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Spore germination and disease development after application of pycnidiospores of *Ascochyta caulina* to *Chenopodium album* plants

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Abstract

Ascochyta caulina is considered a potential mycoherbicide against *Chenopodium album*. Disease development of *C. album* plants and plants of 14 other species after application of pycnidiospores of *A. caulina* was studied in climate chamber experiments. The experiments were carried out to analyse disease development with time, and to recognize factors that may limit disease development. Two time courses of necrosis of *C. album* plants were observed, (1) an increase of necrosis followed by a decline, and (2) an increase of necrosis up to completion with subsequent plant death. Courses of necrosis with time could be described by a non-monotonic, critically damped model when plants survived infection and by a monomolecular model when plants died from infection. Disease development was influenced by interactions between wetness period, density of the spore suspension applied, plant development stage at the time of spore application, and temperature. Disease was favoured by a long wetness period, a high number of spores applied, an early plant development stage at the time of spore application, and temperatures of 18 °C and higher. Disease development was limited to plant species of the genera *Chenopodium*, *Atriplex* and *Spinacia* with differences between the species. Pathogenicity differed significantly between three *A. caulina* isolates tested. Variation in resistance between four source populations of *C. album* was small. Prospects for *A. caulina* as a mycoherbicide are discussed.

Introduction

The mycoherbicide concept was introduced by Daniel *et al.* [1974], who demonstrated that a plant pathogenic, endemic fungus can be utilized to control its weed host by applying a massive dose of inoculum at a particular development stage of the weed. Subsequently, in the last two decades, over 100 fungi have been examined for their ability to control their weed hosts [e.g. Templeton, 1982; Scheepens and Van Zon, 1982; Charudattan, 1991]. Scheepens [1979] suggested to study *Ascochyta caulina* (P. Karst.) v.d. Aa & v. Kest. for the control of *Chenopodium album* L., an annual plant, and word-wide an important weed in many crops [e.g. Holm *et al.*, 1977; Schroeder *et al.*, 1993]. *A. caulina* causes necrotic lesions on leaves and stems of plants of *Chenopodium* L. and *Atriplex* L. species. The fungus is endemic in Europe and central Siberia [Van der Aa and Van Kesteren, 1979].

Eggers and Thun [1988] studied infection of *C. album* plants after application of pycnidiospores of *A. caulina* to the plants. They were not optimistic about the prospects for *A. caulina* as a mycoherbicide, but could not make a thorough evaluation. The objective of the study presented here is to provide a basis for the evaluation of the prospects of *A. caulina* as a post-emergence mycoherbicide against *C. album*. Disease development on *C. album* plants after spray application of suspensions of *A. caulina* pycnidiospores was investigated. Host specificity and some factors that might limit disease development of *C. album* were studied. Holocomb [1982] listed factors that might limit disease development. We studied effects of source population of *C. album*, isolate of *A. caulina*, wet-

ness period after spore application, density of the spore suspension applied, plant development stage at the time of spore application, and temperature on disease developement. In some experiments, observations were made on spore germination and fungal development.

Materials and methods

Plant material

Seeds of *C. album* were collected from mature plants at different locations in Wageningen, The Netherlands, in 1991. Seed samples from four source populations were used, (1) a plant in an arable field with a sugar beet crop, (2) a plant in an arable field with a maize crop, (3) a plant on a ruderal site, and (4) plants in a weed demonstration garden (Department of Agronomy, Wageningen Agricultural University). Seeds of other weed species were harvested from plants in the same weed demonstration garden. Seeds of cultivated species were obtained from commercial seed batches. All seeds were stored in the dark at 5 °C until sowing. Plants to be used in experiments were grown in a climate chamber on a peat soil in plastic pots. Germination of seeds was induced prior to sowing to improve uniformity of emergence. If present, seed coatings were removed from seeds by several washings in water. The seeds were then placed on 1% (w/v) water agar in Petri dishes (9 cm), and incubated in a day-night regime of 14 h light (20 mmol m^{-2} s^{-1}; Philips TL 8W/33) at 25 °C and 10 h dark at 15 °C for 3 days. Germinated seeds were sown in a peat soil, which consisted of a mixture of 10 volumes of peat (Trio b.v., The Netherlands; Triomf no 17) and 1 volume of coarse sand. Soil volume in a pot was 600 ml. The pots were placed in a climate chamber with a day-night regime of 14 h light at 18 °C, 75% RH, and 10 h darkness at 12 °C, 85% RH. Light was obtained from Philips TLD 50W/84 lamps; average photosynthetic active radiation (PAR) at soil level was 240 mmol m^{-2} s^{-1}. The soil was watered twice a week with a nutrient solution [Steiner, 1984]. Plant density was 1 or 4 plants per pot.

Fungal material

Ascochyta caulina was isolated from naturally infected *C. album* plants at different locations in Wageningen, 1990 or 1991. The isolates were axenically maintained on oat meal agar. Three isolates were used in the experiments, (1) an isolate from a leaf of a plant in an arable field (isolate code 90-1), (2) an isolate from a stem of a plant at a ruderal site (91-1), and (3) an isolate from a leaf of a plant from another arable field (91-2). Pycnidiospores of *A. caulina*, to be used as inoculum in experiments, were produced on oat meal agar or on wheat bran medium after Kempenaar [1995]. About 4 h before application of spores to plants, cultures of *A. caulina* on the media were flooded with distilled water with 0.05% (v/v) Tween 80. After 3 h, supernatants with suspended spores were poured into flasks, filtered through cheese cloth, and diluted to required densities.

Spore application and wetness period

When plants of a batch had reached a particular development stage, they were sprayed with a spore suspension until run-off 4 h before the climate chambers went dark. A DeVilbiss atomizer was employed. Sprayed plants were placed in high-humidity chambers for a specified wetness period. Subsequently, they were returned to the climate chamber in which they were originally grown. Conditions in the high-humidity chambers were a near-saturated atmosphere (> 95% RH) with a day-night regime of 14 h light (average PAR 210 mmol m^{-2} s^{-1}; Philips TLD 50W/84) at 18 °C and 10 h darkness at 12 °C. The surface of sprayed plants dried slowly but not completely during exposure to high humidity. When sprayed plants had been returned to the climate chamber at 75/85% RH, the surface of the plants dried within 30 min.

Disease assessment and data analysis

Plants were separated into leaves and stems. For each plant, the proportion of necrotic area of each individual leaf and of the stem was estimated by means of standard diagrams (Fig. 1). The area of each individual leaf was measured using a leaf area meter (LI-COR, USA; Model 3100). The necrotic area of each leaf was calculated by multiplying the leaf area by the estimated proportion of necrosis. The proportion of necrotic leaf area of a plant was calculated by dividing the summarized necrotic leaf area of a plant by the total leaf area of that plant.

A monomolecular model [equation 1; e.g. Campbell and Madden, 1990] and a non-monotonic, critically damped model [equation 2; Gilligan, 1990] were used to describe time courses of necrosis.

$$y = 1 - e^{\{-r \cdot (t-a)\}} \quad (1)$$

$$y = (b + c \cdot (t-a)) \cdot e^{\{-r \cdot (t-a)\}} \quad (2)$$

In both models, y is the proportion of necrotic leaf area or stem area per plant at time t, r is a rate parameter, t is time after spore application in days, and a is the incubation period (intercept of the curve with the x-axis). Parameters b and c in equation 2 are shape parameters. The models were fitted to the data by means of non-linear regression procedures of Genstat 5 [Payne *et al.*, 1987]. Treatment effects on proportions were determined by means of analysis of variance (ANOVA). After evaluation of the residual plots, we decided to angular-transform the proportions to stabilize the variance.

Experiment 1: Observations on spore germination and fungal development

Fungal development on inoculated leaves was assessed by means of the method of Bruzzese and Hasan [1983]. *C. album* plants of population 'arable field 2' were grown at densities of 4 plants per pot. When the plants had reached the 4-leaf stage, they were sprayed with a spore suspension of 10^6 spores ml^{-1} of isolate 90-1. Sprayed plants were placed in a high-humidity chamber. Leaves were harvested from the plants at 3, 7, 11, 24, 48, 72 and 96 h after spore application, 8 leaves per harvest. The leaves were immersed in a clearing and staining solution for two days, transferred to a clearing solution for 1 day, and mounted on microscope slides for observations with a light microscope.

Experiment 2: Effect of source population and isolate on disease development

C. album plants of three populations (arable field 1, arable field 2, and ruderal site) were grown at densities of 4 plants per pot. When the plants had reached the 6-leaf stage, they were sprayed with a suspension of 0 or 5.10^6 spores ml^{-1}. Spore suspensions of three isolates (90-1, 91-1 or 91-2) were applied. Sprayed plants were placed in the high-humidity chamber for a wetness period of 24 h and subsequently returned to the climate chamber at 75/85% RH. The experiment consisted of 12 treatments (3 plant populations * 4 suspensions). A treatment consisted of 2 pots with 4 plants each. The pots were placed in a complete randomized design. Leaf necrosis on individual plants was assessed one week after inoculation. At spraying time, one ml spore suspension of each isolate was plated onto 1% (w/v) water agar in a Petri dish (9 cm). The Petri dishes were placed in the high-humidity chamber for 24 h. Percentages of spore germination were determined by observing 300 spores per isolate. A spore was considered germinated when the germination tube was longer than the width (*c.* 5mm) of the spore.

Experiment 3: Disease development with time

C. album plants of population 'arable field 2' were grown at densities of 1 plant per pot. When the plants had reached the 4-leaf stage, they were sprayed with a suspension of 5.10^6 spores ml^{-1} of isolate 90-1. Nutrients, 0.35% (w/v) Czapek-Dox (Difco, USA) and 0.04% (w/v) yeast extract (Merck, Germany), were added to the spore suspension prior to spraying. Sprayed plants were placed in high-humidity chambers for wetness periods of 8, 16, 24 or 36 h and subsequently returned to the climate chamber at 75/85% RH. At nine dates after spore application, extended over a period of one month, 8 plants per treatment were taken from the climate chamber and assessed for leaf and stem necrosis. The experiment consisted of 36 treatments (4 wetness periods*9 harvest dates). A treatment consisted of 8 pots with 1 plant each. The pots were placed in a randomized block design with 8 replicates.

Experiments 4a, 4b and 4c: Effect of plant development stage, spore density and wetness period on disease development

Three experiments were conducted with three batches of *C. album* plants of population 'arable field 2'. Plant density was 4 plants per pot. Plants of a batch were sprayed at a particular development stage: cotyledonous stage, 2-leaf stage or 6-leaf stage. The factor development stage at the time of spore application was confounded with the experiment number. The three experiments were conducted in a similar way. When the plants of the particular batch had reached the specified development stage, they were sprayed with a suspension of 0, 10^5, 10^6 or 10^7 spores ml^{-1} of isolate 90-1. Sprayed plants were placed in the high-humidity chamber for a wetness period of 0, 4, 8, 16, 28 or 44 h and subsequently returned to the climate chamber at 75/85% RH. Each experiment consisted of 24 treatments (6 wetness periods*4 spore densities). Each treatment comprised 8 plants divided over 8 pots. The pots were placed in a two-factor randomized split-plot block design. The factor wetness period was assigned to the main plot structure (blocks) and the factor spore density was assigned to the sub-plot structure (pots). Leaf necrosis on individual plants was assessed one week after spore application.

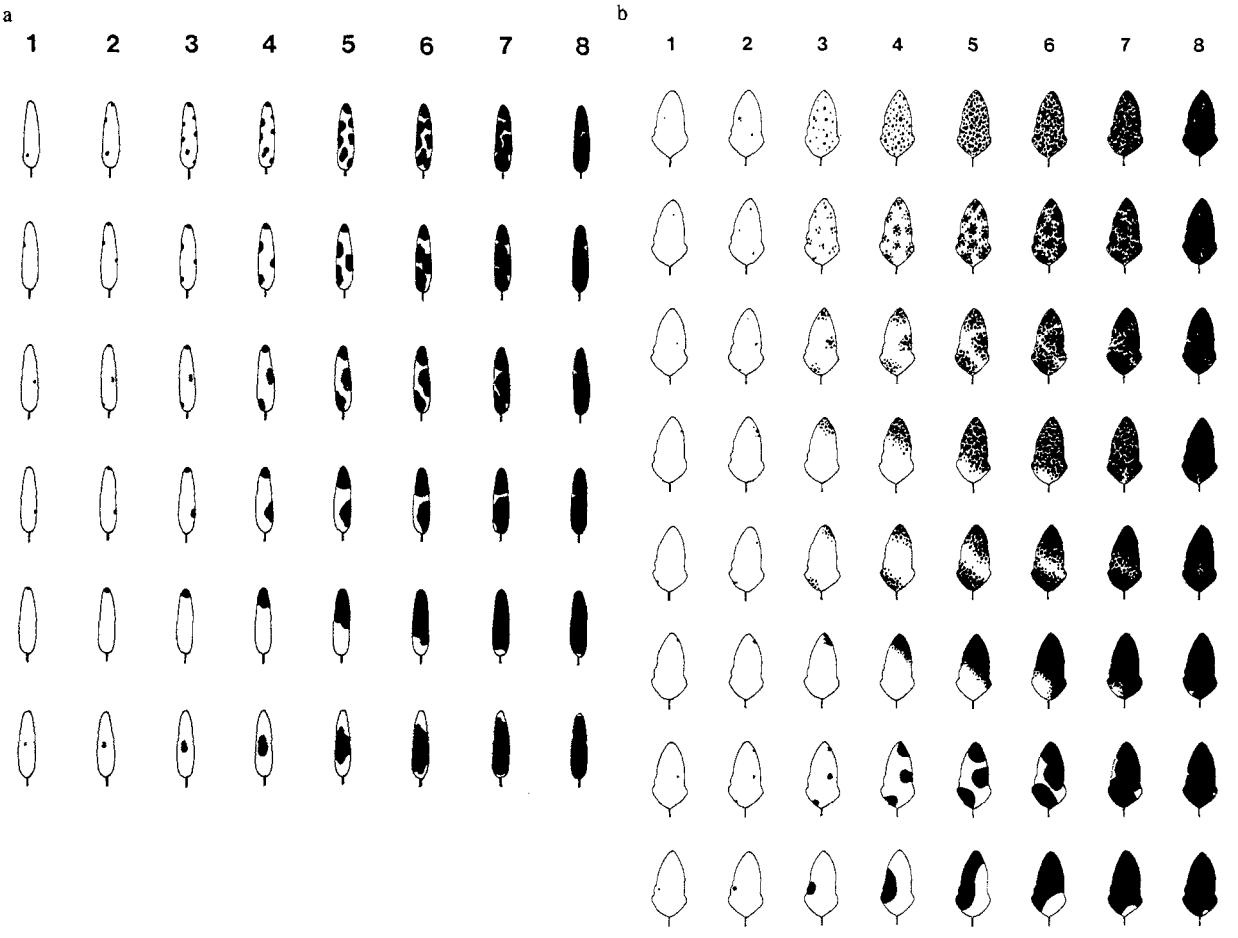

Fig. 1. Standard diagrams for the assessment of proportions of necrotic area on cotyledons (1a), first two leaves (1b), subsequent leaves (1c), and stems (1d). Numbers represent classes of necrosis and refer to proportions of necrotic area: 1 = 0.002, 2 = 0.015, 3 = 0.08, 4 = 0.24, 5 = 0.5, 6 = 0.76, 7 = 0.92 and 8 = 0.985.

Experiment 5: Effect of temperature on disease development

Two batches of *C. album* plants of population 'arable field 2' were sown at a five-day interval. Plant densities were 1 plant per pot. The plants of the two batches were treated at the same time, and sprayed with a suspension of 10^7 spores ml^{-1} of isolate 90-1, when the plants of the two batches had reached the 4-leaf and 2-leaf stage, respectively. Sprayed plants were placed in one of five high-humidity chambers for wetness periods of 16 or 32 h. High-humidity chambers were set at temperatures of 6, 12, 18, 24 or 30 °C. The factor temperature was confounded with the humidity chambers. In each high-humidity chamber, the pots were placed in a two-factor (development stage and wetness period) randomized block design with 8 replicates. After the wetness period, the pots were returned to the climate chamber at 18/12 °C and 75/85% RH. Leaf necrosis on individual plants was assessed one week after spore application. At spraying time, one ml spore suspension was plated on water agar as in experiment 2. The Petri dishes were placed in the high-humidity chambers for periods of 16 or 32 h. The percentage of germinated spores at the five temperatures tested was determined by observing 200 spores per Petri dish.

Experiment 6: Host-specificity

Plants of 22 taxa, mostly *Chenopodiaceae*, were grown at densities of 4 plants per pot. When the plants had reached the 4 to 6-leaf stage, they were sprayed with spore suspensions of 0 or 10^6 spores ml^{-1} of isolate 90-1. Nutrients were added to the suspensions as described for experiment 3. Sprayed plants were placed in high-humidity chambers for a wetness period of 24

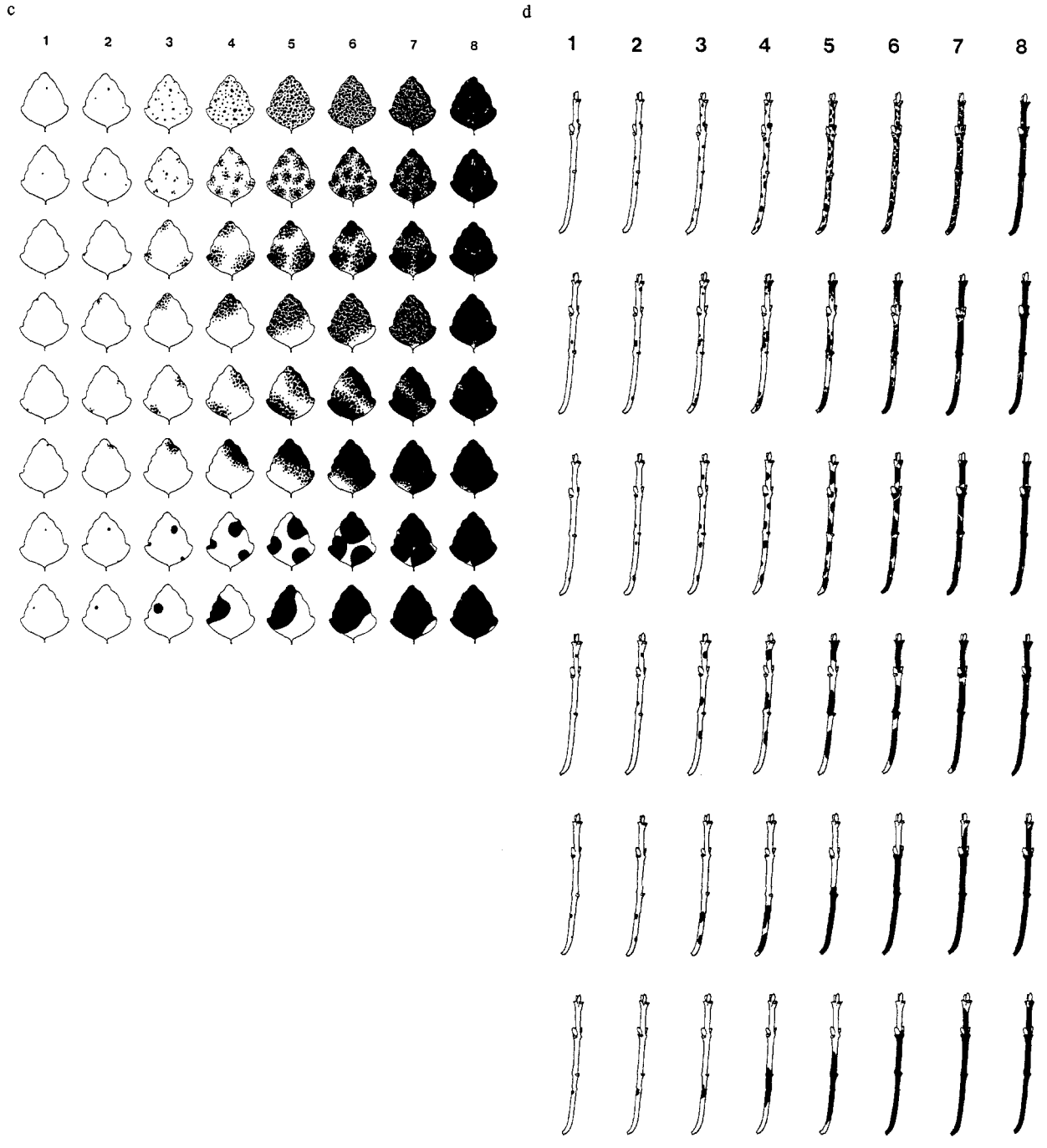

Fig. 1. Continued.

h and subsequently returned to the climate chamber at 75/85% RH. The experiment consisted of 44 treatments. A treatment consisted of 2 pots with 4 plants each. Leaf necrosis on the individual plants of a pot was assessed one week after spore application. The remaining plants were monitored for disease development for a further two weeks.

Results

Experiment 1: Observations on spore germination and fungal development

At 3 h a.i. (after inoculation), early signs of germination were observed. Some spores had developed a short (<10 mm) germination tube. At 7 h a.i., more spores had germinated and germination tubes were longer. At 11 h a.i., some germination tubes had penetrated the leaves through stomata. At that time, appresoria-like structures at the end of some germination tubes were observed on top of stomatal cells and epidermal cells. At 24 h a.i., mycelium was observed in the leaf tissue. At 48 h a.i., more mycelium was observed in the leaf tissue together with disintegrating host cells. At 72 h a.i., necrosis was observed macroscopically and early signs of development of pycnidia microscopically. At 96 h a.i., pycnidia were observed in the leaf tissue.

Experiment 2: Effect of source population and isolate on disease development

No significant differences were observed in the percentages of germinated spores of the three isolates tested. On average, 75% of the spores had germinated on water agar after an incubation period of 24 h. Plants sprayed with spore suspensions developed necrosis in the first week after inoculation while plants sprayed with the spore-free control fluid did not. Leaf necrosis at one week after spore application (Table 1) was influenced by isolates of *A. caulina* ($p < 0.001$), but not by source populations of *C. album* ($p = 0.064$).

Experiment 3: Disease development with time

Average leaf necrosis and average stem necrosis are shown in Fig. 2. Both leaf and stem necrosis showed an interaction ($p < 0.001$) between wetness period and harvest date. Sprayed plants with a wetness period of 36 h showed a rapid increase of necrosis, and complete necrosis of the aerial parts was reached about one week after spore application. These plants died in the second week after spore application. Sprayed plants with a wetness period of 24 h or shorter also showed an increase in necrosis, but it was followed by a decline. The courses of necrosis with time could be described by the monomolecular model or the non-monotonic, critically damped model. The monomolecular model was used when complete necrosis occurred, and fitted well to the data. More than 94% of the variance was always explained by the model. The critically damped model was used when increase of necrosis was followed by

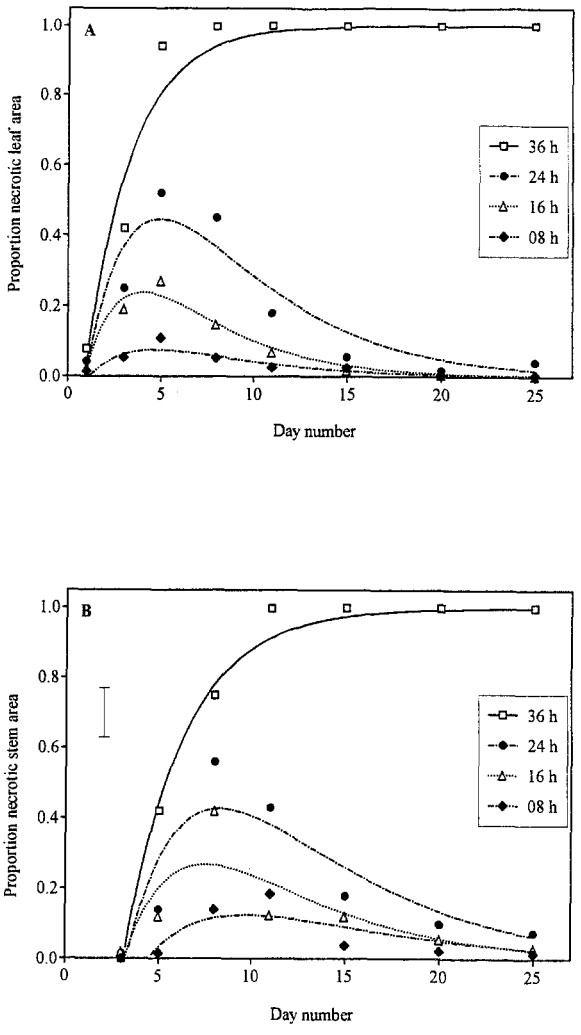

Fig. 2. Experiment 3. Dynamics of leaf necrosis (A) and stem necrosis (B) of *Chenopodium album* plants sprayed with a spore suspension of *Ascochyta caulina*. Sprayed plants were exposed to high humidity (>95% RH) for periods of 8, 16, 26 or 36 h and subsequently exposed to 75/85% RH. Entries are treatment averages. Curves show regressions of a monomolecular model (solid curves) or a non-monotonic, critically damped model (broken curves). Error bars indicate the angular-transformed LSD ($p = 0.05$) divided by 100.

a decline. Regressions with this model could only be made when parameter a was set to a fixed value. Values for a (incubation period) were estimated from daily observations on disease development. The critically damped model underestimated the maximum necrosis levels. Percentages of variance explained by regressions were greater than 50. The parameter estimates of both models are shown in Table 2.

Table 1. Experiment 2. Average proportion of necrotic leaf area of juvenile *Chenopodium album* plants one week after application of pycnidiospores of *Ascochyta caulina* as affected by source population of the weed and isolate of the fungus. Standard errors are in parentheses

Population of *C. album*	Isolate of *A. caulina*			
	91–1	91–2	90–1	Mean
Arable field 1	0.02 (0.03) a	0.09 (0.06) b	0.37 (0.11) cd	0.16 a
Arable field 2	0.03 (0.03) a	0.11 (0.08) b	0.37 (0.11) cd	0.17 a
Ruderal site	0.03 (0.03) a	0.23 (0.15) bc	0.46 (0.14) d	0.24 a
Mean	0.03 a	0.15 b	0.40 c	0.20

Treatment averages and means were separated with different letters according to LSD-tests of angular-transformed data ($p < 0.05$).

Table 2. Experiment 3. Parameters of a monomolecular (mm) or a non-monotonic, critically damped (cd) model, fitted to data of leaf necrosis or stem necrosis of treated *Chenopodium album*. The monomolecular model was used when complete necrosis occurred, the critically damped model when an increase in necrosis was followed by a decline. Estimates of standard errors are in parentheses

Plant part observed/ model fitted	Treatment[1)]	Parameter[2)]			
		b	c	r	a
Leaves/mm	36 h			0.40 (0.08)	0.9 (0.2)
Leaves/cd	24 h	0.01 (0.09)	0.30 (0.08)	0.25 (0.04)	1
Leaves/cd	16 h	0.02 (0.03)	0.20 (0.03)	0.32 (0.03)	1
Leaves/cd	8 h	0.01 (0.02)	0.06 (0.02)	0.28 (0.05)	1
Stem/mm	36 h			0.31 (0.03)	3.1 (0.1)
Stem/cd	24 h	−0.04 (0.11)	0.24 (0.09)	0.20 (0.04)	3.1
Stem/cd	16 h	−0.01 (0.09)	0.17 (0.09)	0.23 (0.07)	3.1
Stem/cd	8 h	−0.03 (0.05)	0.07 (0.04)	0.19 (0.06)	4

1) Wetness period after spore application.
2) Parameters are explained in Materials and methods.

Experiments 4a, 4b and 4c: Effect of development stage, spore density and wetness period on disease development

Average leaf necrosis at one week after spore application is shown in Fig. 3. Plants sprayed with spore-free control liquid did not develop necrosis (not shown in Fig. 3). ANOVA of the combined data of the three experiments showed that leaf necrosis varied significantly between experiments ($p < 0.001$). Experiments were confounded with development stages of the plants at the time of spore application. We ascribe the differences between the experiments to the effect of the development stage. There was a tendency for the effect of the same spore density and the same wetness period to become less at later plant growth stages. Each experiment showed an interaction ($p < 0.001$) between spore density and wetness period. Leaf necrosis was near to zero, and not or hardly influenced by spore density when the sprayed plants had a short wetness period. With increasing wetness periods, leaf necrosis increased significantly and was significantly affected by spore density.

Experiment 5: Effect of temperature on disease development

Spore germination on agar was affected by temperature and incubation period (Fig. 4). Average leaf necrosis one week after spore application is shown in the same figure. Temperatures were confounded with high-humidity chambers. ANOVA of the whole data set showed a significant high-humidity chamber effect ($p < 0.001$). We ascribe the differences between high-humidity chambers to a temperature effect. Leaf necrosis on plants exposed to 6 °C during the wetness period

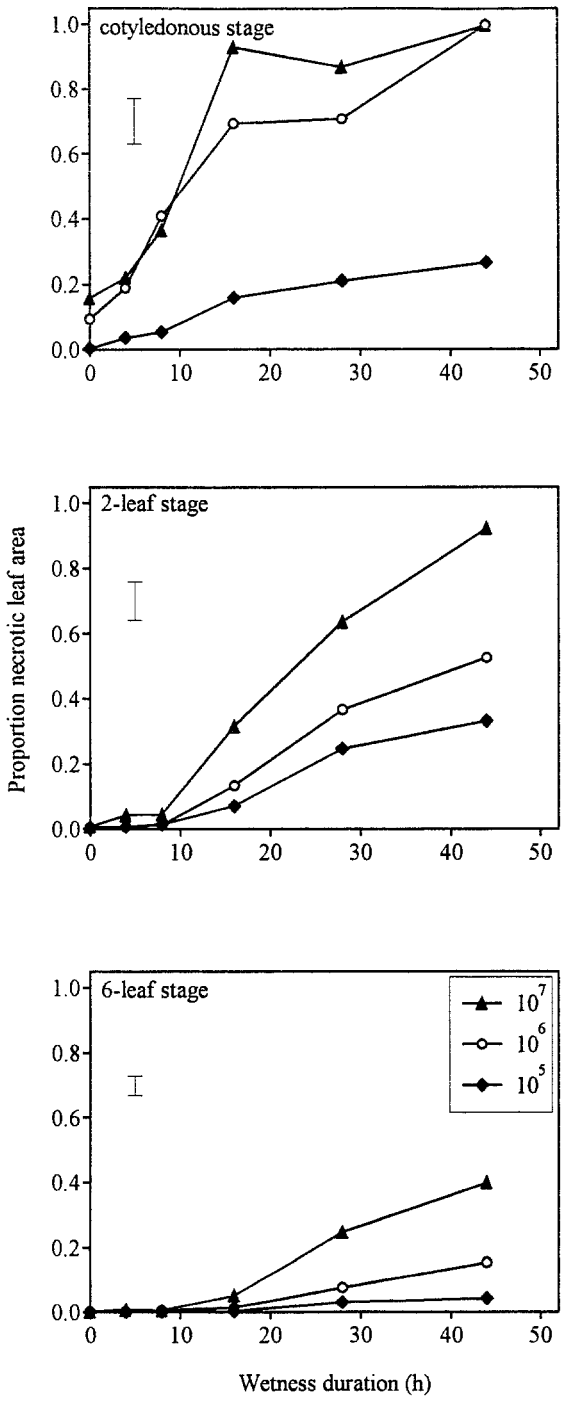

Fig. 3. Experiment 4. Effect of development stage (cotyledonous, 2-leaf or 6-leaf stage), spore density (10^5, 10^6 or 10^7 spores ml^{-1}), and wetness period on leaf necrosis of *Chenopodium album* plants, assessed one week after application of spores of *Ascochyta caulina*. Entries are treatment averages. Error bars indicate the angular-transformed LSD ($p = 0.05$) divided by 100.

showed an interaction ($p < 0.05$) between development stage and wetness period, while leaf necrosis on plants exposed to one of the other temperatures were significantly ($p < 0.05$) affected by both development stage and wetness period.

Experiment 6: Host-specificity

Disease symptoms were observed only on plants of the genera *Chenopodium*, *Atriplex* and *Spinacia* with significant differences between species. Plants sprayed with the spore-free control fluid did not develop disease symptoms. Average leaf necrosis at one week after spore application is shown in Table 3. Plants that had not developed disease symptoms in the first week after spore application remained symptom-free in the two consecutive weeks.

Discussion

Disease development

Spray application of spores of *A. caulina* on *C. album* plants does not unconditionally result in disease development. A period of free moisture on inoculated plants or exposure of inoculated plants to high humidity was required for germination, infection, and development of necrosis. The necrosis development observed is a part of a monocyclic process. *A. caulina* infects the host tissue resulting in necrosis, but mature pycnidia and pycnidiospores are not formed and secondary spread does not occur. The course of necrosis with time could not be described by one model because of a dual response; necrosis either increased and subsequently decreased with time or necrosis increased with time up to complete necrosis. The former could be described by a non-monotonic, the latter by a monomolecular model. The meaning of the parameters of the non-monotonic, critically damped model are poorly understood. Unfortunately. Parameter c was the only parameter affected by treatments (Table 2). Parameter c, the rate of increase of necrosis when the first symptoms appear, is the tangent of the curve at the intercept with the x-axis. Gilligan [1990], who used this model to describe dynamics of fungi in soil, also observed that parameter c was most sensitive to changes in a system.

Disease development was affected by interactions between wetness period, spore density, plant development stage, and temperature. Disease was favoured by a long wetness period after spore application, a high

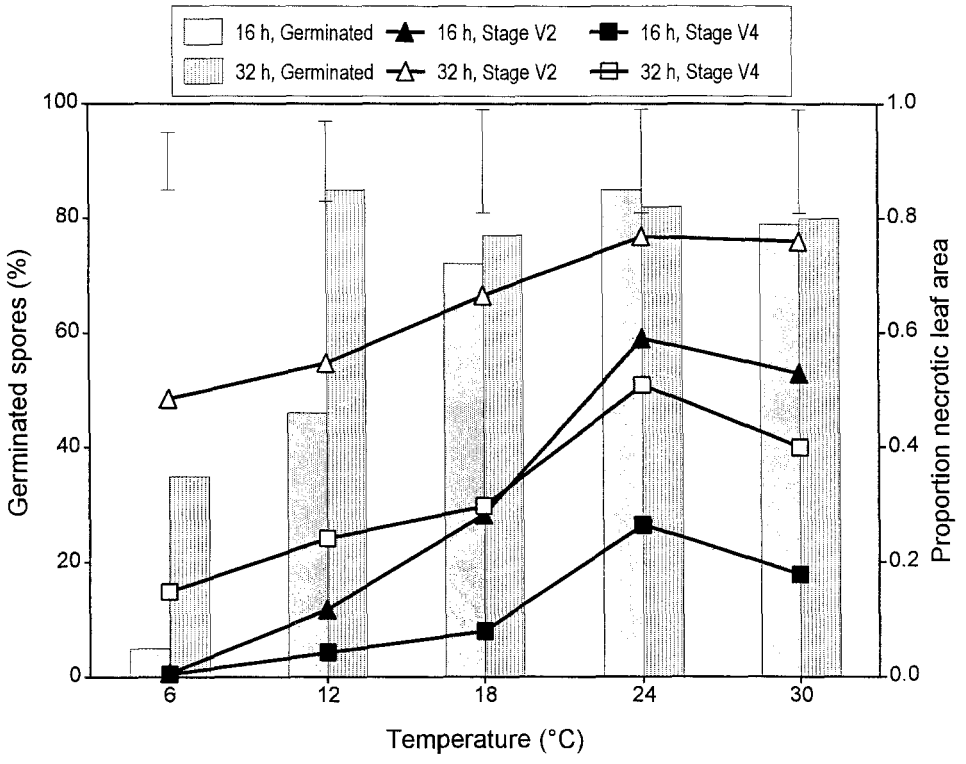

Fig. 4. Experiment 5. Effect of temperature on germination of spores of *Ascochyta caulina*, and of temperature, development stage (2-leaf or 4-leaf stage, V2 and V4 respectively), and wetness period (16 or 32 h) on leaf necrosis of *Chenopodium album* plants, assessed one week after application of spores of *Ascochyta caulina*. Percentages of germinated spores, assessed 16 h or 32 h after plating onto water agar, are shown as columns. Proportions of necrotic leaf area are shown as graph lines and are treatment averages. Error bars indicate the angular-transformed LSD ($p = 0.05$) of proportions divided by 100.

number of spores applied, a young development stage at the time of spore application, and a temperature that allows fast spore germination (20–25 °C). Eggers and Thun [1988] reported 25 °C as the optimum temperature for germination of *A. caulina* spores. Variation in pathogenicity between the *A. caulina* isolates tested was large. We observed only small differences in resistance between source populations of *C. album*. However, this cannot be generalized because of the small number of populations tested.

Wetness period appeared to be the most critical factor for disease development. A minimum wetness period of roughly 8 h was required for penetration of leaves by germination tubes and development of necrosis, under the given temperatures. However, there was one exception. Plants sprayed with spore suspensions at the cotyledonous stage (experiment 4a) developed necrosis, even when they were not exposed to high humidity. This result might suggest involvement of another, minor factor in disease development. Capasso *et al.* [1991] demonstrated that water-soluble toxins can be harvested from cultures of *Ascochyta* species. The necrosis on these young plants could have been caused by the action of a toxin in the suspension. If so, young *C.album* plants are more sensitive to the toxin than older plants.

Prospects for biological control

Charudattan [1989] proposed two major criteria to assess the potential usefulness of plant pathogenic fungi as mycoherbicides: host specificity and efficacy of control. Charudattan's criteria were used to evaluate prospects for *A. caulina* as a mycoherbicide.

Disease development by *A. caulina* was limited to plant species of three genera, *Chenopodium*, *Atriplex* and *Spinacia*. Among the susceptible species, there were two species with some economic importance, *S. oleracea* (a vegetable crop) and *C. quinoa* (an arable crop). We do not consider the susceptiblility of these two crops a serious constraint for the further

Table 3. Experiment 6. Average proportion of necrotic leaf area of juvenile plants of various plant taxa, assessed one week after application of spores of *Ascochyta caulina*. Standard errors are in parentheses

Plant taxon[1)]	Cultivar	Severity of leaf necrosis
Chenopodium album		0.30 (0.11)
Chenopodium ficifolium		0.35 (0.12)
Chenopodium quinoa	Elsevier	0.24 (0.10)
	Wild type	0.06 (0.05)
Chenopodium glaucum		0.11 (0.11)
Chenopodium polyspermum		0.02 (0.02)[2)]
Chenopodium rubrum		0[2)]
Atriplex prostrata		0.35 (0.12)
Atriplex patula		0.27 (0.11)
Spinacia oleracea	Martine	0.02 (0.02)
	Amsterdams reuzenblad	0
Beta vulgaris subspecies *vulgaris*	Carla	0
	Lucy	0
	Univers	0
	Kyros	0
	Egyptische platte ronde	0
Corispernum marschallii		0
Zea Mays	Brazil	0
	Mandigo	0
Pisum sativum	Eminent	0
Triticum aestivum	Arminda	0
Brassica oleacerea ssp. *capitata*	Bartolo	0

[1)] The first 17 taxa are from genera of the plant family of *Chenopodiaceae*.
[2)] Chlorosis on leaves.

development of *A. caulina* into a mycoherbicide. However, more crop species have to be tested for host specificity before a final judgement of the agronomic risks involved with the use of *A. caulina* as a mycoherbicide.

Charudattan [1989] differentiated efficacy of control into amount, speed and ease of weed control. Amount and speed of control required will vary with each weed problem. At the present stage of knowledge, *A. caulina* will meet the demands for amount and speed of control when conditions are favourable for infection. Complete control can be attained within 14 days after spore application. Ease of control was defined by Charudattan as the possibility to produce, store and apply inoculum, and by the independence of disease development from environmental conditions. Disease development strongly depends on environmental conditions, and this dependence might be a limitation to the use of *A. caulina* in practice because long periods of high humidity or free moisture on the target plants required for complete control do not frequently occur under field conditions in western Europe. Thus, either the moisture requirement should be met by appropriate formulation of the spores, or incomplete levels of control should be accepted. We hypothesize that incomplete control of *C. album* (less than 100% plant mortality) could be acceptable under certain conditions. Plants with a severity of leaf necrosis greater than 0.8 one week after spore application all died in the second week after spore application. Plants with a severity of leaf necrosis of 0.6 to 0.8 one week after spore application, either died in the second week after spore application, or were severely retarded in growth. Plants with a severity of leaf necrosis smaller than 0.2 one week after spore application were hardly affected in their growth. Because of the retarded growth of sublethally infected plants, it is probable that *C. album* plants with a leaf necrosis severity greater than 0.6 would not cause competition damage to crops.

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