (table 4). Among the crops tested, spinach (*Spinacia oleracea* L.) was the most susceptible host to *M. acerina* infection. In experiments 3 and 4, the fungus was found frequently on spinach leaves during the growing period, and on plant debris after harvest (table 4). Leaves incubated on moist filter paper in Petri dishes showed heavily sporulating *M. acerina*.

Table 5 Numbers of plants infected by *M. acerina*. Number of plants checked for infection are given between parenthesis. Plant species were collected in inoculated and non-inoculated fields. Other plants were examined after inoculation in the greenhouse (experiment 5). Observations were made in the period from 1990 to 1994.

Plant species	Natural infection		Inoculation				
			Field		Greenhouse		
Anethum graveolens	0	(25)	-		9	(9)	
Anthriscus sylvestris	0	(99)	-		-		
Beta vulgaris	-		-		1	(10)	e
Capsella bursa-pastoris	-		1	(8)	-		d
Carum carvi (crop)	22	(99)	48	(99)	80	(99)	a*
Carum carvi (wild)	1	(5)	-		-		
Chenopodium album	0	(2)	-		-		
Cirsium arvense	-		8	(20)	-		
Daucus carota (wild)	-		-		2	(3)	b
Galium aparine	0	(12)	-		0	(32)	g
Geranium columbinum	-		1	(10)	-		
Hordeum vulgare	-		17	(135)	0	(10)	
Matricaria discoides	1	(10)	-		-		
Phaseolus vulgaris	-		-		1	(10)	c
Pisum sativum	-		5	(126)	1	(10)	c
Plantago major	-		1	(2)	-		
Ranunculus spp.	5	(7)	0	(15)	-		g
Rumex crispus	-		5	(10)	-		
Senecio vulgaris	-		-		13	(27)	g
Sinapis arvensis	-		0	(4)	-		
Spinacia oleracea	0	(14)	52	(164)	17	(25)	c
Stellaria media	-		0	(5)	0	(32)	d
Taraxacum officinale	0	(5)	3	(19)	-		c
Viola arvensis	15	(31)	-		-		f

-: No observations made.

*: Susceptibility previously shown by a: Westerdijk & Van Luijk, 1924; b: Rader, 1945 (in cultivated carrot); c: Tompkins & Hansen, 1950; d: Viennot-Bourgin, 1955; e: Channon, 1965; f: Rintelen & Klewitz, 1976; g: Hermansen, 1992b.

The effect of soil inoculation (experiment 6)

Soil inoculation

In experiment 6, under field conditions, plant density of biennial caraway in autumn significantly decreased when the soil was inoculated with chlamydospores of *M. acerina*. Straw amendments had no significant effect on plant density in autumn (table 6). Mean disease severity (S) per treatment increased significantly ($n = 7$; $r^2 = 0.73$; $P = 0.015$) with mean density of reproductive plants (P) per treatment, according to equation 2 (with standard errors between parentheses).

$$S = - 2.2\ (1.9) + 0.14\ (0.04) \times P \qquad (2)$$

Table 6 Experiment 6. The effect of soil inoculation on anthracnose, plant density and yield of biennial caraway in the field, at Lienden in 1994. Angular or logarithmic means and corresponding LSD's on transformed scale are given between brackets (df=6).

Treatment	Inoculum density	Disease incidence (%)		Disease severity (%)		Plant density (m^{-2})	Seed yield (kg/ha)
control	-	92	(74)	7.7	(1.93)	60	1167
straw	200[a]	92	(73)	6.7	(1.88)	57	1250
straw	400	90	(72)	5.8	(1.72)	71	1269
straw	800	96	(78)	4.6	(1.50)	55	1176
M. acerina	10[b]	87	(70)	3.6	(1.16)	38	1102
M. acerina	100	78	(62)	1.6	(0.41)	35	1176
M. acerina	1000	73	(59)	2.1	(0.67)	33	861
LSD (0.05)		9	(7)	3.5	(0.70)	28	288

a: Weight (g) of infested straw brought into the soil per m^2.

b: Chlamydospore density per ml in the top soil layer (2.5 cm).

The effect of soil inoculation under greenhouse conditions (experiments 7 & 8)

Results on seedling emergence, seedling infection and dry weight were averaged over two experiments (table 7), since no significant effects between experiments were found. Six days after sowing the first caraway seedlings emerged. No seedling infection was observed in the control treatment. In experiment 7 no seedlings treated with pea debris were infected. All other treatments resulted in seedling infection (table 7). Application of high chlamydospore density led to significantly lower seedling emergence and higher infection percentage than plant debris amendments.

In experiment 7, caraway seedlings in the pea treatment were attacked by an unidentified micro-organism. A great number of seedlings showed damping-off, but none of these plants was infected by *M. acerina*. The dry weight was therefore underestimated.

Table 7 Experiments 7 and 8. Effects of *M. acerina* on seedling emergence, seedling infection (including damping-off) and dry weight of the surviving caraway plants when seeds were sown in soil containing plant debris or chlamydospores as inoculum source of *M. acerina*. Angular means and corresponding LSD's on transformed scale are given between brackets.

Inoculum source	Seedling emergence (%)		Seedling infection (%)		Dry weight (g)
Control	80	(64)	0.0	( )	3.4
Spinach	78	(62)	0.7	(3.4)	4.3
Barley	79	(63)	0.7	(2.7)	5.1
Peas	78	(62)	0.2	(1.1)	3.2
Caraway	72	(58)	9.0	(16.8)	2.8
Chlamydospores[a]	70	(57)	61.8	(55.6)	0.7
Chlamydospores[b]	69	(58)	97.2	(83.5)	0.2
LSD (0.05)	10	(8)	15.6	(12.0)	1.4
Degrees of freedom	30		29		29

a: Inoculum density adjusted to 100 chlamydospores per gram soil.

b: Inoculum density adjusted to 1000 chlamydospores per gram soil.

Discussion

The effect of the cover crop and preceding crop on anthracnose

Cover crops and preceding crops compared

In the Netherlands, biennial caraway is usually sown under a cover crop. The effect of the agronomical suitability of the cover crop and the effect of the cover crop on disease development will inevitably influence each other. To try and avoid effects of cover crops on other parameters than disease development, such as shading, crop establishment and root growth, these cover crops were also sown as preceding crops. Effects of preceding crops on nutrient availability for following spring caraway may exist, but these effects will be less profound than the effects of cover crops. Thus, effects on *M. acerina* inoculum build-up and disease development in caraway are estimated better by the combination of a preceding crop followed by spring caraway than by the alternative combination of a cover crop with biennial caraway. Susceptibility to and disease expression by *M. acerina* of stems, umbels and seeds of spring caraway is much the same as in biennial caraway.

Cover crop (experiments 1 & 2)

The cover crops were inoculated and must have been infected by *M. acerina*, since the same crops sown as preceding crops were infected by *M. acerina* (table 4). Inoculation of cover crops and preceding crops took place on the same day, with the same inoculum, and the inoculated areas were only 9 m apart. On peas and barley inoculum build-up was negligible. Spinach was more susceptible, and might have added to the build-up of inoculum in the first year. Without a cover crop, biennial caraway forms many leaves, providing a dense and moist substratum, with considerable build-up of *M. acerina* inoculum. With a cover crop, caraway forms fewer leaves so that less substratum is available for *M. acerina* development. Apparently, a susceptible cover crop is less conducive to anthracnose development in biennial caraway than no cover crop, compare treatments spinach and 'none' in tables 2 and 3.

Possibly, an exception has to be made for the highly susceptible spring caraway as a cover crop (not tested here). *M. acerina* inoculum build-up on spring caraway will

probably be high, and undersown biennial caraway might be heavily attacked in the next year.

The cover crops affected plant density and root development of caraway. Low plant densities disfavour *M. acerina* (Evenhuis *et al.*, 1995). The effect of the cover crops on lodging of the biennial caraway crop in the next year were probably caused by differences in nutrient availability after the harvest of the cover crops. High nitrogen availability may stimulate anthracnose development in caraway, by making the crop more dense or by furthering lodging which, in turn, stimulates *M. acerina* development (Evenhuis *et al.*, 1995).

Preceding crop (experiments 3 & 4)

Plant density of spring caraway was not significantly influenced by preceding crops (table 4). Differences in anthracnose development in spring caraway after different preceding crops might be explained by different inoculum build-up on these crops. Spinach preceding caraway caused significantly more anthracnose in spring caraway than bare soil. The relatively poor suitability of spinach as a host of *M. acerina* was confirmed by other experiments (tables 6 and 7). Other combinations of treatments had no significantly different effects on disease severity of spring caraway. Disease levels in spring caraway were high and hardly influenced by the preceding crops. Therefore, the possibilities for control of *M. acerina* by crop rotation are limited, at best. The high disease level of spring caraway might have been caused by survival of chlamydospores for more than a year after inoculation. In carrot, chlamydospores of *M. acerina* survived at least two years (Wall & Lewis, 1980c).

Host plants

Crops

Dill and barley have not yet been recorded as host plants of *M. acerina*. Infection of dill in the field was not found, but no inoculations were made. Dill was infected under greenhouse conditions. If dill will be grown on a large scale problems might arise due to *M. acerina*. In the greenhouse, spring barley was not infected after inoculation but in the field it was. The finding of *M. acerina* on a monocotyledonous species was unexpected because the host range of *M. acerina* described so far consisted of

dicotyledonous species only. As infection was found only early after inoculation and no conidia were produced on decaying leaves of barley using the filter paper method, barley is a poor host plant, at best. Perhaps *M. acerina* grew saprophytically on decaying barley tissue. Maybe the fungus survives on any decaying plant tissue irrespective of species. In carrot, the fungus survives easily on decaying leaves (Wall & Lewis, 1980b). Records on infection of sugar beet (Channon, 1965), spinach (Tompkins & Hansen, 1950) and peas (Tompkins & Hansen, 1950) by *M. acerina* under greenhouse conditions were confirmed. Infection of peas and spinach in the field, after inoculation, is reported for the first time.

Weeds

New records of host weeds are *Cirsium arvense* (L.) Scop., *Plantago major* L., *Rumex crispus* L. and *Geranium columbinum* L. These weeds, found in caraway crops were shown to be infected by *M. acerina* several months after inoculation of the crop. As the plants were not present at the moment of inoculation, infection must have occurred spontaneously, under circumstances which might be found in any naturally infested field. In Denmark, *Geranium sanguineum* L. was reported as a host plant of *M. acerina* (Neergaard & Newhall, 1951). *Matricaria discoides* DC. was found to be naturally infected by *M. acerina*. Other *Matricaria* spp. were shown to be susceptible by Hermansen (1992b). Carrot (*Daucus carota* L.) grown from seed collected in verges was infected by *M. acerina* after inoculation. In cultivated carrot, *M. acerina* causes liquorice rot, an important post-harvest disease (Rader, 1945; Årsvoll, 1969).

Susceptibility of the weed species *Capsella bursa-pastoris* (L.) Med. (Viennot-Bourgin, 1955), *Ranunculus* spp. (Hermansen, 1992b), *Senecio vulgaris* L. (Hermansen, 1992b), *Taraxacum officinale* Weber (Tompkins & Hansen, 1950), and *Viola arvensis* Murr. (Rintelen & Klewitz, 1976; Hermansen, 1992b) was confirmed.

No infection of *M. acerina* was found on *Chenopodium album* L. and *Sinapis arvensis* L. Infection of *C. album* was found by Rintelen & Klewitz (1976), but not by Hermansen (1992b). Inoculation of *S. arvensis* with *M. acerina*, carried out by Hermansen (1992b), produced no infection either. *Galium aparine* L. (Hermansen, 1992b) and *Stellaria media* (L.) Vill. (Viennot-Bourgin, 1955; Rintelen & Klewitz, 1976) were reported susceptible, but we could not confirm these observations.

Straw management under field conditions.

Caraway straw

In experiment 6 and two other straw management experiments (Evenhuis & Verdam, 1995), no significant effects of amendments with infested straw on plant density, disease incidence and yield were found. Possibly, other inoculum sources masked the effect of bringing infested straw into the soil. An alternative explanation might be that germination of *M. acerina* was hampered by other micro-organisms colonizing the straw.

Chlamydospores

The plant density decreased by 40% when caraway was inoculated with chlamydospores (table 6). If we compare the chlamydospore treated plots with the other plots, the average plant densities are 35 (n=9) and 61 (n=12), respectively, a highly significant difference ($P < 0.001$). Therefore, in the second year, an open caraway canopy was formed and subsequently *M. acerina* development was hampered due to lack of moisture (Evenhuis *et al.*, 1995). In carrot, until three weeks after emergence, seedlings showed damping-off due to *M. acerina* infection. Plant density was reduced by 60% (Årsvoll, 1969; Wall & Lewis, 1980a). Plant density reduction in caraway was of a similar magnitude.

Straw inoculum under greenhouse conditions.

Inoculum sources

The infestation levels of straw and debris of caraway and cover crops were not established. Infection of seedlings by *M. acerina*, after soil inoculation, was found in the greenhouse tests (table 7). As the soil was sterilized, it was not a source of inoculum. The seeds used in the experiments were obtained from a seed lot free of infection, as established by the freezing blotter method (Evenhuis & Verdam, 1995). It is unlikely that conidia were blown in from outside, since plastic covers were placed over the containers in experiments 7 and 8. The covers were only removed during disease assessments. Dispersal of the fungus from one diseased seedling to another within an experimental unit, i.e. a container, is improbable as conidia of *M. acerina* are mainly splash dispersed. During the experiment no water was added over the top of the

plants, thus minimizing the risk of splash dispersal infection among neighbouring plants. Transport of conidia by water running over the soil is not impossible, but infection of seedlings neighbouring a diseased seedling was not found. It is concluded that plant debris and chlamydospores brought into the soil must have been the inoculum sources.

Inoculum density

Caraway sown in soil treated with infested caraway, spinach, spring barley and pea debris became infected by *M. acerina* (table 7). Though the disease incidence was not significantly different from the control, its non-zero value suggests that plant debris transmitted *M. acerina*. Disease incidence of caraway seedlings in the debris treatments was low in comparison to the chlamydospore treatments. The difference suggests that only a limited amount of inoculum can be brought into the soil with infested plant debris. Other micro-organisms on decaying straw might hamper *M. acerina* infection of caraway seedlings.

In the field, straw will decay rapidly but chlamydospores of *M. acerina* may survive in the soil. These chlamydospores might supplement inoculum sources in the soil for future caraway fields. In experiments 7 & 8 the load of applied chlamydospores was probably much higher than in the field, though high field values have been found with a dilution plating technique. Between 90 and 700 colony forming units, probably chlamydospores, per ml soil were found in a sample taken from a heavily infested focus in a caraway field (Evenhuis & Verdam, 1995). Davies *et al.* (1981) found approximately 30 chlamydospore chains per gram soil adhering to roots of carrot, which they thought to be a high level.

Crop rotation, weeding and straw management to limit anthracnose development

Inoculum potential

Growing peas, spinach and spring barley as preceding crops had little effect on anthracnose development in caraway in the next year (table 4). It may be expected that these crops will have as little effect on inoculum build-up when used as a cover crop. If so, effects on disease development in biennial caraway must be caused by the suitability of the crop as a cover crop (tables 2 & 3). From the results on cover crops

and preceding crops we conclude that inoculum build-up on other crops is limited, but that these crops may play a role in the survival of *M. acerina* levels in the soil. We surmise that chlamydospores infect host plants and that resulting colonies form new chlamydospores.

From the results presented in table 5 it is hard to rank plant species according to their susceptibility. The importance of weeds and crops for the survival of *M. acerina*, when no caraway is grown, is not fully understood. The evidence suggests that the level of *M. acerina* in the soil may be maintained because several weed and crop species which can host *M. acerina*. Hermansen *et al.* (1997) showed that a three year crop rotation was slightly effective against liquorice rot in carrots, caused by *M. acerina*. The disease pressure decreased most significantly by growing red clover (*Trifolium pratense* L.), grass (*Festuca pratensis* Huds., *Phleum pratense* L., and *Lolium perenne* L.), or potato (*Solanum tuberosum* L.) in rotation with carrot. Barley was less effective and onion (*Allium cepa* L.) was ineffective. A rotation with celery (*Apium graveolens* L.) once in 3 years was not long enough to eliminate *M. acerina* (Newhall, 1944). Celery preceding parsnip (*Pastinaca sativa* L.) gave problems with *M. acerina* anthracnose in parsnips (Klewitz, 1972). The results of crop rotation experiments with carrot in Norway showed only a slight decrease of liquorice rot during a period of three years, which indicated that inoculum levels in the soil only decrease slowly (Hermansen *et al.*, 1997).

Although, crop rotations differed little in their effects on *M. acerina* development in carrot, growing non-susceptible crops might help to prevent a 'clean soil' from becoming infected (Hermansen *et al.*, 1997). Weeding of susceptible weed plants might help to control *M. acerina* survival or build-up, although the effect will probably be small. Growing a susceptible crop in autumn and winter as green manure might enhance survival of *M. acerina* or even stimulate inoculum build-up, but this is not a very common practice on clay soils. Susceptibility of green manure crops has not been determined yet.

The field experiment (6) and the greenhouse experiments (7 & 8) suggest that the inoculum brought into the soil by caraway straw or crop debris was rather small, since the effect of adding chlamydospores to the soil were much larger than that of infested straw. Perhaps, germination of *M. acerina* was hampered by other micro-organisms colonizing the straw. Once straw has decayed, the chlamydospores will be left naked in

the soil. Chlamydospores in soil do not lose their viability for at least two years (Wall & Lewis, 1980c). Ploughing infested caraway straw into the soil leads to an increase of the inoculum potential of the soil (table 6). Even caraway grown in a six years rotation (the usual caraway rotation period, pers. comm. Vreeke) may suffer from a previous caraway crop since inoculum levels decrease slowly. As a safety precaution, the authors recommended to remove infested caraway straw from the field, although the amount of inoculum carried off might be small (table 6).

Agronomical value of the cover crop

The effect of the cover crop on plant density and yield of biennial caraway is more important than the effect on anthracnose development. In experiments by Bernelot Moens (1968), Vreeke (1989) and ourselves, great differences in caraway yield were found using different cover crops. The effect can be explained by the light interception by the cover crop and the time at which the cover crop is harvested. If more light is available to the caraway crop, more seedlings will survive and growth will be improved. Consequently the tap roots will be larger and a higher proportion of the plant will flower in the second year (Wander, 1994). A positive relation between root diameter of caraway in autumn and yield was found by Weglarz (1983). Consequently, a farmer should choose a cover crop which is harvested early in the season or has an open character, such as peas, spinach for seed production or seed poppy. From these crops spinach is the most susceptible to *M. acerina* and therefore the least desirable. Unfortunately, the afore mentioned cover crops have only limited marketing potential. Cereals provide an alternative, but are less suitable when crop management aims at high grain yields (Bernelot Moens, 1968; Vreeke, 1989).

Conclusions

Anthracnose management by crop rotation, including the choice of cover crop, contains little promise, but crop rotation and weed control might help to avoid problems with *M. acerina*. The choice of the cover crop should be based primarily on its agronomical values as a cover crop. *M. acerina* can survive in crop debris. Removal of infested straw from the field might be considered as a safety measure, though with limited effect.

CHAPTER 7

Crop management and anthracnose development in caraway (*Carum carvi* L.)

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Abstract

Anthracnose of caraway is caused by the fungus *Mycocentrospora acerina* (Hart.) Deighton. A reduction of leaf wetness duration was positively correlated ($r^2 = 0.71$; $P < 0.001$) with a decrease of disease severity. Lodging and higher plant density prolonged leaf wetness duration. Disease incidence and severity of anthracnose were reduced by crop management activities minimizing leaf wetness duration. Reduction of nitrogen levels reduced the risk of anthracnose development in spring and biennial caraway. Decreasing the sowing rate from 8 to 4 kg ha^{-1} resulted in a lower disease severity and an increase of seed yield in spring caraway, but not in biennial caraway. In biennial caraway disease severity decreased with wider row spacing. A damage threshold between 6% and 12% disease severity is proposed. Positive financial results of crop management activities are indicated.

Introduction

Caraway (*Carum carvi* L.) is an arable crop grown for seeds and essential oil, especially carvone. A commercial sprout inhibitor of potatoes, based upon carvone, was brought to the market in 1995 (Hartmans *et al.*, 1995). In the Netherlands, farmers usually grow biennial or winter cultivars. In the first year caraway is grown under a cover crop. If the root collar reaches a diameter of about 6 mm at the end of the growing period of the first year, the plant will bolt in April and flower in May of the following year. If the required root diameter is not reached, the plants will remain vegetative for one more year. On heavy clay soils a high sowing rate is usually applied to ensure crop establishment under difficult conditions. When, however, conditions are favourable for germination more plants than necessary will emerge and competition among caraway plants may occur, and root growth may be hampered.

Spring caraway is an annual type, grown without a cover crop. In the Netherlands, spring caraway has only been grown by a few farmers. From bolting until harvest spring caraway is very similar to biennial caraway. Spring caraway flowers in July and is harvested in September, approximately two months later than biennial caraway.

Caraway yields vary from 600 to 2500 kg ha^{-1}, depending on year, region and field (Bouwmeester *et al.*, 1995). Part of this variation may be caused by *Mycocentrospora acerina* (Hart.) Deighton, the causal agent of anthracnose in caraway. Crop and disease management, essential to increase the crop's yield stability, demand better knowledge of pathogen/crop interactions. In this paper, experiments to study the effect of nitrogen rate, sowing rate and row spacing on anthracnose are described.

Materials and methods

General information on thirteen field experiments is given in table 1. The locations of the experimental sites are shown in figure 1. Information on inoculation, disease assessment, leaf wetness duration and agronomical observations is given first and in general applies to all experiments. Treatments per experiment are described later.

Inoculation

Spring caraway was inoculated at the end of spring, just before bolting. Biennial caraway was inoculated in March, also just before bolting. Inoculum was produced by blending a 3 to 4 week old culture of *M. acerina* on Potato Dextrose Agar (18 °C) in tap water. The suspension was sieved through double cheesecloth. The final inoculum density was adjusted to 10^4 chlamydospore chains per ml suspension. Inoculum or water (500 l ha^{-1}) was added to each plot using a knapsack sprayer. Inoculations were performed on rainy days.

Disease assessment

In biennial caraway disease levels were estimated weekly from bolting till harvest. Disease incidence was observed on 25 plants per plot and expressed as the percentage of caraway plants infected by *M. acerina*. Disease severity was determined by estimating the percentage of the main stem surface covered with lesions caused by *M. acerina*, on the same 25 plants per plot. Final disease assessments were made about four weeks before harvest, sampling 25 plants at 5 positions per plot (125 plants per plot). In spring caraway, only the final disease assessments were made.

Leaf wetness duration

In 1993 and 1994, `Lufft' leaf wetness sensors (G. Lufft Meß- und Regeltechnik GmbH, Stuttgart) were used to assess daily leaf wetness duration, from bolting until harvest, in the nitrogen experiments. Each week, the sensors were moved from one block to another and from one nitrogen level to another. Displacements were made to avoid confounding effects of sensors and sensor positions on the evaluation of leaf wetness duration. In Wageningen, leaf wetness duration was estimated by 'De Wit' leaf wetness recorders. Differences in leaf wetness duration between treatments were

analysed by considering daily leaf wetness durations to be sequential replicates. Analysis of variance was applied to leaf wetness data.

Table 1. General information on field experiments. Biennial caraway is sown one year before the harvest year.

Experiment	1	2	3	4	5	6	7	8	9	10	11	12	13
Harvest year	1992	1993	1994	1992	1992	1993	1993	1993	1993	1994	1994	1993	1994
Location[a]	L	L	R	NB	W	NB	W	NB	W	NB	W	W	Ln
Soil type	clay	clay	clay	clay	sand	clay	sand	clay	sand	clay	sand	clay	clay
Caraway crop[b]	S	S	B	S	S	S	S	B	B	B	B	B	B
Cover crop[c]	none	none	SB	none	none	none	none	OSF	SB	SB	SB	P	P
Sowing date	9/3	5/4	28/4	9/4	22/4	15/4	21/4	-/4	22/4	-/4	21/4	-/4	-/4
Sowing rate (kg/ha)[d]	7.5	7.5	8	t	t	t	t	t	t	t	t	t	t
Row spacing (cm)[d]	25	25	25	t	t	t	t	t	t	t	t	t	t
Nitrogen rate (kg/ha)[d,e]	t	t	t	100	100	100	100	100	100	100	100	100	70
Inoculation date	18/6	16/6	6/4	24/7	24/7	16/6	-[f]	10/92	-	9/93	-[f]	-	-
Lodging assessment	17/6	6/7	7/7	-	-	13/8	-	-	9/6	-	30/6	-	23/6
Disease assessment	31/8	17/8	16/6	20/8	7/9	13/8	25/8	-	9/6	-	15/6	17/6	30/6
Harvest date	31/8	6/9	18/7	9/9	12/10	20/9	29/10	none	8/7	none	19/7	-	20/7
(Sub) Plot size (m)	6*6	6*6	3*15	6*18	6*15	6*18	6*15	6*18	6*15	6*18	6*15	5*5	5*5

a: Field experiments were located at Lelystad (L), Lienden (Ln), Nieuw Beerta (NB), Randwijk (R), and Wageningen (W).

b: S and B are spring caraway and biennial caraway, respectively.

c: SB spring barley, P = peas, OSF = oil seed flax.

d: For t = treatments, see Materials and Methods.

e: Nitrogen rate applied in spring, for biennial caraway nitrogen rate applied in autumn depended on soil type and cover crop.

f: Not relevant.

Plant density

Plant density of caraway was determined by counting caraway stem bases in two or four, depending on row spacing, adjacent rows of one meter length at four positions in each plot (approximately 2 m^2 per plot), directly after harvest.

Lodging

Lodging of caraway was assessed whenever it occurred (table 1). The percentage of lodged crop area was estimated per plot. Plants were considered to be lodged when the stem's angle of inclination deviated more than 45 degrees from the upright position.

Yield

Caraway was harvested using a combine harvester (Wintersteiger, Nurserymaster Elite, Austria) designed for field experiments. Seed lots per subplot were weighed after drying to 12% water content and removal of trash. The result was converted to seed yield in kg per hectare.

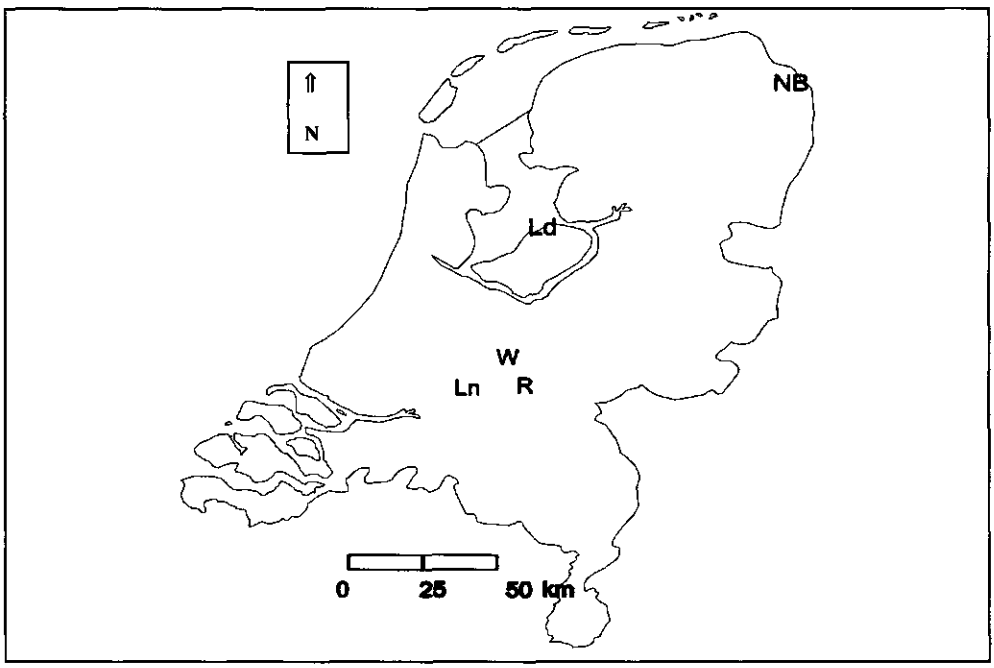

Figure 1. Map of the Netherlands with the sites of the field experiments; NB: Nieuw Beerta; Ld: Lelystad; Ln: Lienden; R: Randwijk; W: Wageningen

Effect of nitrogen rate on anthracnose (experiments 1, 2 & 3)

Treatments

In spring 1992 either 40 or 100 kg ha^{-1} fertilizer nitrogen was given to spring caraway. Nitrogen fertilization of spring caraway was 40, 100 and 160 kg ha^{-1} in 1993. Nitrogen levels applied to biennial caraway in March 1994 were 40, 100 and 160 kg ha^{-1}. Each of the three experiments consisted of four blocks in a split-plot design. A block consisted of two plots, which were either inoculated with *M. acerina* or treated with water. Each plot consisted of two (1992) or three (1993 & 1994) subplots to which the nitrogen level was allotted randomly. The plots and subplots were surrounded by a 3 m buffer zone of barley in 1992 and of caraway in 1993 and 1994. The buffer zone was intended to reduce the spread of the fungus from inoculated to non-inoculated subplots.

Effect of sowing rate and row spacing on anthracnose (experiments 4 to 13)

Treatments in spring caraway (experiments 4 to 7)

The row spacing was 12.5, 37.5 and 50 cm at Nieuw Beerta and 12, 36 and 48 cm at Wageningen. The sowing rates were 4 and 8 kg seed per hectare. Each combination of sowing rate and row spacing (plot) was applied in a block, four blocks per experiment. Each plot had two subplots of which one was inoculated as described above. Inoculations were carried out at the end of spring, just before bolting of the caraway crop. No inoculation was carried out in experiment 7 at Wageningen in 1993, because a natural inoculum source was present in the field. No buffer zones between plots were applied in these experiments.

Treatments in biennial caraway (experiments 8 to 11)

The cover crops were sown with a row spacing of 25 cm. The row spacing of caraway was 12.5, 37.5 and 50 cm at Nieuw Beerta (experiments 8 & 10) and 12, 36 and 48 cm at Wageningen (experiments 9 & 11). The sowing rates were 6 and 12 kg ha^{-1}. The experimental design was as in spring caraway. At Nieuw Beerta half of the plots were inoculated with *M. acerina* in October 1992 (experiment 8) and September 1993 (experiment 10). No inoculations were carried out at Wageningen (experiments 9 & 11), since a natural inoculum source was present.

Table 2. *Experiments 1 & 2.* Effect of three nitrogen levels and inoculation with *M. acerina* (*M.a.*) on lodging, plant density, disease incidence and severity of *M. acerina*, yield and daily leaf wetness duration in spring caraway at Lelystad in 1992 and 1993. Data from the two experiments were averaged.

Nitrogen rate (kg ha^{-1})	Replicates	*M.a.*[a]	Lodging (%)	Plant density (m^{-2})	Disease incidence (%)	Disease severity (%)	Yield (kg ha^{-1})	Mean[b] daily leaf wetness duration (h.min day^{-1})
40	8	-	5	77	37	3.2	884	--[c]
100	8	-	34	75	45	4.6	907	--
160	4	-	61	75	54	7.6	873	--
40	8	+	4	76	56	16.2	674	--
100	8	+	29	72	64	24.1	601	--
160	4	+	66	73	60	24.3	462	--
LSD i*n (0.05)[d]			19 (25)[e,f]	6 (10)[f]	5 (6)	3.9 (8.7)	161 (241)	--
all	20	-	26	76	44	4.5	893	--
all	20	+	25	74	60	20.9	607	--
LSD i (0.05)[g]			12[f]	6[f]	3	10.8	242	--
40	16	-/+	4	76	47	9.7	779	14h.14
100	16	-/+	31	74	55	14.4	754	15h.20
160	8	-/+	63	74	57	15.9	667	15h.55
LSD n (0.05)[h]			14 (17)	4 (5)[f]	4 (5)	2.9 (3.4)	-[f]	55 min

a: Caraway sprayed with water (-) or inoculated with *M. acerina* (+).

b: Leaf wetness was measured at Lelystad in 1993, only.

c: No data available.

d: LSD given for the inoculation and nitrogen interaction.

e: LSD given when data are compared with the maximum number of replicates, between brackets the LSD is given for the comparison of data with fewer replicates.

f: No significant differences at $P < 0.05$.

g: LSD given for the inoculation effects.

h: LSD given for the nitrogen effects.

Supplementary experiments (12 & 13)

Disease assessments were made in two field experiments not specifically designed to investigate the effect of row spacing on *M. acerina* development. The experiments were located on clay soils at Wageningen (experiment 12) and Lienden (experiment 13) in 1992/93 and 1993/94, respectively (table 1). In these experiments row spacings were 25, 37.5 and 50 cm at Wageningen and 25 and 50 cm at Lienden.

Statistics

Data were analysed with Genstat 5, release 3.1. Analyses of variance were conducted on disease assessment data and agronomical data. Least significant differences (lsd) at $P = 0.05$ were calculated from s.e.d. values generated in the analyses. Logit transformation of disease incidence and disease severity usually improved the discriminative capacity of the analyses. Disease severity was square root transformed in experiment 12.

Data from nitrogen experiments 1 and 2 were analysed with Genstat 5 directive REML (residual maximum likelihood), since one more nitrogen level was tested in 1993 than in 1992.

Linear regression was conducted to demonstrate the effect of lodging on disease severity in the nitrogen experiments (experiments 1 to 3). To compare these experiments, partial normalisation of data was applied. Instead of the values x_{ij}, with x for the variate, i for the experiment and j for the j^{th} observation in experiment i, we used the deviation from the mean per experiment, $y_{ij} = x_{ij} - x_i$, with y for the partially normalised variate (complete normalisation requires division of y_{ij} by σ_i). The effect of disease severity on caraway yield was also described by linear regression. Yield was log transformed. Leaf wetness duration was measured in six experiments (experiments 2, 3, 5, 7, 9 & 11). The effects of leaf wetness duration on disease severity was studied by linear regression, after partial normalisation of the data.

Gompertz curves were calculated to describe disease development with time (Evenhuis & Verdam, submitted) in experiment 3.

Table 3. *Experiment 3*. Effect of three nitrogen levels and inoculation with *M. acerina* (*M.a.*) on lodging, disease incidence and severity of *M. acerina*, yield and leaf wetness duration in biennial caraway at Randwijk in 1993/94.

Nitrogen rate ($kg\ ha^{-1}$)	*M.a.*[a]	Lodging (%)	Plant density (m^{-2})	Disease incidence (%)	Disease severity (%)	Yield ($kg\ ha^{-1}$)	Mean total leaf wetness duration (h.min day^{-1})
40	-	0	46	16	0.3	2105	13h.40
100	-	19	50	23	0.7	2621	--[d]
160	-	53	51	35	1.2	2304	16h.02
40	+	0	56	86	3.3	2065	--
100	+	13	51	96	10.5	1384	--
160	+	85	51	99	14.2	1292	--
LSD n (0.05)[b]		14	9[c]	11	2.2	433[c]	57 min
LSD i (0.05)		19[c]	8[c]	18	3.6	383	--
LSD i*n (0.05)		21	12[c]	17[c]	3.5	565	--

a: Caraway sprayed with water (-) or inoculated with *M. acerina* (+).

b: LSD given for the nitrogen effects, the inoculation effects and the interaction, respectively.

c: No significant differences at $P < 0.05$.

d: No data available

Results

Effect of nitrogen rate on anthracnose

Experiments 1 and 2 were carried out with spring caraway and experiment 3 with biennial caraway (table 1). The soil nitrogen rate (0-90 cm) in early spring was 20, 18, and 30 kg ha^{-1} in experiments 1 to 3, respectively.

Disease incidence and disease severity increased significantly with the nitrogen level applied in the inoculated plots (experiments 1 to 3; tables 2 & 3). In the control plots no effect of nitrogen rates on disease incidence and disease severity was found in experiment 1. In experiments 2 and 3 disease incidence and disease severity increased significantly with nitrogen rate in the control plots, although the effect was less pronounced than in inoculated plots (table 3).

In experiment 1, seed germination was moderate (65 plants m^{-2}) and growth was rather poor which resulted in an open crop and no lodging. In experiments 2 and 3, the caraway crops lodged after heavy rainfall in early July and June, respectively. Lodging increased with the nitrogen rate applied. Inoculation had no effect on lodging. Disease severity increased with lodging and N-fertilization (tables 2 & 3). To be able to compare the effect of lodging on disease severity in spring (experiment 2) and biennial caraway (experiment 3) partial normalisation was applied. Figure 2 shows that after normalisation an increase of lodging led to an increase of disease severity, of the same magnitude both in spring and biennial caraway.

M. acerina disease progress curves were fitted to Gompertz equation. Disease incidence increased to 100% in inoculated plots, though with lower nitrogen level the epidemic was a little slower. In the control plots, *M. acerina* disease incidence increased with increasing nitrogen rate (figure 3).

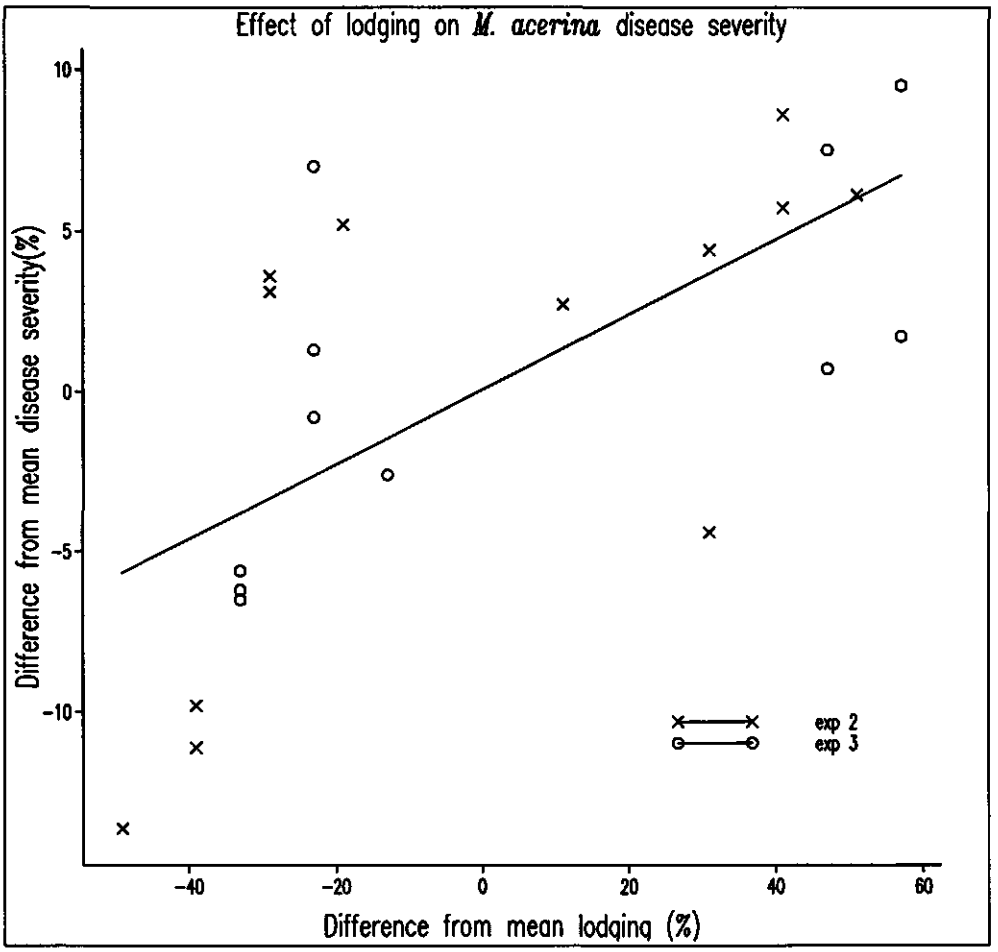

Figure 2. The effect of lodging (L) on disease severity (S) of anthracnose in caraway at Lelystad (experiment 2) in 1993 and Randwijk (experiment 3) in 1994 (n=24, $P < 0.001$, $r^2=0.42$), with standard errors between brackets:

S = 0.06 (1.06) + 0.117 (0.028) * L

S and L are rendered as partially normalized variates (see text).

Yield was negatively correlated with disease severity in experiments 2 and 3. Caraway yield of inoculated plots was 44% (experiment 2) and 33% (experiment 3) lower than of non-inoculated plots. In experiment 1, disease severity levels were less than 2%, too

low to cause yield loss by anthracnose. Under low disease pressure, an increase of the spring caraway yield from 540 to 740 kg ha^{-1} was found with an increase of nitrogen rate from 40 to 100 kg ha^{-1}. A similar effect was found in experiment 3 in the control plots. Yield of biennial caraway increased from 2100 kg ha^{-1} to 2600 kg ha^{-1} with an increase of N-fertilization of 40 to 100 kg ha^{-1}. Under high disease pressure (experiment 2 and inoculated plots of experiment 3) yield dropped with N-fertilization due to increased anthracnose development in lodged plots.

Lower nitrogen levels led to shorter leaf wetness duration in spring caraway (table 2) and biennial caraway (table 3).

Effect of sowing rate and row spacing on anthracnose in spring caraway

Experiment 4

The disease severity (*M. acerina*) was 0.2% in 1992 at Nieuw Beerta, and the experiment was therefore excluded from the analysis. Under low disease pressure a reduction of the sowing rate from 8 to 4 kg ha^{-1} led to a small (9%) but significant decrease ($P = 0.001$) of the caraway yield from 1304 to 1186 kg ha^{-1}. Plant density was 53 and 100 plants m^{-2} at a sowing rate of 4 and 8 kg ha^{-1}, respectively. No significant effect of row distance on yield was found.

Experiments 5, 6, & 7

Disease incidence and severity of *M. acerina* were higher in inoculated spring caraway than in the uninoculated controls of experiments 5 and 6. No interaction was found between inoculum level and sowing rate or row spacing. No significant interactive effects of sowing rate and row spacing on disease incidence, disease severity, plant density, lodging and yield were found. Therefore the aspects of sowing rate and row spacing in spring caraway were analysed separately.

In experiment 5, a low sowing rate resulted in lower plant densities, shorter leaf wetness duration, and lower disease levels in spring caraway. Therefore, the low sowing rate had a significant positive effect on yield (table 4). Averaged over experiments 5, 6 & 7 similar results were obtained (Evenhuis *et al.*, 1995). At Wageningen (experiments 5 & 7), daily leaf wetness duration was approximately one

hour shorter in caraway with a sowing rate of 4 kg ha^{-1} compared to a sowing rate of 8 kg ha^{-1}.

Table 4. *Experiment 5.* Effect of sowing rate on lodging, plant density, disease incidence and mean severity of *M. acerina* and yield in spring caraway. Observations were averaged over row spacings at Wageningen in 1992. Row spacings varied from 12 to 48 cm.

Sowing rate	Lodging	Plant density	Disease incidence	Disease severity	Yield
(kg ha^{-1})	(%)	(m^{-2})	(%)	(%)	(kg ha^{-1})
4	20	67	44	4.3	483
8	33	112	58	6.3	370
LSD (0.05)	16[a]	12	11	1.9	99

a: No significant difference at $P < 0.05$.

In experiment 5, plant density and lodging were significantly affected by row spacing, but not yield, disease incidence and severity (table 5). But, averaged over experiments 5, 6 & 7 yield increased significantly with smaller row spacing.

Yield was significantly affected by disease severity in experiment 6 at Nieuw Beerta, 1993. Yield in inoculated plots was approximately 75% lower than yield of non-inoculated plots. Disease severity had no significant effect on yield, analysed by linear regression in experiments 5 and 7, at Wageningen, in 1992 and 1993.

Table 5. *Experiment 5.* Effect of row spacing on lodging, plant density, disease incidence and severity of *M. acerina* and yield in spring caraway. Observations were averaged over sowing rates at Wageningen in 1992. Sowing rates were 4 and 8 kg ha^{-1}.

Row spacing (cm)	Lodging (%)	Plant density (m^{-2})	Disease incidence (%)	Disease severity (%)	Yield (kg ha^{-1})
12	6	108	56	6.4	406
36	24	80	52	5.2	404
48	50	82	45	4.2	469
LSD (0.05)	19	14	13[a]	2.4[a]	121[a]

a: No significant differences at $P \leq 0.05$.

Effect of sowing rate and row spacing on anthracnose in biennial caraway

Experiments 8 & 10

The experiments 8 & 10, on sowing rate and row spacing at Nieuw Beerta, met with misfortune. In autumn/winter of 1992/93 (experiment 8), many caraway plants did not produce a root large enough (diameter 6 mm) to permit bolting and flowering. Between 5 and 50% of the plants flowered in 1993, depending on sowing rate and row spacing (table 6). The higher sowing rate and the smallest row spacing gave the highest number of flowering plants. A significantly ($P < 0.001$) higher percentage of flowering plants was achieved with a sowing rate of 6 compared to 12 kg seed ha^{-1}. The poor bolting was probably caused by the cover crop. Oil seed flax was harvested late, and started regrowth, and thus hampered caraway root growth. In 1993/94 (experiment 10), the number of caraway plants was very low after harvest of the cover crop and plant densities patterns were irregular throughout the field. Nevertheless the highest numbers of plants were found with the higher sowing rate and the smallest row spacing. The effect of plant density on disease severity could not be assessed at these low plant densities and irregular plant density pattern. Both experiments were excluded from the analysis of the relationship between disease development and plant density.

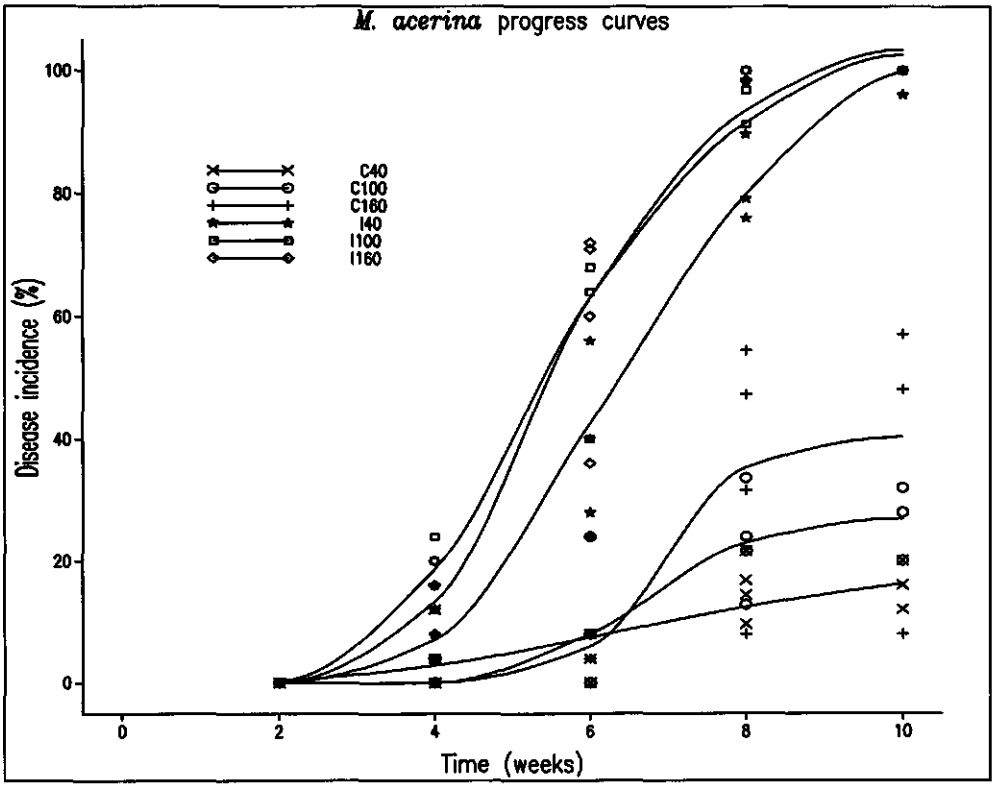

Figure 3 Disease incidence progress curves for *M. acerina* in caraway at Randwijk in spring 1994 were fitted to Gompertz curves (Y = A + C * EXP (EXP (-B*(X-M)))). At X = 0 bolting just started. Disease incidence was assessed in plots treated with water (C) or inoculated with *M. acerina* (I) with nitrogen rates of 40, 100 and 160 kg ha^{-1}. Parameter A was set to zero. Least significant differences for the parameter C was 14.

$Y_{C40} = 22 * EXP(-EXP(-0.33 * (X-6.2)))$

$Y_{C100} = 35 * EXP(-EXP(-0.60 * (X-6.6)))$

$Y_{C160} = 42 * EXP(-EXP(-1.22 * (X-6.5)))$

$Y_{I40} = 116 * EXP(-EXP(-0.50 * (X-6.0)))$

$Y_{I100} = 108 * EXP(-EXP(-0.59 * (X-5.0)))$

$Y_{I160} = 107 * EXP(-EXP(-0.69 * (X-5.1)))$

Experiments 9 & 11 to 13

The sowing rate had no effect on disease incidence and disease severity of reproductive plants at Wageningen. Mean densities of total plants were 122 and 198 plants m^{-2} in 1993 (experiment 9) at sowing rates of 6 and 12 kg ha^{-1}, respectively, and 67 and 101 plants m^{-2} in 1994 (experiment 11). Mean plant densities of reproductive plants were 91 and 114 in 1993 and 56 and 58 in 1994 at sowing rates of 6 and 12 kg ha^{-1}, respectively. At the higher sowing rate a smaller percentage of the plants flowered (table 6).

Table 6. *Experiments 8 to 11.* The interactive effect of sowing rate and row spacing on plant density of biennial caraway at Nieuw Beerta and Wageningen.

Row spacing (cm)	Sowing rate (kg ha^{-1})	Total plant density (m^{-2})				Reproductive plant density (m^{-2})			Percentage reproductive plants (%)		
		8	9	10	11	8	9	11	8	9	11
12	6	120	117	29	72	30	98	72	25	85	100
36	6	94	125	20	66	27	85	55	29	69	87
48	6	88	123	26	62	12	89	41	13	72	68
12	12	178	213	42	132	35	136	75	20	65	61
36	12	149	183	29	96	14	109	48	9	60	51
48	12	137	199	27	76	2	98	51	1	50	71
LSD r (0.05)		27	29[a]	6[a]	19	17	22[a]	10	13[a]	8	20[a]
LSD s (0.05)		22	24	5[a]	15	14[a]	18	8[a]	11	6	17
LSD r*s (0.05)		37[a]	42[a]	10[a]	26[a]	21[a]	21[a]	14[a]	19[a]	11[a]	29[a]

a: No significant differences at $P < 0.05$.

Since the effect of sowing rate on reproductive plant density was non-significant, only the effect of row spacing was analysed. In both experiments (9 & 11), disease incidence and disease severity decreased with increasing row spacing (table 7). In experiment 12, disease severity of biennial caraway was significantly lower at 37.5 cm row spacing than at 50 cm. A row spacing of 25 cm led to intermediate levels of *M. acerina* infection. Row spacing of 48 cm seemed to increase the risk of lodging (experiments 9, 11 & 13), though the effect on lodging was non-significant in these experiments.

Table 7. *Experiments 9 & 11.* Effect of row spacing on mean lodging, plant density, disease incidence and severity of *M. acerina*, yield and leaf wetness duration in biennial caraway at Wageningen in 1992/93 and 1993/94, analysed together. Data from plots with sowing rates of 6 and 12 kg ha^{-1} were taken together.

Row spacing (cm)	Lodging (%)	Plant density (m^{-2})	Disease incidence (%)	Disease severity (%)	Yield (kg ha^{-1})	Leaf wetness duration (h.min.day^{-1})
12	23	92	75	12.3	1274	9h.13
36	23	71	70	8.3	1372	--[a]
48	28	64	67	6.9	1300	8h.20
LSD (0.05)	11[b]	14	4	3.0	84[b]	28 min

a: No data available.

b: No significant differences at $P < 0.05$.

Effect of M. acerina disease severity on caraway yield

In five experiments (2, 3, 6, 11 & 13) yield decreased with increasing disease severity. Linear regression of yield on disease severity gave a coefficient of determination r^2 = 0.51 with n = 128 and $P < 0.001$. Linear regression of the log transformed yield on disease severity gave $r^2 = 0.71$ (n = 128; $P < 0.001$; figure 4). In three experiments (8, 10 & 12) yield was not determined. In experiments 1, 4 and 9 disease severity was less then 2% and only small effects on disease severity were found. In experiments 5 and 7, mean disease severities were 5.3 and 15.8 %, respectively, with very poor yields and no correlation between disease severity and yield.

Table 8. *Experiment 12.* Effect of row spacing on plant density (reproductive plants), disease incidence and severity of *M. acerina* and on yield of biennial caraway at Wageningen in 1993. The sowing rate was 8 kg per ha^{-1}. Lodging was not assessed.

Row spacing (cm)	Plant density (m^{-2})	Disease incidence (%)	Disease severity (%)	Yield (kg ha^{-1})
25	50	62	4.4 ab	1398
37	43	51	2.6 a	1689
50	40	68	6.1 b	1498
LSD (0.05)	6	13	2.3[a]	232

a: Different characters indicate significant differences at $P \leq 0.05$. Disease severity was square root transformed before analysis of variance.

Effect of leaf wetness duration on anthracnose

Leaf wetness duration was measured in six experiments (2, 3, 5, 7, 9 & 11), including two nitrogen experiments (annual/biennial), two experiments on sowing rate in spring caraway and two experiments on row spacing in biennial caraway. In all experiments longer leaf wetness duration coincided with higher disease incidence and disease severity levels (tables 2, 3, and 7). Figure 5 shows the effect of leaf wetness duration on disease severity ($n=15$, $r^2=0.71$; $P<0.001$), when for each experiment deviations from the means were calculated.

Table 9. *Experiment 13.* Effect of row spacing on means of lodging, reproductive plant density, disease incidence and severity of *M. acerina*, and yield in biennial caraway at Lienden in 1994. The sowing rate was 8 kg ha^{-1}.

Row spacing (cm)	Lodging (%)	Plant density (m^{-2})	Disease incidence (%)	Disease severity (%)	Yield (kg ha^{-1})
25	19	94	95	13.6	1947
50	23	54	89	10.0	1996
LSD (0.05)	22[a]	11	6.7[a]	9.0[a]	298[a]

a: No significant difference at $P \leq 0.05$.

Discussion

Spring caraway and biennial caraway

Crop management experiments were carried out with spring caraway and biennial caraway. Growth and development of a spring caraway crop is largely comparable to the growth and development of biennial caraway in the second year. However, in spring caraway there is no build-up of inoculum on vegetative plants in autumn, because the crop is sown in spring and harvested in September. Infested soil must be the main inoculum source for infection of spring caraway plants in spring and summer. In biennial caraway inoculum build-up takes place in autumn (Evenhuis & Verdam, 1995). Infection of stems and flowers of biennial caraway takes place from April the next year. When no natural inoculum source was suspected caraway crops were inoculated to create some disease pressure. Both caraway types are highly susceptible to *M. acerina* (Evenhuis & Verdam, 1997). Disease development due to *M. acerina* on stems, umbels and seeds of spring caraway is much the same as in biennial caraway. A difference is that spring caraway bolts, flowers and matures approx. 2 months later in the season than biennial caraway. Whether *M. acerina* infection will be severe or not depends upon weather conditions. From 20 experiments, including biennial and spring caraway a positive correlation (r^2=0.61; P <0.001) was found between mean disease severity and rainfall during the month of flowering (Evenhuis & Verdam, 1995). The crop management activities studied were expected to reduce anthracnose in the reproductive phase. Therefore it was expected that the experiments could be performed with spring caraway as well as with biennial caraway.

Caraway is an arable crop with only a short past concerning domestication and breeding. Most of the varieties grown in the Netherlands are selections from landraces. At present only three Dutch biennial varieties and one annual variety are available. Caraway is predominantly a cross-fertilizer (protandry) and the crop is genetically very heterogeneous. The plant to plant variation in growth and plant structure is large and caraway canopies are irregular. Unfortunately the effect in field experimentation is that standard errors tend to be large. On top of that, caraway is by nature biennial. Field experiments with small plots and few replications are not desirable, especially for biennial caraway, but usually inevitable. By analysing many experiments part of this problem can be overcome.

Buffer zones

Inter-plot interference between inoculated and non-inoculated plots was not found when buffer zones were used. This is in accordance with the short distance dispersal of *M. acerina* found in other experiments (Evenhuis *et al.*, 1997). Usually a large inoculation effect was found on disease development. In 1993, weather conditions were conducive to *M. acerina* development. At Nieuw Beerta (experiment 6), no buffer zones were applied and non-inoculated plots became infected, although less severely than inoculated plots. At Lelystad (experiment 2), buffer zones were applied but non-inoculated plots became infected anyhow by *M. acerina*, probably by an unidentified, natural source of inoculum.

Yield

In spring caraway, yield loss due to anthracnose was found to be significant at Lelystad in 1993 (experiment 2; 44%) and Nieuw Beerta in 1993 (experiment 6; 75%). In biennial caraway, yield was significantly reduced by *M. acerina* at Randwijk (experiment 3; 33%), Wageningen (experiment 11; 30%) and Lienden (experiment 13; 30%) in 1994. The mean disease severity in these experiments varied from 12 to 28%. No relationship between yield and disease severity was found in experiments with mean disease severity levels below approximately 6%. We conclude that the damage threshold must be between 6 and 12% for both biennial and spring caraway. The damage threshold is defined as the minimum level at which disease adversely affects yield (Zadoks & Schein, 1979). No significant effect of disease severity on yield was found in experiment 7, notwithstanding a mean disease severity of 16%. Disease severity varied from 6 to 39 % between plots and corresponding yields varied from 200 to 800 kg ha^{-1}. On average yields were low, even for spring caraway. This is an indication that a disease severity of 6% is already higher than the damage threshold.

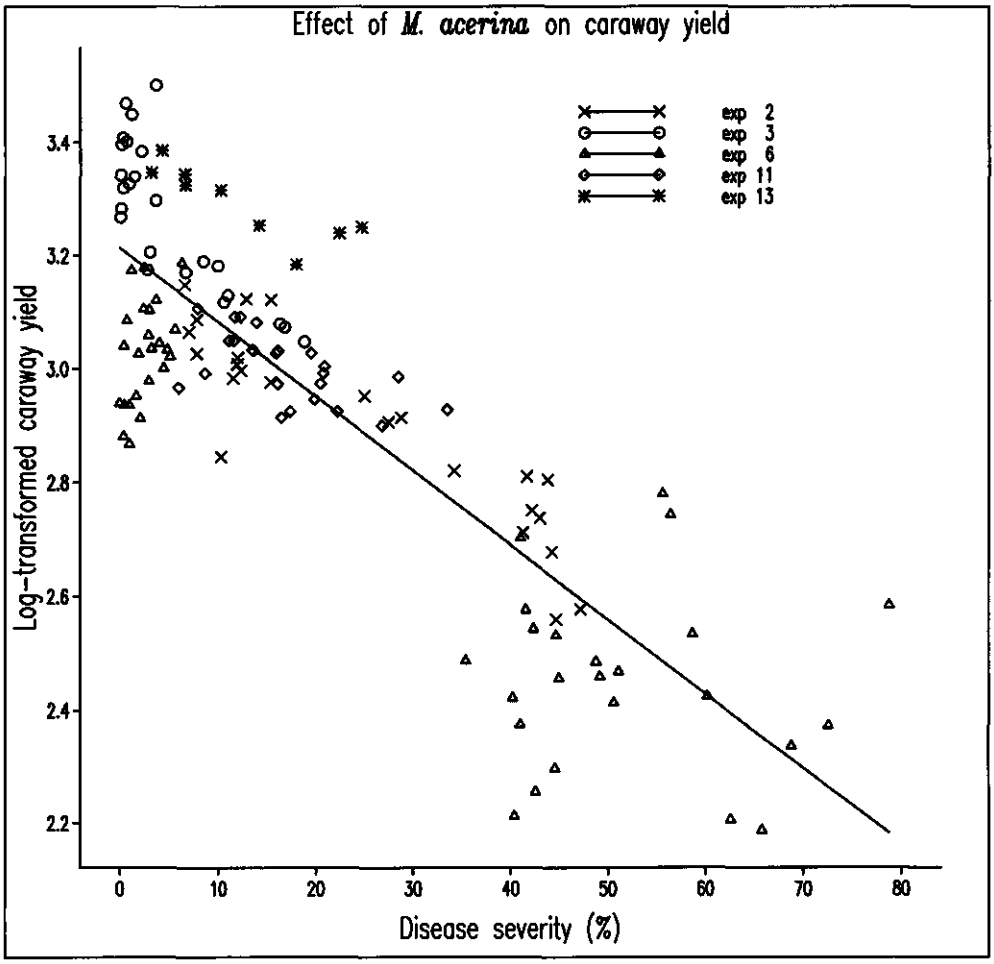

Figure 4. The relationship between disease severity (S) and log transformed yield (Y) of caraway in five experiments (n=128; r^2=0.71), P < 0.001) with standard errors between brackets:

Log(Y) = 3.21 (0.02) - 0.013 (0.0007) * S

Leaf wetness duration

Leaf wetness duration was measured with `Lufft' recorders in the nitrogen experiments (2 & 3) and with `De Wit' leaf wetness recorders in the plant density experiments (5, 7, 9 & 11). Differences in leaf wetness duration due to cultivation treatments within field experiments were significant. To compare the effect of leaf wetness duration on disease severity between experiments and between types of leaf wetness sensor, data were partially normalized. After normalization, an increase in leaf wetness duration was clearly correlated ($n=15$; $r^2=0.71$; $P < 0.001$) with an increase in disease severity. Lodging and higher plant density prolonged leaf wetness duration. In plots with these features conditions favouring *M. acerina* last longer. Spore production by *M. acerina* infection and colonization of the crop are facilitated. The damage threshold is reached earlier under these circumstances. Yield loss may be severe, especially if the crop lodges. Similarly, in carrot (*Daucus carota* L; *Umbelliferae*) the number of lesions increased with prolonged leaf wetness durations after inoculation with *Cercospora carotae* (Carisse & Kushalappa, 1992). *Mycocentrospora* spp. are related to *Cercospora* spp., and *Mycocentrospora acerina* was first described as a member of *Cercospora* family (Sutton & Gibson, 1977). In crops with higher plant densities, more infection sites are available, which may also accelerate the rate of an epidemic. In agricultural practice, activities to reduce the daily leaf wetness duration contribute to minimize yield loss due to *M. acerina* by slowing down the epidemic in the reproductive phase of the caraway crop.

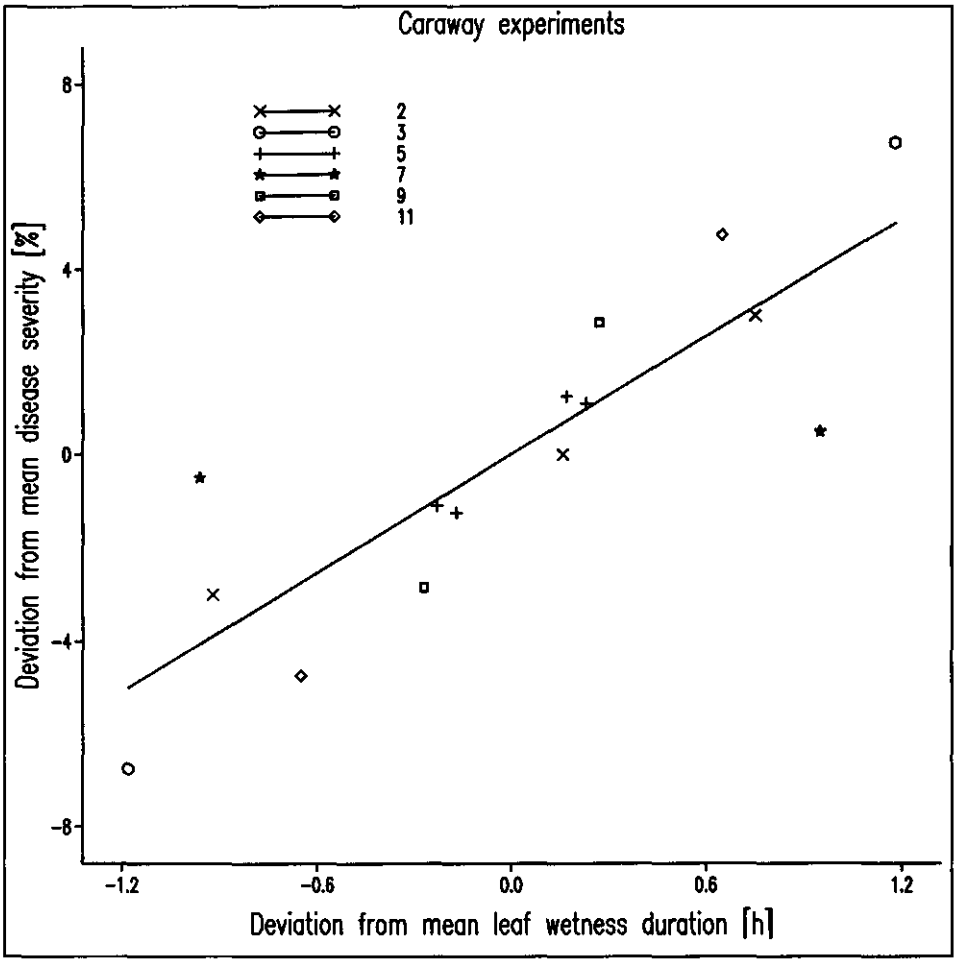

Figure 5. The effect of mean daily leaf wetness duration (W) on disease severity (S; *M. acerina*) of caraway in six caraway experiments (n=15, r^2=0.71, P<0.001) with standard errors between brackets:

S = 0.006 (0.49) + 4.24 (0.71) * W

S and W are rendered as partially normalized variates.

Nitrogen

For good caraway growth and development, an adequate supply of nitrogen is necessary. However, over-application can easily result in lodging, as was found in experiments 2 and 3. Lodging in combination with rainfall stimulated *M. acerina* development in caraway. In 1992, disease severity levels remained low in spring caraway due to a non-lodged crop and a dry and sunny summer (experiment 1). In 1993, heavy summer rainfall stimulated anthracnose development in a lodged spring caraway crop (experiment 2). The disease apparently spread into the non-inoculated plots, or a natural inoculum source was already present, since disease severity levels were rather high in these plots. Thus the interaction between nitrogen level and inoculation could not be demonstrated in 1993. The interactive effect was obvious in 1994, experiment 3. High nitrogen application in the presence of *M. acerina* resulted in severe yield loss. Where no natural inoculum source was present and spread of anthracnose into non-inoculated plots was limited, yield increased up to an optimum nitrogen level.

From these results we conclude that prevention of lodging also prevents *M. acerina* to develop towards levels causing yield loss. Lodging can be prevented by avoiding high nitrogen rates. Observations in Germany suggested that problems with *M. acerina* in caraway occurred in wet and cool years, especially when crops were over-fertilized (Müller *et al.*, 1989), which is in accordance with our results. In Poland, up to 200 kg N ha^{-1} is recommended for biennial caraway (Weglarz, 1983). The continental climate of Poland is in general dryer than the Atlantic climate of the Netherlands. Therefore under Polish conditions the microclimate in a caraway crop is less conducive to *M. acerina* development, so that higher nitrogen levels are less risky than under Dutch conditions. In the Netherlands, a nitrogen rate of 100-125 kg ha^{-1} minus the nitrogen available in the soil is recommended (Wander, 1994). From the experiments discussed in this paper it was concluded that 125 kg N ha^{-1} should be considered the upper limit to nitrogen fertilization. Modest nitrogen application meets government policy, aimed at reducing environmental pollution by means of reducing agricultural inputs (here N), and promotes an environmentally sound image of caraway and its derivates such as a sprout inhibitor of potatoes based upon carvone.

Sowing rate

In biennial caraway manipulation of the sowing rate had little effect on anthracnose development. The effect of sowing rate was largely eliminated by competition between plants for light and nutrients in the autumn leading to nearly equal numbers of reproductive plants (table 6). In contrast, spring caraway flowers without the need of producing a minimal root diameter. Therefore, in spring caraway manipulation of sowing rate is a good means to influence plant density (table 4) and, indirectly, *M. acerina* development.

Row spacing

In biennial caraway row spacing is a means for indirect control of *M. acerina*. Narrow row spacing (12 cm) led to a denser crop, whereas a wide row spacing (48 cm) increased risk of lodging (experiments 9, 11, 12 & 13). Thus, intermediate row spacing (25 - 36 cm) minimizes the risk of anthracnose development. These observations apply also to spring caraway. In experiment 9, disease severity was relatively low and seed yield increased with narrower row spacing, probably because the distribution of the plants was better at a row spacing of 12 cm than at 48 cm. In experiment 11, disease severity was higher and the effect of a better plant distribution on yield was counteracted by anthracnose development (tables 6 & 7).

The percentage of biennial caraway plants remaining vegetative depends on plant density and root size before onset of winter. Plant density is influenced by sowing rate and row spacing (table 6). Factors involved in root growth are the cover crop used, the harvest date of the cover crop and the weather conditions between harvest of the cover crop and the onset of winter. Vegetative plants do not contribute to seed production, but *M. acerina* infects these plants too. Thus, vegetative plants may enhance inoculum build-up in autumn and serve as inoculum sources for the reproductive plants in spring. The farmer should minimize the percentage of vegetative plants by lowering the sowing rate. But to guarantee plant establishment on heavy clay soils, work to ensure germination percentages to be adequate under all circumstances is still needed. Results from our study indicate that on a sandy soil a sowing rate of 6 kg ha^{-1} suits this purpose better than a sowing rate of 12 kg ha^{-1}. Experiments on clay soils in the Netherlands show that when caraway has to be harvested only once a sowing rate of 5 kg ha^{-1} results in an adequate plant density and a good yield (Bernelot Moens, 1968).

Experiments in Denmark with biennial caraway sown under spring barley pointed to an optimal sowing rate as low as 4 kg ha^{-1} and a nitrogen application in spring of approximately 90 kg ha^{-1} (Nordestgaard, 1986). In Hungary optimum yield was achieved at a row spacing of 30 cm and a sowing rate of 6-8 kg ha^{-1} (Hornok & Csáki, 1982). In Poland a sowing rate of 5 kg ha^{-1} is recommended (Weglarz, 1983). Probably the results in Hungary and Poland were obtained with biennial caraway sown without a cover crop. Our results are in accordance with the results obtained by Bernelot Moens (1968) and Nordestgaard (1986).

The effect of *M. acerina* susceptible cover crops on caraway establishment is more profound than the effect of these cover crops on disease development in caraway (Evenhuis & Verdam, 1997) The choice of cover crop and the preparation of the land show to be more critical on clay than on sandy soils, as was observed in the establishment of the caraway crops in experiments 10 & 11. A dry period after sowing resulted in poor germination and emergence and a very low plant density if biennial caraway is grown under spring barley on a clay soil. Instead of using a higher sowing rate to meet with adverse conditions, as suggested by Bernelot Moens (1968), efforts should be made to improve caraway crop establishment. The effect of the species and the variety of the cover crop on the establishment of the undersown caraway has to be investigated further. Seed dressing improved crop establishment under adverse conditions, but is not permitted in the Netherlands (Evenhuis & Verdam, 1995). However, importation of caraway seeds dressed with fungicides is accepted.

Inoculum build-up

The results obtained in this study show that in spring caraway lower inoculum levels at the start of the reproductive phase led to lower disease severity levels and reduced risk of yield loss. Therefore, supplementary crop protection activities in biennial caraway should be aimed at the reduction of inoculum build-up in autumn, because a low inoculum level will delay the epidemic in spring. Furthermore, plant breeders should develop cultivars with higher levels of partial resistance to anthracnose, to reduce the rate of development of an epidemic. Some preliminary work was done to develop a method for the selection of partial resistance to anthracnose in caraway (Evenhuis & Verdam, 1995).

Economics of crop management activities

Experiments in which nitrogen levels varied from 75 to 165 kg N ha^{-1} had no significant effect upon caraway yield (Floot, 1990). From these results it is concluded that nitrogen application can be reduced from 160 to 100 kg ha^{-1}, leading to a saving in expenditure of approximately 50 Dutch guilders ha^{-1}. Further reduction might lead to yield loss, since a nitrogen application of 75 kg ha^{-1} seemed to be sub-optimal for seed production (Floot, 1990).

Lowering the sowing rate from 10-15 kg ha^{-1} to 5 kg ha^{-1} did not result in smaller yields in biennial caraway (Bernelot Moens, 1968; Nordestgaard, 1986). Lowering the sowing rate by 5 kg ha^{-1} saves the expenditure of 5 * 15 = 75 Dutch guilders ha^{-1}. In anthracnose management, reduction of sowing rate is especially appropriate for spring caraway. An indication was found (experiment 4) that by lowering the sowing rate the yield of spring caraway decreases under disease free conditions, but the effect was small. The optimum sowing rate of spring caraway must be between 4 and 8 kg ha^{-1}, depending on seedbed conditions and sowing date.

With an increase of the row spacing the plant density decreases. With lower plant densities the root diameter will be larger and yield will increase (Weglarz, 1982). In biennial caraway, the negative effect on yield of low plant density is compensated by larger roots (Wander, 1997). Therefore, medium row spacing as a tool to reduce anthracnose development in caraway is economically relevant.

Conclusions

A damage threshold between 6 and 12% disease severity level is proposed, which provides a range of disease severities for actions to prevent yield loss of caraway due to *M. acerina*.

Activities preventing prolonged leaf wetness duration help to decrease the disease severity of *M. acerina* and consequently to increase yield stability. Reduction of nitrogen application and sowing rate, and a medium row spacing are therefore relevant management activities.

Lodging enhances the conditions favouring *M. acerina* and increases the probability of severe yield loss in the presence of *M. acerina*. In agricultural practice lodging should and can be prevented by avoiding over-fertilization.

CHAPTER 8

General discussion

Introduction

Caraway yield varies strongly from year to year. Part of this variation is ascribed to the variation in photosynthetic active radiation during flowering of the secondary and tertiary umbels (Bouwmeester *et al.*, 1995). Anthracnose caused by *Mycocentrospora acerina* (Hart.) Deighton, is a major constraint in caraway production (Chapter 7). In chapter 8 the effect of weather conditions on anthracnose was studied, and yield loss due to anthracnose was investigated. Control measures to reduce yield loss due to anthracnose are discussed. These features are illustrated with data from 13 field experiments involving biennial and 8 field experiments involving spring caraway. Most experiments were described in the previous chapters, some are described elsewhere (Evenhuis & Verdam, 1995). From each experiment agronomical, disease and weather data were assessed. Assessment of plant density, lodging, disease incidence and disease severity were described in Chapter 5. Assessment of seed infection levels was described in Chapter 4. Weather data were obtained from nearby weather stations. Data were analyzed together to find effects on both the host and the pathogen.

Nomenclature

The term anthracnose is refers to the appearance of the symptoms in the form of 'burnt black lesions' which may appear on all parts of the plant (Butler & Jones, 1955). *M. acerina* causes brown to black lesion on stems. Therefore the term anthracnose used for the disease in caraway caused by *M. acerina* is correct.

Sources of inoculum for *M. acerina*

Soil inoculum

Wet sieving and dilution plating is a technique to estimate the inoculum potential of *M. acerina* in soil (Evenhuis & Verdam, 1995). There is probably a straightforward relationship between inoculum density and disease development. Preliminary results showed that between 95 (30) and 650 (110) colony forming units per ml soil were isolated from two different samples from a soil with a caraway crop heavily diseased at the end of autumn (Evenhuis & Verdam, 1995). The standard errors are given between brackets. Davies *et al.* (1981) found approximately 30 chlamydospore chains per gram

soil adhering to roots of carrot, which they thought to be a high level. The results indicate that soil infestation can be quite high. However the wet sieving and dilution plating technique must be refined before it can be used as a technique to predict soil infestation levels before sowing susceptible crops. When infection levels in the soil are known, fields with a heavy *M. acerina* infestation may be avoided in future.

Seed infection level

M. acerina was proven to be seed-borne. The seed infection levels of caraway samples were usually between 0% and 3% (Chapter 4). Seed infection levels appeared to increase up to 50% when the crop had lodged and/or when precipitation during or after flowering was high. An average transmission efficiency of 43% was found in the greenhouse (Chapter 4). Growing caraway from a *M. acerina* infested seed lot resulted in higher levels of anthracnose development than from a non-infested seed lot (Chapter 5). Thus infested seeds may be an inoculum source for *M. acerina*. When the soil is already contaminated by *M. acerina* low levels of seed-transmitted inoculum would probably not add much to the soil-borne inoculum. A seed test was developed to determine the infestation level of seed lots (Chapter 4). A seed test enables the development of a certification scheme to promote the use of healthy seed in the future.

Dispersal of *M. acerina* conidia

Liberation and dispersal of conidia

The importance of wind seems to increase with the height of the trap. Wind does not play a role in the liberation of conidia but may contribute to the impaction of spore carrying droplets on spore traps and the impaction of *M. acerina* conidia on stems and umbels of caraway in spring. Spore catches increased with increasing rainfall. Therefore, we assume that water is necessary for the formation and/or liberation of conidia of *M. acerina*. We surmise that rain splash is the most important take-off mechanism of *M. acerina* conidia. Conidia of *M. acerina* are carried in droplets larger than 480 µm in diameter, and mainly larger than 900 µm (Darby, 1977), which only travel short distances (Ingold, 1965). Apparently, effective dispersal of *M. acerina* conidia vertically (at least 70 cm) and horizontally over medium distances (i.e. a few metres) of open area occurs on rainy days with high wind speed and turbulence and

low solar radiation. Disease incidence of the trap plants decreased steeply with distance from the inoculum source. Our results imply that a caraway crop is usually infected by an inoculum source within the field, i.e. infested soil, other host plants and/or infected seeds (Chapter 4). We surmise that inoculum sources from outside caraway fields such as non-caraway host plants and other caraway crops play no substantial part in the onset of an epidemic. This conclusion is in accordance with data from Norway, where Hermansen (1992a) trapped only a few spores just outside carrot plots.

Inoculum build-up

Spring caraway

In spring caraway there is no build-up of inoculum on vegetative plants in autumn, because the crop is sown in spring and harvested in September. Infested soil must be the main inoculum source for infection of spring caraway plants in spring and summer. Potentially the time for inoculum build-up in spring caraway is shorter than for biennial caraway. Therefore it might be expected that in normal agricultural practice problems with anthracnose in spring caraway are less severe than in biennial caraway. However, spring caraway has hardly been grown as a crop in the Netherlands until 1998. Data on anthracnose development in commercial fields of spring caraway and subsequent yield loss are not available. Disease incidence and severity levels were found to be significantly higher when spring caraway had been inoculated with *M. acerina* than in the non-inoculated control. This result suggests that increase of inoculum potential during autumn and winter is important for the levels of anthracnose expected in the second year in biennial caraway. Therefore, supplementary crop protection activities in biennial caraway should be aimed at the reduction of inoculum build-up in autumn, because a low inoculum level will delay the epidemic in spring.

Inoculum build-up due to sporulation

Abundant sporulation of *M. acerina* on senescent leaves of biennial caraway may account for a considerable inoculum build-up, especially during a wet autumn and winter (Evenhuis & Verdam, 1995). Focus expansion primarily takes place during autumn and winter in the first growing year, when the crop is in a rosette stage and

weather conditions are cool and moist. Sporulation continued even when temperatures dropped to almost 0 °C and infection was found at least until 5 °C (Chapter 2; Gündel 1976). In vitro growth was found between -3°C and 27°C (Gündel, 1976). Dispersal of conidia is limited both in quantity and in distance (Chapter 2), but a low spore production is associated with large spores and with high efficiency of spore dispersal (Ingold, 1953). The number of propagation cycles may be high, because under optimal conditions new dispersal units are produced within 3 days after infection. Probably spore production also depends on the availability of substratum. During long periods of frost leaves decay completely, but after a short period of frost decayed leaves still allow *M. acerina* to sporulate. Especially during a mild autumn and winter most of the caraway plants may become infected before spring. In carrot, the fungus survives easily on decaying leaves (Wall & Lewis, 1980b). Conidia of *M. acerina* are able to survive for at least six months (Wall & Lewis, 1978b) and probably initiate another phase of infection on senescent leaves, stems and umbels of caraway in spring. Thus, in caraway, sporulation of *M. acerina* during autumn and winter may increase the inoculum potential and supplement the other inoculum source, chlamydospores surviving in soil (Evenhuis & Verdam, 1995).

Inoculum build-up on other host plants

Several dicotyledonous species are susceptible to *M. acerina*, including crops and weeds (Newhall, 1944; Rader, 1945; Tompkins & Hansen, 1950; Rintelen & Klewitz, 1976; Hermansen, 1992b; Chapter 6). The finding of *M. acerina* on a monocotyledonous species was unexpected. In barley, infection was found only shortly after inoculation and no conidia were produced on decaying leaves. Barley is a poor host plant, at best. Perhaps *M. acerina* grew saprophytically on decaying barley tissue. Maybe the fungus survives on any decaying plant tissue irrespective of species. The function of weeds and crops for the survival of *M. acerina*, when no caraway is grown, is not fully understood. We surmise that chlamydospores infect (senescent) host plants and that resulting colonies form new chlamydospores. Therefore the level of *M. acerina* in the soil may be maintained by several weed and crop species which can host *M. acerina*. Biennial caraway is usually grown under a cover crop, which might host *M. acerina* too. On peas and barley inoculum build-up was negligible. Spinach was

more susceptible, and might have added to the build-up of inoculum in the first year (Chapter 6).

Without a cover crop biennial caraway forms many leaves, providing a dense and moist substratum, with considerable build-up of *M. acerina* inoculum. With a cover crop, caraway forms fewer leaves so that less substratum is available for *M. acerina* development. Figure 1 summarizes the estimated percentage of the plot area covered by infected leaves (assessed by vertical projection of those leaves on the plot surface) with *M. acerina* of caraway fields with different cover crops in autumn 1992 and 1993 (Chapter 6). Anthracnose development in biennial caraway was significantly (n=24; P <0.001) higher when no cover crop was used than in caraway sown under a cover crop. Spinach preceding caraway caused significantly more anthracnose in spring caraway than bare soil. Peas and spring barley as preceding crops had no effect on disease severity of spring caraway.

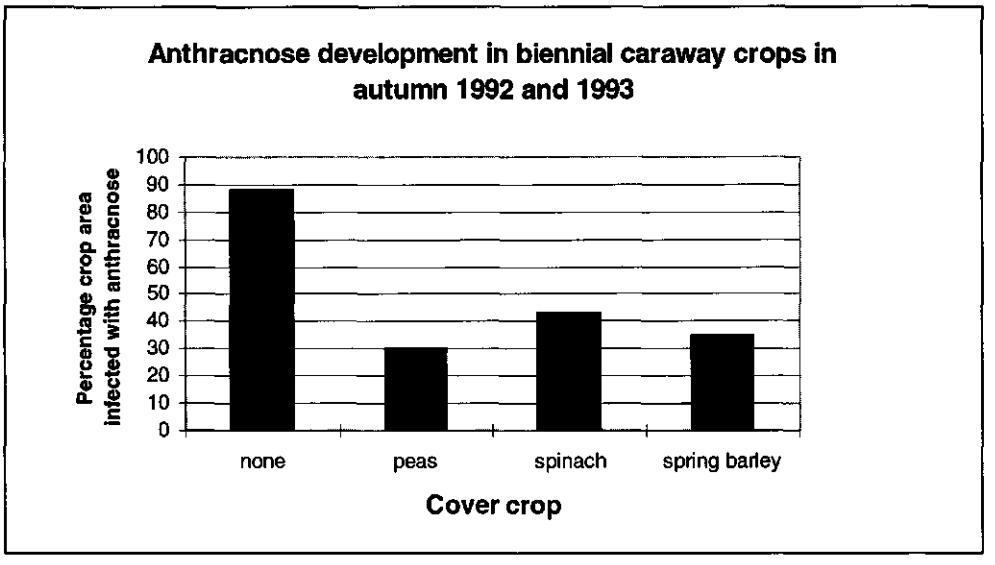

Figure 1. Caraway was sown with (peas, spring barley and spinach) and without a cover crop in 1992 and in 1993. The percentage of the plot area with *M. acerina* infected leaves was estimated at the end of autumn, and given as a mean over both years.

Crop rotation experiments with carrot in Norway showed only a slight decrease of liquorice rot during a period of three years, which indicated that inoculum levels in the soil only decrease slowly (Hermansen, 1992a). Among the rotations tested, only clover and grass reduced the infection of *M. acerina* significantly compared to a monoculture with carrots. A 3 year rotation with grass or barley was as effective as 6 and 8 year rotation with the same crops (Hermansen *et al.*, 1997). Nevertheless, growing non-susceptible crops might help to prevent a 'clean soil' from becoming infested (Hermansen, 1992a). Weeding might help to control *M. acerina* build-up or survival, although the effect will probably be small. Growing a susceptible crop in autumn and winter as green manure might enhance survival of *M. acerina* or even stimulate inoculum build-up. Therefore, the possibilities for control of *M. acerina* by crop rotation are limited, at best. From the results on cover crops and preceding crops we conclude that inoculum build-up on other crops is limited, but that these crops may play a role in the survival of *M. acerina* in the soil. An exception has to be made for the highly susceptible spring caraway as a cover crop. *M. acerina* inoculum build-up on spring caraway will probably be high, and undersown biennial caraway may be heavily attacked in the next year.

Straw inoculum

Chlamydospores in soil stem from conidia in run-off water, which form chlamydospores (Wall & Lewis, 1978a) or by ploughing infested caraway straw into the soil after harvest. These chlamydospore chains in the soil may infect a new caraway crop or other host plants and increase inoculum levels in the soil. Caraway sown in soil treated with infested caraway, spinach, spring barley and pea debris became infected by *M. acerina* under greenhouse conditions (Chapter 6). Though the disease incidence was not significantly different from the control, the non-zero values suggests that plant debris of caraway and the tested cover crops transmitted *M. acerina*. Disease incidence of caraway seedlings in the debris treatments was low in comparison to the chlamydospore treatments. The difference suggests that only a limited amount of inoculum can be brought into the soil with infested plant debris. In the field, chlamydospore chains were formed in affected plant tissue. In the soil, chlamydospores are viable for at least two years (Wall & Lewis, 1980c). Even caraway grown in a six years rotation may suffer from a previous caraway crop since inoculum levels decrease

slowly, in part due to the broad host range of *M. acerina*. As a safety precaution, infested caraway straw might be removed from the field, although the amount of inoculum carried off is probably be small.

Crop establishment

The percentage of biennial caraway plants remaining vegetative depends on plant density and root size before onset of winter. Plant density is influenced by sowing rate, row spacing, preparation of the soil and seed infection levels. Factors involved in root growth are plant density (Hornok & Csáki, 1982), the cover crop used, the harvest date of the cover crop and the weather conditions between harvest of the cover crop and the onset of winter. A positive correlation was found between root diameter of caraway on the one hand and percentage of flowering plants and seed yield on the other hand (Weglarz, 1983). For bolting and flowering of biennial caraway the root diameter of the plants in the autumn of the first year must be at least 6 mm (Bernelot Moens *et al.*, 1973).

Cover crops

The effect of *M. acerina* susceptible cover crops on caraway establishment was more profound than the effect of these cover crops on disease development in caraway (Chapter 6). The choice of cover crop and the preparation of the land were more critical on clay than on sandy soils. In experiments by Bernelot Moens (1968), Vreeke (1989) and ourselves, great differences in caraway yield were found using different cover crops. The effects can be explained by the light interception by the cover crop and the time at which the cover crop is harvested. If more light is available to the caraway crop, more seedlings will survive and growth will be improved. Consequently the tap roots will be larger and a higher proportion of the plant will flower in the second year (Wander, 1994). A farmer should choose a cover crop which is harvested early in the season or has an open character, such as peas, spinach for seed production or seed poppy. From these crops, spinach is the most susceptible to *M. acerina* and therefore the least desirable. Unfortunately, the afore mentioned cover crops have only limited marketing potential. Cereals provide an alternative, but are less suitable when crop management aims at high grain yields (Bernelot Moens, 1968; Vreeke, 1989). The

effect of the species and the variety of the cover crop on the establishment of the undersown caraway has to be further investigated.

Effect of seed infection level on crop establishment

In caraway, seed-borne *M. acerina* delayed emergence and caused damping-off and death of emerged seedlings. High seed infection levels reduced total emergence and subsequent density of flowering plants and seed yield. At higher seed infection levels the percentage of senescent leaves was higher at any time, and leaf senescence occurred earlier in autumn, thus restricting the growth of the caraway roots. A similar effect was found in carrot where plants surviving an early *M. acerina* attack usually senesced earlier than healthy plants (Wall & Lewis, 1980a).

Effect of infected seeds on crop establishment and anthracnose epidemics

High seed infection levels potentially initiate an anthracnose epidemic in caraway (Chapter 5). The follow-up depends largely on the weather during flowering of the crop. Leaf senescence and plant density in autumn respond to seed infection indicating that seed infection has an impact on the first year's events. Yield, disease incidence and severity, and AUDPC observed in the second year respond far less to seed infection. The relatively small effect of seed infection on the second year's events may be due to the effect on plant density in the first year, which levels off some of the effects of seed infection. However, given suitable conditions for *M. acerina*, the effect of seed-borne inoculum might become far more pronounced and the loss due to a decrease in reproductive plants might well exceed the 20% level of the 1993/94 experiment. Accordingly, we believe that high seed infection levels make it more difficult to establish a caraway crop with sufficient plants to flower the next year. Since crop establishment is already a bottleneck in the growing of caraway under cover crop, damage by *M. acerina* should be avoided by sowing healthy seed. In the greenhouse and under adverse conditions in the field, seed dressing of caraway seeds improved total emergence (figure 2) and crop establishment. With increasing soil inoculum density, emergence percentage of caraway decreased significantly in the untreated control and seed dressed with fosetyl-Al. Fosetyl-Al proved to be phytotoxic. A non-significant increase in emergence was found in caraway treated with carbendazim or

iprodione (figure 2). Some of the fungicides used as seed dressing controlled infection of seedlings significantly as illustrated in figure 3 (Evenhuis & Verdam, 1995).

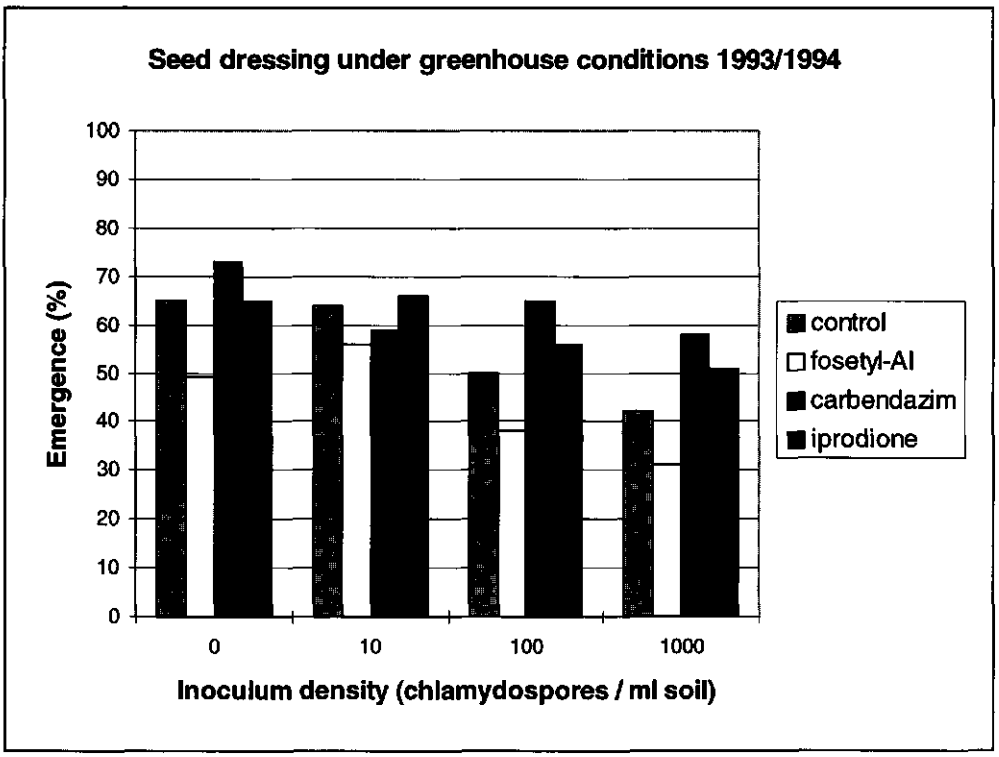

Figure 2. Caraway seed lots were dressed with fungicides and sown in the greenhouse in autumn 1993, spring 1994 and autumn 1994. Pasteurized soil was inoculated with *M. acerina*. Inoculum densities were 0, 10, 100 and 1000 chlamydospore chains per ml soil. Emergence of caraway treated with fungicides was rendered as the mean of three experiments.

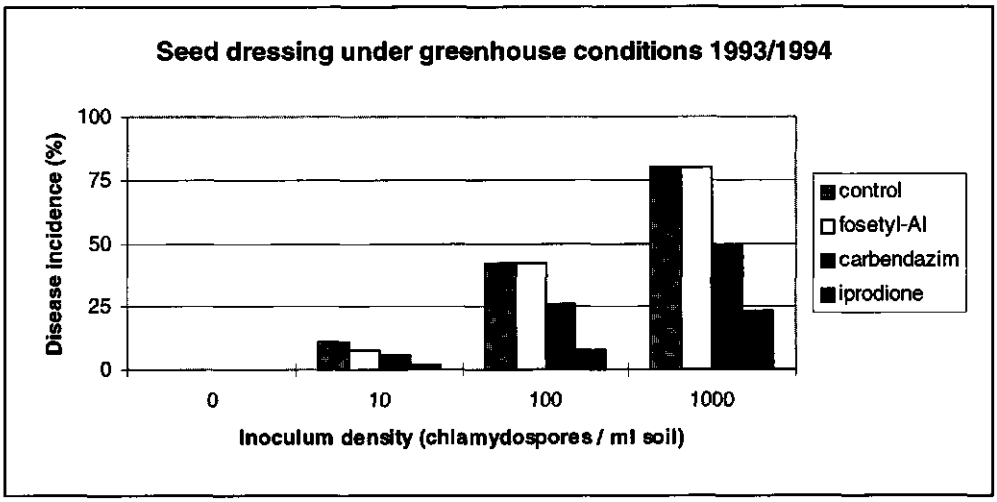

Figure 3. Caraway seed lots were dressed with fungicides and sown in the greenhouse in autumn 1993, spring 1994 and autumn 1994. Pasteurized soil was inoculated with *M. acerina*. Inoculum densities were 0, 10, 100 and 1000 chlamydospore chains per ml soil. Disease incidence of caraway treated with fungicides was rendered as the means of three experiments. In the control treatment no infection of seedlings by *M. acerina* was found.

Sowing rate.

Vegetative plants do not contribute to seed production, but *M. acerina* infects these plants too. Thus, vegetative plants may enhance inoculum build-up in autumn and serve as inoculum sources for the reproductive plants in spring. Lowering the sowing rate to approximately 6 kg ha^{-1} is a means to minimize the percentage of vegetative plants. But to guarantee plant establishment on heavy clay soils, more work to ensure adequate germination and emergence under adverse circumstances is needed. Under greenhouse conditions, seed treatment provided a good protection for seedlings against anthracnose. Seed dressing improved crop establishment under field conditions too, but seed dressing of caraway is not permitted in the Netherlands (Evenhuis & Verdam, 1995). However, importation of caraway seed dressed with fungicides is accepted.

Eventual control measures to prevent inoculum build-up

In Norway, carrots are treated four times with iprodione in autumn shortly before harvest (Netland, 1993). In winter caraway, a single application of iprodione in autumn

had no effect on disease severity in spring (Evenhuis & Verdam, 1995). Repeated application of fungicides in caraway is economically and environmentally undesirable. Biological control might reduce inoculum build-up. *Trichoderma harzianum* (Tronsmo, 1989) and *Pythium oligandrum* (Lutchmeah & Cooke, 1984) were found to control *M. acerina* in stored carrots to some extent. Antagonists such as *Trichoderma* spp. which should be adapted to cold weather might reduce the build-up of inoculum in autumn. Biological control must be developed and tested for its agricultural value.

Infection of roots

Wounding and ageing of caraway roots

In carrot (Davies *et al.* 1981) and celery (Day *et al.*, 1972) *M. acerina* was facilitated by wounding of the plant, but in parsnips (Channon, 1965) and celery roots in storage (Gündel, 1976) wounding had no effect on *M. acerina*. In caraway, *M. acerina* does not need visible wounds to infect roots in cold storage, although both disease incidence and severity increased when roots were artificially injured. A chemical component like falcarindiol, which is probably the resistance factor in the outer tissues of the carrot roots (Davies & Lewis 1981; Garrod & Lewis 1982), may exist in caraway roots too. A decrease in the concentration of such a component in the inner tissues would explain why injury of caraway roots leads to the formation of progressive lesions, as in carrots, whereas only restricted lesions are formed on roots with no or minute wounds (Chapter 3). For breeding purposes, identification and measurement of the chemical involved could be interesting.

An alternative explanation might be that the mechanical resistance to penetration is much higher in the epidermal layer than in tissues beneath. Minute injuries especially on or near small lateral roots and root hairs may be the points of entry for *M. acerina*. Organisms of the soil fauna can cause minute to medium sized wounds. Some agricultural practices cause injuries, such as weeding. Under cool conditions wound healing will be slow, as indicated by the fact that a period of high temperatures before cold storage accelerated wound repair of harvested carrot roots and diminished infection by *M. acerina* (Lewis *et al.*, 1981). The development of caraway slows down and growth almost ceases as the temperature falls during autumn. In such a period the defence of the roots probably weakens, so that *M. acerina* could penetrate and colonize the roots.

Especially in winter, conditions are relatively favourable for *M. acerina* development since the fungus is able to grow at low temperatures (Newhall, 1944; Neergaard & Newhall, 1951; Channon, 1965; Gündel, 1976) so that the fungus gains an advantage over the host in winter time. Any injury during such a period might lead to successful penetration and colonization of the caraway root.

Roots, of caraway to be harvested for a second time in succession, tend to be more injured than roots of a first time caraway crop. This finding indicates that a second harvest caraway crop runs a higher risk to become seriously infected by *M. acerina* than a first harvest crop. In addition an indication was found that the incubation period of *M. acerina* on caraway roots lifted 100 days after sowing was much longer than on caraway roots lifted 227 to 373 days after sowing. Caraway roots in crops were frequently injured at the end of autumn. These injuries increase the probability of roots becoming infected by *M. acerina*. In several individual fields positive relationships were found between root injury and root rot in cold storage. However, taking the fields together, linear regression showed no significant relation between mean root injury index in autumn and mean root rot index after six months of cold storage. Apparently root injury can play a role in the loss of caraway plants through root rot, but that role is not very prominent. Root injury assessment in autumn cannot be used as an indication for anthracnose development on roots.

Infection of stems and umbels

Environmental conditions

The progress of disease incidence of caraway from bolting until approximately three weeks before harvest can be described by Gompertz curves, as shown in Chapters 5 and 7. It is an indication that an epidemic of *M. acerina* in caraway is a polycyclic process. *M. acerina* progress curves for biennial caraway inoculated in autumn, spring and non-inoculated crops shows that inoculum build-up in autumn gave stronger epidemics (figure 4).

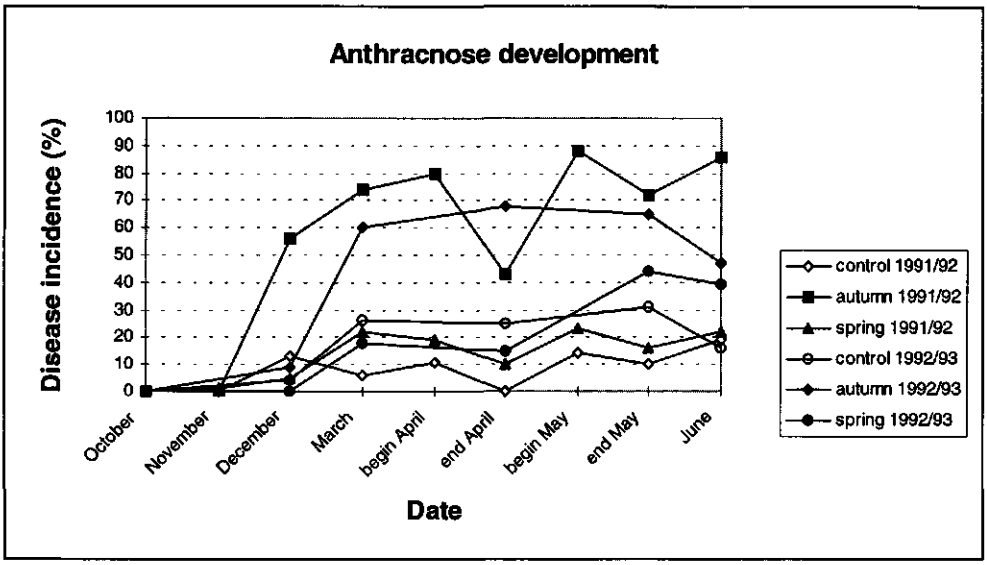

Figure 4. Inoculation of caraway in autumn resulted in higher disease severity levels in caraway than inoculation in spring and the water sprayed control treatment.

Disease severity (S) of biennial caraway assessed in spring increased significantly ($n=13$, $r^2=0.80$, $P<0.001$) with higher amount of rainfall (R_{nd}) in November and December (equation 1). Probably, inoculum build-up on senescent leaves of the rosette was stimulated by moist conditions due to heavy rainfall in autumn. An increase of inoculum in autumn leads to a higher risk of *M. acerina* infection of stems and umbels in the following spring, if weather circumstances permit anthracnose development. In 1991 and 1992, disease severity levels of biennial caraway were significantly higher after inoculation in the autumn of the previous year than after inoculation in the spring (Evenhuis & Verdam, 1995).

$$S = -28.3\ (5.0\ ) + 0.17\ (0.02) * R_{nd} \qquad (1)$$

The amount of rainfall in spring had an effect on disease incidence and disease severity levels approx. 3 weeks before harvest. Rainfall in the period of flowering (R_f) and

disease severity (S) of biennial and spring caraway were correlated (n=21, r^2=0.62, P<0.001; equation 2)

$$S = -8.1\ (2.9) + 0.20\ (0.04) * R_f \qquad (2)$$

Disease severity (S) was also correlated with lodging (L) of the crop (n=21, r^2=0.57, P<0.001), equation 3.

$$S = 2.7\ (1.5) + 0.35\ (0.07) * L \qquad (3)$$

Whether *M. acerina* infection will be severe or not depends partly on weather conditions. *M. acerina* was prevalent in fields with dense canopies. Reduction of sowing rate, wider row spacing and reduction of nitrogen application diminished disease severity. Lodging of the crop should be avoided under any circumstance, because lodging favours anthracnose in caraway (equation 3). Selection for short straw varieties might help to prevent lodging and still allow nitrogen application to obtain a reasonable yield.

Spatial patterns of anthracnose severity

Disease incidence and disease severity of three biennial caraway crops were assessed as described in Chapter 5. The caraway crops were grown on a sandy soil situated at Wageningen. Row distances of the caraway fields were 25 cm in 1991, 25 cm in 1992 and 12.5 in 1993. Observations were made in caraway fields infested by a natural inoculum source. Disease assessments on caraway were made about three weeks before harvest. All plants were inspected within a 6 by 6 m area. The area was subdivided in quadrats of 25 by 25 cm, so that disease assessments were available for 576 quadrats. The position of the quadrats was mapped. Morisita's index of dispersion (equation 4) was used to determine distribution and spatial pattern of disease severity (Morisita, 1959; Schuh *et al.*, 1986). However, Schuh *et al.* (1986) used disease incidence where we used disease severity to calculate the index of dispersion.

$$I\delta = [(\Sigma(S^2) - \Sigma S) / ((\Sigma S)^2 - \Sigma S)] n \quad (4)$$

In equation 4, $I\delta$ = index of dispersion, S = disease severity and n = the number of sampling units.

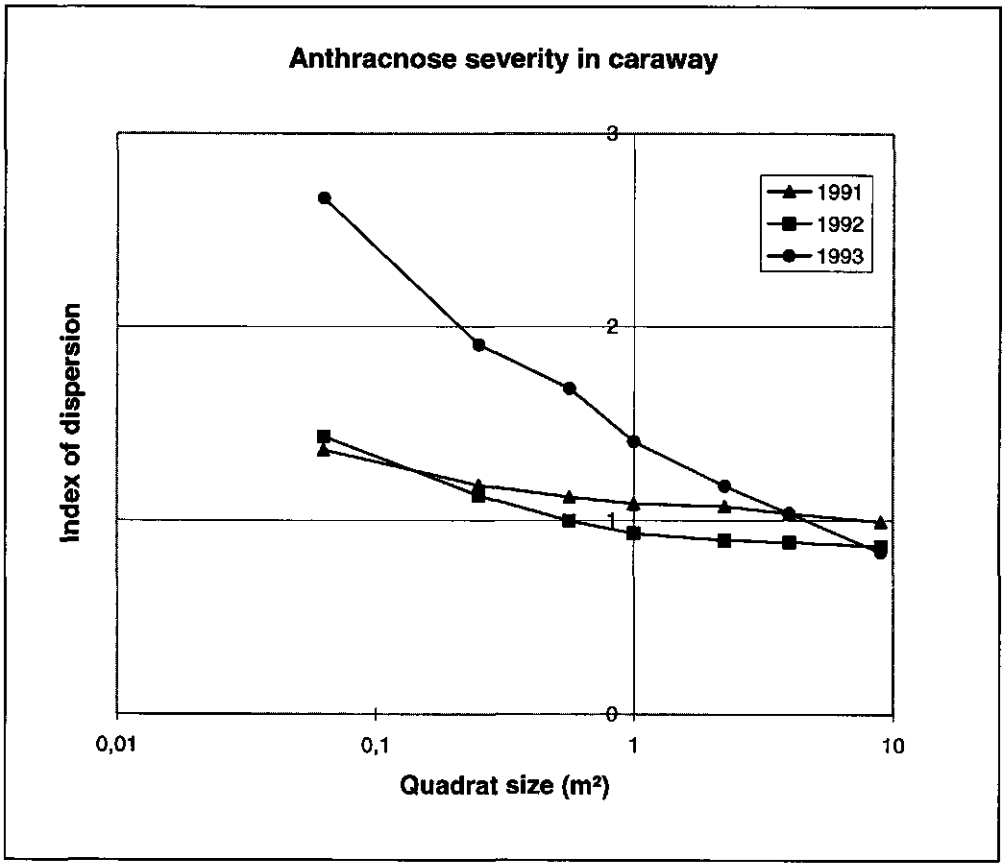

Figure 5 Morisita index at different quadrat sizes for anthracnose disease severity in caraway at Wageningen in 1991 to 1993.

Figure 5 shows a reduction of indices (Iδ) with larger quadrat sizes. In small quadrats, Iδ was higher than 1 indicating a clumped distribution. When quadrat size was increased, Iδ decreased and eventually was less than 1 which suggests an uniform distribution of disease severity (Schuh *et al.*, 1986). Morisita index was highest in 1993, probably because disease severity was rather low, especially compared to disease severity of biennial caraway in 1991. In 1993, anthracnose development was mildest on average, but with a few clusters of quadrats with $S \geq 15\%$ and a majority of quadrats with $S \leq 2\%$. Median disease severity was only 1.8 % in 1993. In 1991 median disease severity (32.2 %) was much higher than in 1993 and the disease had spread through the field, thus lowering the Morisita index. In 1992, median disease severity (4.3 %) was two and a half times higher than in 1993, but maximum disease severity was much smaller (25 % in 1992 versus 35 % in 1993). We surmise that many small local inoculum sources were present with a uniform distribution over the field. Figures 6, 7 & 8 show the patterns of disease severity in these caraway crops in 1991 to 1993 at Wageningen.

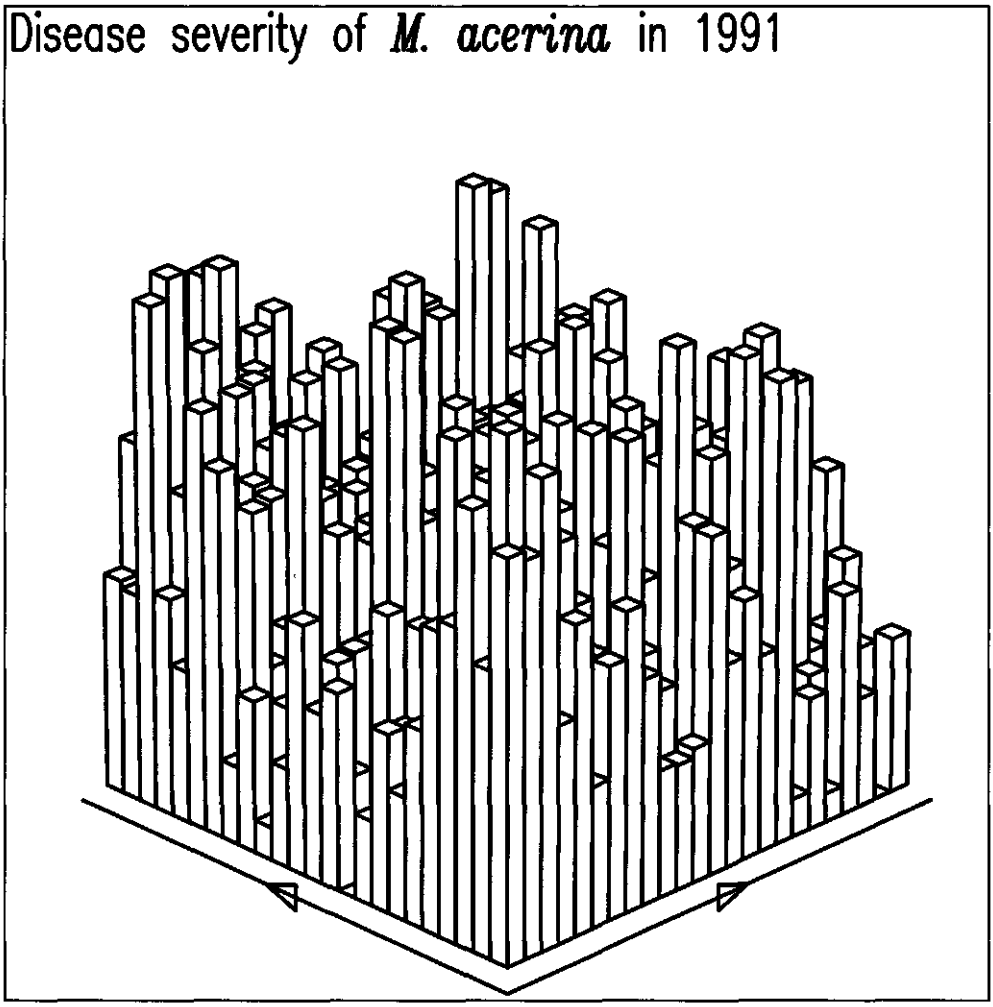

Figure 6. Disease severity pattern of *M. acerina* in biennial caraway on a sandy soil at Wageningen in 1991. The maximum disease severity was 93%, mean and median disease severities were 36 and 32 %. The field was divided in 256 quadrats of 25 cm * 25 cm.

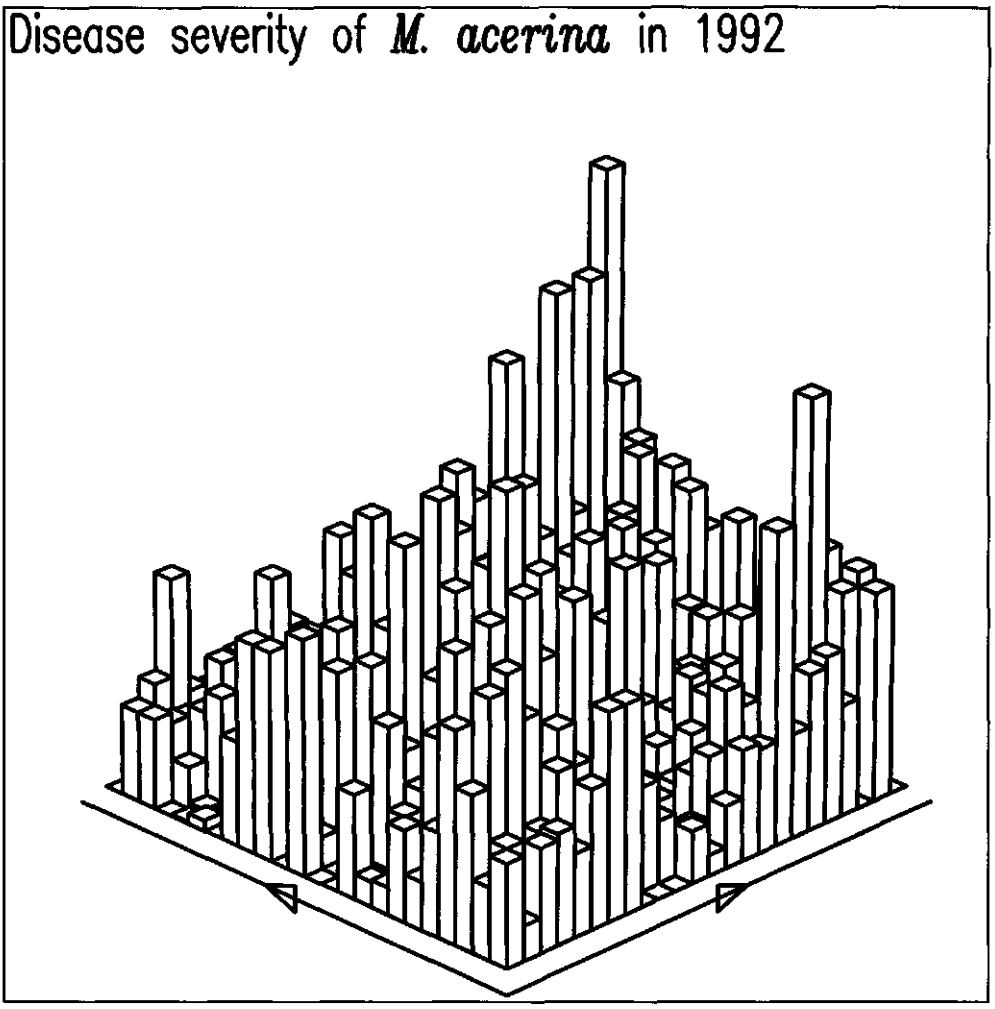

Figure 7. Disease severity pattern of *M. acerina* in biennial caraway on a sandy soil at Wageningen in 1992. The maximum disease severity was 25%, mean and median disease severities were 5.0 and 4.3 %. The field was divided in 256 quadrats of 25 cm * 25 cm.

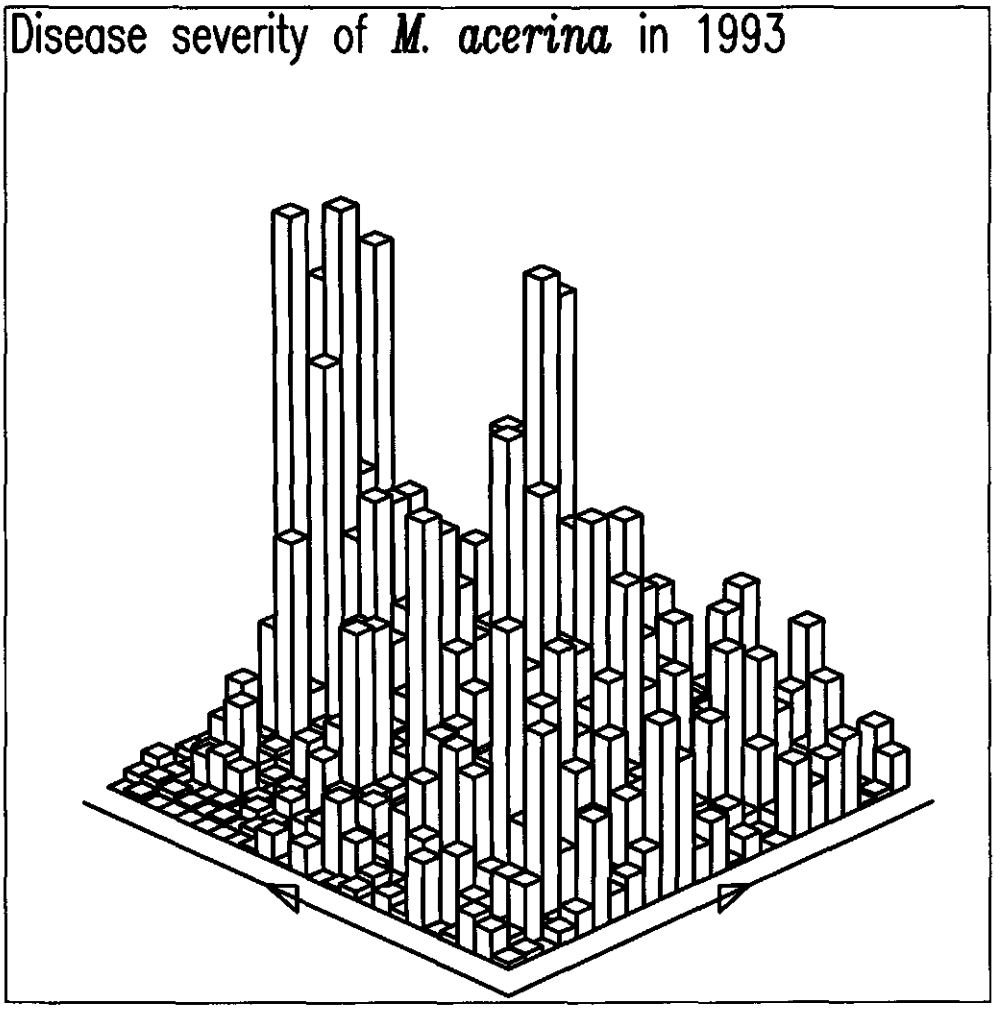

Figure 8. Disease severity pattern of *M. acerina* in biennial caraway on a sandy soil at Wageningen in 1993. The maximum disease severity was 35%, mean and median disease severities were 3.9 and 1.8. The field was divided in 256 quadrats of 25 cm * 25 cm.

Yield loss

Lesions formed on stems could become so large that parts above the infection site died, causing yield loss. In spring caraway, yield loss due to anthracnose was found to be significant at Lelystad in 1993 (Chapter 7, exp. 2; 44%) and Nieuw Beerta in 1993 (Chapter 7, exp. 6; 75%). In biennial caraway, yield was significantly reduced by *M. acerina* at Randwijk (Chapter 7, exp. 3; 33%), Wageningen (Chapter 7, exp. 11; 30%) and Lienden (Chapter 7, exp. 13; 30%) in 1994. The mean disease severity in these experiments varied from 12 to 28 %. In order to compare biennial and spring caraway, relative seed yield (RY) per field experiment was calculated. Relative seed yield was based upon the seed yield of plots not or slightly infected with *M. acerina*. Disease severity (S) had a significant (n=21, r^2=0.63, P<0.001) effect on relative seed yield (RY) of spring caraway and biennial caraway as described by equation 5 (Evenhuis & Verdam, 1995).

$$RY = 99.5\ (1.9) - 1.03\ (0.17) * S \qquad (5)$$

However, a regression with actual yield as the dependent variable is more interesting. Since the potential yield of biennial and spring caraway differ much, the caraway type has to be introduced in the regression. A significant effect of both crop type and disease severity were found on yield (n=21, r^2=41.5, P<0.001), as described in equation 6. In equation 6 the parameter C is 0 when biennial caraway is grown and 1 when spring caraway is grown. No interaction was found between crop type and disease severity. The lack of interaction indicates that spring and biennial caraway are equally susceptible to *M. acerina* and respond with yield loss in the same manner and with the same magnitude. Apparently in our experiment the potential yield of biennial caraway is 2109 kg ha^{-1} and exceeds spring caraway with 709 kg ha^{-1}.

$$Y = 2109\ (257) - 31.8\ (25.0) * S - 709\ (318) * C \quad \begin{array}{l|l} & C = 0 \text{ biennial caraway} \\ & C = 1 \text{ spring caraway} \end{array} \qquad (6)$$

No relationship between yield and disease severity was found in experiments with mean disease severity levels below approximately 6%. We conclude that the damage threshold must be between 6 and 12% for both biennial and spring caraway. No significant effect of disease severity on yield was found in an experiment at Wageningen in 1993, notwithstanding a mean disease severity of 16% (Chapter 7, exp. 7). Disease severity varied from 6 to 39 % between plots and corresponding yields varied from 200 to 800 kg ha^{-1}. On average yields were low, even for spring caraway grown on a sandy soil. On clay soils the average yield of spring caraway was 1436 kg ha^{-1} (Toxopeus & Bouwmeester, 1993). We found on average 1315 kg ha^{-1}. We interpret the low yields at Wageningen in 1993 as an indication that a disease severity of 6% already exceeds the damage threshold, although part of the yield decrease is explained by the fact that the crop was grown on a sandy soil.

Leaf wetness duration

Over-application of nitrogen can easily result in lodging and anthracnose development (Chapter 7). In Germany, an increase of anthracnose development in caraway was found in a cool and wet season in combination with overfertilization (Müller *et al.*, 1989). Lodging and higher plant density prolonged leaf wetness duration. In plots with these features, conditions favouring *M. acerina* lasted longer and an increase in disease severity was found. Spore production by *M. acerina*, infection and colonization of the crop are facilitated. In crops with higher plant densities, more infection sites are available, which may accelerate the rate of an epidemic. The damage threshold is reached earlier under these circumstances. Yield loss may be severe, especially if the crop lodges. Any measure to reduce the daily leaf wetness duration will contribute to minimize yield loss due to *M. acerina* by slowing down the epidemic in the reproductive phase of the caraway crop. Prevention of lodging by modest nitrogen application also prevented *M. acerina* from developing to levels causing yield loss.

Manipulation of the sowing rate had little effect on anthracnose development in biennial caraway. The effect of sowing rate was largely eliminated by competition between plants for light and nutrients in the autumn leading to nearly equal numbers of reproductive plants. In contrast, nearly all plants of spring caraway are reproductive. Therefore, in spring caraway manipulation of sowing rate is a good means to influence plant density and, indirectly, *M. acerina* development (Chapter 7). In biennial caraway,

narrow row spacing (12 cm) led to a denser crop whereas wide row spacing (48 cm) increased risk of lodging. Thus, intermediate row spacing (25 - 36 cm) minimized the risk of anthracnose development. These observations apply also to spring caraway. In general, when disease severity is relatively low, seed yield increases with narrower row spacing, probably because the distribution of the plants is better at a row spacing of 12 cm than at 48 cm. When disease severity is higher than the damage threshold the effect of a better plant distribution on yield is counteracted by anthracnose development.

Recommendation for plant protection

Problems with anthracnose of caraway occur after long periods of rainfall. The disease cannot be prevented by any single crop protection measure. However, a number of measures reduces the occurrence of *M. acerina*. Reducing the risk of yield loss due to this pathogen should begin with: 1) A disease-free start to delay the build-up of inoculum (disease-free seeds, choice of an uncontaminated field). 2) Slowing down the epidemic in spring by taking measures to avoid lodging and to limit leaf wetness duration in a crop (reduction of sowing rate, intermediate row spacing and reduction of nitrogen fertilization). 3) Seed dressing can retard the first infection of seedlings, but it is illegal in the Netherlands. Fungicides against white mould (*Sclerotinia sclerotiorum*) sometimes reduce anthracnose in caraway, but results are not guaranteed.

Plant breeders should develop cultivars with higher levels of partial resistance to anthracnose, to reduce the rate of progress of an epidemic. Some preliminary work was done to develop a selection method for partial resistance to anthracnose in caraway. Genetic diversity for resistance against anthracnose seems to be present in caraway populations (Evenhuis & Verdam, 1995).

Conclusions

1. Spore dispersal can be quantified satisfactorily by using inverted Petri dishes or trap plants. Spore dispersal is stimulated by rainfall and moderate (15-20°C) temperatures, whereas solar radiation has a negative effect on spore catches. Dispersal gradients of *M. acerina* are so steep that the primary inoculum sources must be present within the caraway fields themselves. Field verges and nearby diseased crops hardly play a role as an inoculum source.
2. Root injury increases the risk of root infection by *M. acerina*. An indication was found that a caraway crop, which is to be harvested a second time, runs a higher risk of becoming seriously infected by *M. acerina* than a first harvest crop.
3. *M. acerina* is seed-borne on caraway. For at least three years, *M. acerina* remains viable on caraway seeds in storage. To overcome part of the problems in caraway due to anthracnose and to establish a viable crop (sufficient plant density and root diameter), seeds should be free from *M. acerina*.
4. For rapid screening of seed-borne *M. acerina* the freezing-blotter method is suggested as the most suitable and reliable method. Formal introduction of this technique allows to set an upper limit to the percentage of caraway seed infected by *M. acerina* in certified seed lots.
5. When a seed test cannot be performed it is recommended to use seed with minimum exposure to *M. acerina*, e.g. seed from fields with an open stand and without lodging, and/or seeds from years with low rainfall during bolting, flowering and ripening.
6. Seed infection hampers caraway crop establishment and promotes anthracnose development in caraway.
7. Anthracnose management by crop rotation, including the choice of cover crop, contains little promise, but crop rotation in combination with weed control might help to suppress inoculum build-up. The choice of the cover crop should be based primarily on its agronomical value as a cover crop. *M. acerina* can survive in crop debris. Removal of infested straw from the field might be considered as a safety measure, though with limited effect.

8. A damage threshold between 6 and 12% disease severity level is proposed. Thus, for anthracnose a zero-tolerance is not required, which provides a margin to prevent yield loss of caraway due to *M. acerina*.

9. Measures preventing long leaf wetness duration help to decrease the disease severity of *M. acerina* and consequently increase yield stability. Reduction of nitrogen levels and sowing rate, and intermediate row spacing are therefore relevant management methods.

10. Lodging enhances the conditions favouring *M. acerina*, therefore increasing the probability of severe yield loss in the presence of *M. acerina*. In agricultural practice lodging should and can be prevented.

11. Research on *M. acerina* was conducted with caraway as host plant. Results obtained might help to combat this polyphagous fungus in other economically interesting crops (e.g. carrots, celery).

CHAPTER 9

Summary

Summary

This thesis describes aspects of the biology of *Mycocentrospora acerina* (Hart.) Deighton and their implications for caraway (*Carum carvi* L.) cultivation. The biology of *M. acerina* under field conditions was not yet described satisfactorily. More is known of *M. acerina* as the causal agent of liquorice rot, a post-harvest disease in carrots. Various studies in caraway were performed in the period from 1990 until 1995 with the aim to obtain information on *M. acerina* and to generate a set of crop protection methods based on this information. Chapter 1 provides an introductory overview.

Chapter 2 reports on the dispersal of conidia of *M. acerina* in caraway field trials. A Burkard spore trap, rotorods, inverted Petri dishes containing sucrose agar and rain gauges were used to trap conidia of *M. acerina*. Sporulation was stimulated by rainfall (≥ 2 mm) and moderate temperatures (around 15°C). Solar radiation had a negative effect on sporulation. Hardly any conidia were found in the spore traps on rainless days. Short distance (≤ 9 m) spread of *M. acerina* was mainly caused by splash dispersal of its conidia. Trap plants at 0 to 4 m from the inoculum source were readily infected under moist conditions. Beyond 9 m from an inoculum source no infection of caraway trap plants was found. Trap plants at 9 m from an inoculum source were infected in one out of three seasons only. Long distance (> 9 m) spread could not be demonstrated by the techniques used in this study. The results suggest that, usually, a caraway field is infected by inoculum sources within that field.

Chapter 3 deals with the effect of mechanical injury on disease incidence, incubation period and lesion development rate on caraway roots following infection by *M. acerina*. After inoculation with *M. acerina*, disease incidence of injured roots was significantly higher than of non-injured roots under laboratory conditions. The incubation period of *M. acerina* was significantly shorter on injured roots than on non-injured roots. The incubation period shortened with increasing root injury level. Younger injured roots tended to be more resistant to *M. acerina* infection than older injured roots, as expressed by longer incubation periods. The lesion development rate was, on average, higher on heavily injured roots than on non-injured or slightly injured roots. The lesion development rate remained fairly constant after the first appearance of the symptoms on

the caraway root, until the whole root was colonized. Caraway roots carefully dug up in autumn frequently showed injuries enabling *M. acerina* to penetrate the roots. However, the correlation between root injury and root rot after cold storage was weak. Injury of roots had a stimulating effect on infection by and development of *M. acerina*, but roots without wounds could be infected too.

Seed transmission of *M. acerina* in caraway is demonstrated in Chapter 4. Seed transmission efficiency varied between 27 and 62%. Methods to quantify *M. acerina* seed infection in caraway are presented and discussed. Seed infection levels were usually less than 3%, but infection levels up to 50% were found. *M. acerina* remained viable in caraway seeds for at least three years. Results suggest that infected caraway seed can be an inoculum source for the development of anthracnose in caraway crops. The importance of seed infection for long distance dissemination and for the onset of an epidemic is discussed.

The effect of seed infection of caraway by *M. acerina* on crop establishment and yield was studied in field experiments described in Chapter 5. High seed infection levels hampered crop establishment of caraway and limited the number of plants producing a root diameter large enough to permit flowering in the next year.

Chapter 6 reports on the search for inoculum sources of *M. acerina* on caraway. Obvious suspects are cover crops of biennial caraway and preceding crops of annual caraway. Other suspects are weeds in or alongside the field. Finally, survival structures of the fungus, chlamydospore chains, packed in plant debris or naked, are suspected. *M. acerina* is able to infect many plant species, including cover crops of caraway such as spinach for seed production and peas. However, the agronomical suitability of a crop to serve as a cover crop of biennial caraway proved to be a more important factor in determining caraway yield than the susceptibility of the cover crop to *M. acerina*. This finding was corroborated by the fact that spinach and peas as preceding crops had no significant effects on *M. acerina* development in spring caraway sown the next year. Dill, barley and four weed species were found as new hosts of *M. acerina*. The role of weed hosts, susceptible crops and plant debris in the survival of the fungus in years without caraway is discussed. Caraway sown on soil containing infested caraway straw, infested debris of other plant species or chlamydospores grown in pure culture, became infected by *M. acerina*. Only high inoculum densities of chlamydospores in the soil caused severe

damping-off of caraway seedlings. The opportunity for disease management by agronomical means is quite limited.

Chapter 7 describes the effect of some cultivation measures on anthracnose development in caraway. A reduction of leaf wetness duration was positively correlated with a decrease of disease severity. Lodging and higher plant density prolonged leaf wetness duration. Disease incidence and severity of anthracnose were reduced by crop management activities minimizing leaf wetness duration. Reduction of nitrogen levels reduced the risk of anthracnose development in spring and biennial caraway. Decreasing the sowing rate from 8 to 4 kg ha^{-1} resulted in a lower disease severity and an increase of seed yield in spring caraway, but not in biennial caraway. In biennial caraway disease severity decreased with wider row spacing. A damage threshold between 6% and 12% disease severity is proposed. Positive financial results of crop management activities are indicated.

Relationships between scientific aspects described in the previous chapters and the applied research described elsewhere are discussed in Chapter 8. Problems with anthracnose of caraway occur after long periods of rainfall. The disease cannot be prevented by any single crop protection measure. However, some measures reduce the occurrence of *M. acerina*. Reducing the risk of yield loss due to this pathogen should begin with 1) a disease-free start to delay the build-up of inoculum (disease-free seeds, choice of an uncontaminated field), 2) slowing down the epidemic in spring by taking measures to avoid lodging and to limit leaf wetness duration in a crop (reduction of sowing rate, intermediate row spacing and reduction of nitrogen fertilization), and 3) seed dressing can retard the early infection of seedlings, but it is illegal in the Netherlands. Fungicides against white mould (*Sclerotinia sclerotiorum*) sometimes reduce anthracnose in caraway, but results cannot be guaranteed.

CHAPTER 10

Samenvatting

Samenvatting

De schimmel *Mycocentrospora acerina* (Hart.) Deighton veroorzaakt verbruiningsziekte van karwij (*Carum carvi* L.). De leefwijze van de schimmel in het veld was bij aanvang van het onderzoek onvoldoende bekend. Dezelfde schimmel veroorzaakt een bewaarziekte van wortelen, waarvan uit het buitenland reeds het een en ander bekend was. In de jaren 1990 tot 1995 werden verschillende onderzoeken uitgevoerd aan *M. acerina*, met het doel mogelijkheden te vinden voor een aantal gewasbeschermingsmaatregelen. Hoofdstuk 1 geeft een inleidend overzicht.

In hoofdstuk 2 wordt het onderzoek naar de verspreiding van de schimmel in het veld beschreven. *M. acerina* kan zich verspreiden door de vorming van sporen. Om een inzicht te krijgen onder welke omstandigheden sporen gevormd en verspreid worden, werden deze gevangen en geteld. Het bleek dat sporenverspreiding vooral plaats vond tijdens regenval (> 2 mm) en bij gematigde temperatuur (15°C). Zonnige omstandigheden waren ongunstig voor sporenverspreiding en infectie. Vrijwel geen enkele spore werd gevonden als het niet had geregend. Verspreiding van de sporen over korte afstand (≤ 9 m) werd vooral veroorzaakt door het spatten van waterdruppels. Uit het onderzoek bleek dat de sporen op deze wijze over een afstand van maximaal 9 meter verspreid konden worden. Het is onwaarschijnlijk dat een gewas karwij geïnfecteerd wordt door ziektebronnen van buiten het veld.

Hoofdstuk 3 behandelt het effect van wortelbeschadiging op infectie door *M. acerina*. De schimmel werd aangebracht op onbeschadigde en kunstmatig beschadigde karwijwortels. Deze wortels werden vervolgens in een koelcel opgeslagen. Op beschadigde wortels leidde dit vaker tot aantasting dan op intacte wortels. De eerste ziekteverschijnselen werden op beschadigde wortels sneller zichtbaar dan op onbeschadigde wortels. Naarmate de wortel zwaarder beschadigd werd kon de schimmel zich sneller vestigen en uitbreiden. De snelheid waarmee de schimmel de wortel koloniseerde bleef constant nadat de eerste symptomen waren gevonden. De weerstand van de karwijwortel tegen *M. acerina* leek minder te worden naarmate de wortels ouder waren. In het veld zorgvuldig opgegraven wortels waren vaak beschadigd, waardoor de schimmel de wortel makkelijk kon infecteren. Desondanks was er slechts een gering verband tussen wortelbeschadiging en infectie onder gecontroleerde omstandigheden. Hoewel het erop leek dat beschadigde wortels

makkelijker geïnfecteerd kunnen worden bleken ook niet beschadigde wortels ziek te worden.

Karwijzaad bleek geïnfecteerd te kunnen zijn door *M. acerina*, zoals werd aangetoond in hoofdstuk 4. Tussen de 27 en 62% van de zieke zaden gaf onder kasomstandigheden een zieke zaailing. Een methode werd ontwikkeld om het percentage zieke zaden in zaaizaad vast te stellen. In zaadpartijen afkomstig van karwijtelers bleek het percentage geïnfecteerde zaden meestal tussen de 0 en 3% te liggen. Karwijzaad verzameld uit een zwaar aangetast gewas bleek voor 50% besmet te zijn. Deze waarneming geeft aan dat ziek karwijzaad een besmettingsbron voor verbruiningsziekte kan zijn. Hieruit valt af te leiden dat besmet karwijzaad een mogelijkheid is voor de schimmel om zich van veld tot veld te verspreiden.

Het effect van de mate van zaadbesmetting op gewasontwikkeling en opbrengst van karwij was onderwerp van studie in hoofdstuk 5. Naarmate het zaad zwaarder besmet was met *M. acerina* werd de opkomst slechter. Hierdoor verminderde ook het aantal bloeiende planten in het tweede jaar.

In hoofdstuk 6 werden mogelijke besmettingsbronnen onderzocht. In aanmerking hiervoor kwamen de dekvruchten van tweejarige karwij en voorvruchten van eenjarige karwij. Daarnaast speelden onkruiden mogelijk een rol bij de overleving en uitbreiding van de schimmel. Infectie van karwij kon ook voortkomen uit overlevingsstructuren (chlamydosporen) van de schimmel in de grond en in gewasresten. De waardplantenreeks van *M. acerina* is zeer groot. Naast diverse onkruiden bleken ook veel gebruikte dekvruchten als spinazie voor zaadproductie en erwt vatbaar te zijn voor de schimmel. De vatbaarheid voor *M. acerina* van spinazie en erwt bleek minder doorslaggevend voor de geschiktheid als dekvrucht dan de lichtdoorlatendheid en de mogelijkheid tot een vroege oogst. Dit resultaat werd bevestigd door de waarneming dat spinazie en erwt als voorvruchten van eenjarige karwij geen effect hadden op de ziekteontwikkeling in dit gewas. Aan de lijst met waardplanten konden dille, zomergerst en vier onkruidsoorten worden toegevoegd. De rol van onkruiden en gewassen voor de overleving van de schimmel in het veld tussen twee opeenvolgende karwijteelten werd besproken. Karwij werd ziek als deze werd geteeld op grond besmet met overlevingstructuren van de schimmel, besmet karwij stro en besmette gewasresten van andere gewassen. Alleen bij een hoge besmettingsgraad leidde dat tot uitval van kiemplanten. De brede waardplantenreeks en de langdurige

overleving van de schimmel in de grond belemmeren de bestrijding van de schimmel via vruchtwisseling.

In hoofdstuk 7 wordt het effect van enkele teeltmaatregelen beschreven op de ontwikkeling van verbruiningsziekte in karwij. De mate van aantasting nam toe als het gewas gemiddeld genomen langer nat bleef. Legering van het gewas en hoge plantdichtheden zorgden voor een vertraagde opdroging van het gewas. Maatregelen die leidden tot een open stand gaven een vermindering van de aantasting van karwij door *M. acerina*. Vermindering van de stikstofgift leidde tot een geringere kans op legering en daarmee werd het optreden van de verbruiningsziekte belemmerd. In eenjarige karwij leidde een halvering van de zaaizaadhoeveelheid van 8 tot 4 kg per hectare tot een lagere aantastingsgraad en een verhoging van de opbrengst. Dit bleek niet het geval in tweejarige karwij, waarin bij gebruik van meer zaaizaad een groter percentage van de planten vegetatief bleef. Daardoor veranderde de plantdichtheid van die planten in tweejarige karwij die bijdroegen aan de zaadzetting maar weinig, ongeacht de hoeveelheid zaaizaad die werd gebruikt. Vergroting van de rijenafstand verminderde de aantasting van karwij door *M. acerina*, hoewel bij zeer wijde rijenafstand de kans op legering toenam. De schadedrempel voor verbruiningsziekte in karwij ligt waarschijnlijk tussen de 6 en 12 % aantasting.

In hoofdstuk 8 worden relaties gelegd tussen de resultaten beschreven in de voorgaande hoofdstukken en resultaten beschreven in praktijkgerichte publikaties. De verbruiningsziekte geeft problemen als tijdens het schieten en de bloei van karwij veel regen valt. De ziekte kan tot op heden niet curatief bestreden worden. Als een aantal preventieve maatregelen worden genomen kan het optreden van de verbruiningsziekte in karwij beperkt worden. Vermindering van opbrengstverlies als gevolg van deze schimmel begint met 1) een gezonde uitgangssituatie (gezond zaaizaad, keuze voor een onbesmet veld), 2) vertraging van de epidemie in het voorjaar door het voorkómen van legering en verkorten van de periodes waarin het gewas nat is (lagere zaaizaadhoeveelheid, niet te nauwe rijenafstand en een beperkte stikstofgift), en 3) ontsmetting van zaaizaad verbetert de opkomst van het gewas en vermindert de vroege aantasting door de ziekte. Zaadontsmetting is echter in Nederland niet toegestaan. Bespuiting met een fungicide tegen *Sclerotinia* heeft een gunstige nevenwerking op verbruining, maar het effect bleek in de praktijk zeer wisselvallig te zijn.

CHAPTER 11

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Curriculum vitae

Bert Evenhuis werd geboren op 2 mei 1964 te Kibbelveen, gemeente Sleen (Dr.). In 1981 haalde hij het HAVO diploma aan de Gemeentelijke Scholen Gemeenschap te Emmen. Twee jaar later werd het VWO-diploma behaald aan dezelfde school. Een van de meest fascinerende facetten van de agrarische bedrijfsvoering is voor mij de strijd tegen ziekten en plagen. Vooral de niet chemische gewasbescherming had en heeft mijn belangstelling. In 1983 werd begonnen met de studie Plantenziektenkunde aan de Landbouw Universiteit Wageningen met daarin veel aandacht voor plantenveredeling. Een stage werd uitgevoerd op het H.L. Hilbrands Laboratorium voor bodemziekten te Assen onder leiding van Dr. ir. A. Mulder, gericht op biologische bestrijding van *Rhizoctonia solani*. Het toen nog vrij onbekende maïswortelknobbelaaltje was onderwerp van studie op de vakgroep Nematologie bij ing. J.J. s'Jacob. Hierna volgde een afstudeervak Plantenveredeling, gericht op morfologische aspecten van resistentie van tomaat tegen *Cladosporium fulvum*, begeleid door Dr. ir. R.E. Niks. Epidemiologische aspecten kwamen aan de orde in een afstudeervak Fytopathologie, betreffende *Mycosphaerella brassicicola* in spruitkool, onder leiding van ir. J.E. van den Ende. In 1989 werd de studie aan de Landbouw Universiteit afgerond met een diploma in de plantenziektenkunde en een diploma in de plantenveredeling. Vervolgens werd een half jaar gewerkt aan een literatuur recherche naar de effecten van introductie van genetisch gemodificeerde organismen bij de vakgroep Fytopathologie van de LUW. Van mei 1990 tot augustus 1995 werden de data verzameld die hebben geleid tot dit proefschrift. Het werk werd uitgevoerd op het Proefstation voor de Akkerbouw en Groenteteelt in de Vollegrond (PAGV) te Lelystad in nauwe samenwerking met het Instituut voor Plantenziektenkundig Onderzoek (IPO-DLO) te Wageningen. Op het PAGV werd in 1995 gewerkt aan bestrijding van *Phytophthora infestans* in aardappelen en in 1996 aan het rassenonderzoek van asperge, peen en stamslaboon. Sinds maart 1997 is de auteur in dienst als regionaal onderzoeker vollegrondsgroenteteelt bij het Praktijkonderzoek voor de Akkerbouw en Vollegrondsgroenteteelt Zuidoost Nederland (PAV-ZON) te Horst-Meterik.

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