Modulation of primary and secondary infections in epidemics of carrot cavity spot through agronomic management practices

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The relative importance of primary and secondary infections (auto- and alloinfections) in the development of a carrot cavity spot (CCS) epidemic caused by *Pythium* spp. were investigated. Three cropping factors: fungicide application, soil moisture and planting density, were selected as the key variables affecting the disease tetrahedron. Their effects on: (i) disease measurements at a specific time, (ii) the areas under the disease progress curves (AUDPCs) and (iii) a time-dependent parameter in a pathometric incidence-severity relationship, were studied. Mefenoxam applications 5 and 9 weeks after sowing reduced the intensity of a field CCS epidemic that involved both primary and secondary infections. In microcosm experiments, mefenoxam reduced secondary infections by *Pythium violae* obtained by transplanting infected carrot roots and slowed disease progress (1·6 lesions per root in treated versus 5·8 lesions in non-treated microcosms). A deficit of soil moisture limited the movement of *Pythium* propagules to host tissue, and thus reduced primary infections in the field; it also promoted the healing of lesions, limiting lesion expansion and the potential for alloinfections (6·8–7·5 lesions per root in irrigated plots compared with 2·4 lesions in non-irrigated plots). A negative relationship between the mean root-to-root distance and the rate of alloinfections was established in microcosms; a reduction in mean planting density was also effective in limiting CCS development (0·5, 1·6 and 2·0 lesions per root in microcosms containing 8, 16 and 31 roots, respectively). An integrated disease management system based on a combination of cultural methods, such as optimized fungicide application, date of harvest versus soil moisture content, and host density versus planting pattern, may make a useful contribute to the control of CCS.

Keywords: carrot cavity spot, mefenoxam, planting density, *Pythium violae*, secondary infection, soil moisture

Introduction

Carrot cavity spot (CCS) is one of the most damaging fungal diseases of carrot worldwide, causing yield losses as badly affected roots are unmarketable (Guba *et al.*, 1961; Hiltunen & White, 2002). This soilborne disease is characterized by small sunken elliptical lesions on the taproot. In France, CCS is mostly caused by *Pythium violae* and *P. sulcatum* (Montfort & Rouxel, 1988; Breton & Rouxel, 1993; Suffert & Guibert, 2007). Over the last 20 years, information about the aetiology of the disease has

been gathered, but few epidemiological studies have investigated the dynamics of the disease in the field.

The occurrence of primary and secondary infections in CCS epidemics was recently demonstrated experimentally (Suffert & Montfort, 2007). The fitting of simulation models to disease progress curves also supports the occurrence of both processes (Suffert, 2007). The decrease over time of the time-dependent parameter $a(t)$ in the pathometric relationship $i = b \cdot (1 - \exp(-a(t) \cdot tda)$, where i is the disease incidence, tda the total diseased area and t the thermal time (accumulated degree days from sowing, base 3·5°C), indicated that CCS epidemics were driven by (i) mobilization of soil inoculum (primary infection), (ii) disease intensification (autoinfection, on the same root) and (iii) disease spread (alloinfection, i.e. root-to-root contamination) (Suffert, 2006). The polycyclic nature of CCS epidemics was thus firmly established: soil inoculum of *Pythium* sp. is the source of primary infections and diseased roots then provide inoculum for secondary infections.

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The experimental use of fungicide is a classical way of identifying the importance of different phases in an epidemic. This is also obviously useful to optimize agricultural practices (dose and date of application). The fungicide, mefenoxam (R-isomer of metalaxyl), provides a mean of controlling CCS (Hiltunen & White, 2002). The timing of application was shown to be important for effective disease control (Lyshol et al., 1984; Gladders & McPherson, 1986; McDonald, 1994). Although effects on the different epidemiological processes were not considered separately, several observations raised the possibility that the fungicide acts predominantly on either primary or secondary infections. While seed treatments and early season application were generally found to be most effective (Hiltunen & White, 2002), the hypothesis that mefenoxam could reduce both primary and secondary infections rates should be tested.

Soil moisture is one of the most important environmental factors known to favour the expression of soilborne diseases (Griffin, 1969; Cook & Papendick, 1972) and the seasonal activity of *Pythium* spp. (Stanghellini, 1974). An increase of CCS is generally associated with high soil moisture (Guba et al., 1961), for example after rainfall (McDonald, 1994) or irrigation periods (Perry & Harrison, 1979) or in poorly drained or flooded soils (Soroker et al., 1984; Vivoda et al., 1991). As *Pythium* species are zoosporic organisms, the effect of soil moisture on CCS development could be the result of enhanced secondary infection rates and increased lesion size; however, this hypothesis has not yet been confirmed experimentally.

Planting density can affect the number of primary infection foci, as established for *P. irregulare* in cress seedlings (Burdon & Chilvers, 1975), but mainly the rate and time of alloinfections. This effect was described for some soilborne pathogens, such as *Sclerotium rolfsii* infecting carrot (Smith et al., 1988), *Sclerotium cepivorum* infecting onion (Scott, 1956; Crowe & Hall, 1980; Littley & Rahe, 1987), *Sclerotinia sclerotiorum* infecting sunflower (Huang & Hoes, 1980), *Phymatotrichum omnivorum* infecting cotton (Koch et al., 1987), and *Helminthosporium solani* and *Rhizoctonia solani* infecting potato (Firman & Allen, 1995). White (1988) suggested that the high plant densities in later carrot crops, where most taproots were touching neighbours, enhanced the potential for plant-to-plant spread. Although Vivoda et al. (1991) found no significant differences in the incidence of CCS among three densities of 57, 115 or 230 plants m^{-2} in the field, this hypothesis should still be tested in experimental controlled conditions (microcosms).

The aim of the present study was to assess the relative importance of primary and secondary infections in the development of a CCS epidemic. The work was based on analysis of the modulation of disease progress curves, in whole epidemics in the field and in microcosms where alloinfections only were generated using the transplantation method described by Suffert & Montfort (2007), by various cropping factors postulated to influence infection mechanisms. The research strategy was to select cropping factors acting on separate components of the disease

tetrahedron involving the pathogen, the environment and the host defined by Zadoks & Schein (1979), such as fungicide application (effect on pathogen inoculum), soil moisture (effect on abiotic environment) and planting density (effect on host availability). Each factor that potentially affects independently or concomitantly primary infections, auto- and alloinfections, and lesion expansion, is an experimental tool to elucidate the processes occurring in CCS epidemics.

Materials and methods

Field experiment

The effects of fungicide applications and soil moisture contents on the development of a whole CCS epidemic were tested in plots located at the INRA Station of Le Rheu (Ille-et-Vilaine, France; 48°01′ N, 1°43′ W). The silt loam soil (16·3% clay, 62·5% silt, 21·2% sand and 2·4% organic matter) was infested with *P. violae* (isolate Pv490, CBS 102·609, obtained in 1994 from CCS lesions in Normandy) on 29 April 2002, 3 weeks before sowing carrots (cv. Nanco). To produce inoculum, bags containing 240 mL of dry barley grains and 300 mL of distilled water were autoclaved twice at 120°C for 1 h at 24-h intervals, and inoculated with plugs of *P. violae*, then incubated for 3 weeks at 20°C in a dark room. The inoculum was applied on the soil surface at 50 g m^{-2} (dry grain) (dose D10; Suffert, 2007). *Pythium sulcatum*, *P. intermedium*, *P. sylvaticum*, *P. coloratum* and *P. ultimum*, known to be pathogenic on carrot, were found in the field area (Suffert & Guibert, 2007). Experimental units were 2- × 6-m plots and consisted of five rows 50 cm apart with 80 plants per linear metre. Cultural practices applied uniformly to all plots were similar to those described by Suffert & Guibert (2007). Disease was assessed about every month on samples consisting of all carrot roots present along a 50-cm segment of any of the three central rows of each plot. Each sample typically included 30–40 roots. Air temperature 1 m above soil level and soil temperature at a depth of 20 cm were measured with a Hobo H8® (Prosensor) at 15-min intervals throughout the experiments.

Effect of fungicide application on a whole epidemic

The experiment was set up as a randomized block design (four blocks), with timing of fungicide application as one factor with four treatments: untreated control (NT), early application (P$_1$) immediately after sowing, intermediate application (P$_2$) 5 weeks after sowing (4 July 2002), and late application (P$_3$) 9 weeks after sowing (21 August 2002). Plots were artificially infested with *P. violae* as described above and sown to carrot on 29 May 2002. Each fungicide spray application consisted of 0·75 L ha^{-1} Santhal TC® (480 g L^{-1} mefenoxam; Syngenta Agro). This rate is 1·5 times the recommended rate; it was chosen to compensate for the loss of active ingredient applied on leaves, as the product is normally applied directly to the seed bed (Hiltunen & White, 2002). Five samples were harvested from each plot (S1, 08 July 2002, 591 degree

days from sowing (dd); S2, 29 July 2002, 903 dd; S3, 19 August 2002, 1197 dd; S4, 30 September 2002, 1715 dd; and S5, 18 November 2002, 2103 dd) and scored for disease intensity. Areas under the disease progress curves (AUDPCs) were calculated for three different intervals: $AUDPC_1$ from sowing to 1197 dd (S3), $AUDPC_2$ from 1197 dd (S3) to 1715 dd (S4), and $AUDPC_3$ from 1715 dd (S4) to 2103 dd (S5).

Effect of soil moisture on a whole epidemic

The experiment was set up as a randomized block design (four blocks), with soil moisture levels generated by manipulating irrigation intensity with sprinklers as one factor with three treatments: dry (NI; non-irrigated), medium (I_1; about 200 L m^{-2} over 5 months) and wet (I_2; about 600 L m^{-2} over 5 months). Natural rainfall amounted to about 400 mm during the experiment. Plots were artificially infested with *P. violae* as described above and sown to carrot on 1 June 2003. Nightly irrigation periods were activated every second or third day in July and August with a Toro GreenKeeper 212® controller; each irrigation period lasted 2 h in I_1 and 4 h in I_2 plots. The flow was measured as 4 L m^{-2} h^{-1}. Soil moisture content was monitored weekly from 24 June 2003 to 18 November 2003 by measuring the wet weight (*WW*) and dry weight (*DW*) of soil cores collected weekly at random locations from the topsoil (0- to 15-cm depth) in rows of a plot of each treatment (three weekly cores per treatment). Soils cores were weighed before (*WW*) and after desiccation at 120°C for 48 h (*DW*), and the gravimetric soil moisture (*GSM*) was calculated as:

$$GSM = \frac{WW - DW}{WW} \times 100 \qquad (1)$$

Disease was assessed on six sets of carrot samples (S1, 1 July 2003, 601 dd; S2, 21 July 2003, 924 dd; S3, 5 August 2003, 1181 dd; S4, 28 August 2003, 1605 dd; S5, 30 September 2003, 2027 dd; and S6, 24 November 2003, 2329 dd). AUDPCs were calculated for two intervals: $AUDPC_1$ from sowing to 1181 dd (S3) and $AUDPC_2$ from 1181 dd (S3) to 2329 dd (S6).

Controlled-conditions experiments

Microcosm experiments were carried out to generate CCS epidemics from secondary infections only. They were conducted in pots in growth chambers where climatic conditions (16 h daylight at 20°C and 8 h night at 12°C) were regulated and compatible with both carrot growth and *P. violae* development (Van der Plaats-Niterink, 1981; Suffert & Guibert, 2007) and in greenhouses where temperature was maintained in the range 17–27°C and the light was natural. Secondary infections were obtained by transplanting infected carrot (cv. Nanco) roots ('donor plants') into a population of healthy mature carrots ('receptor plants') grown in a steam-sterilized soil mixture (50% sand, 25% compost, 25% organic soil) (Suffert & Montfort, 2007). PVC tubes (32 mm diameter, 230 mm long) were introduced into each pot, and seeds destined to

generate the receptor plants were sown around the tubes. Twelve weeks after sowing the tubes were replaced by donor roots artificially inoculated with *P. violae* (Pv490). Donor roots were prepared by washing fresh carrot roots in 0·3% bleach for 1 min, rinsing them three times with sterile water, wounding the epidermis of the roots at two or four spots (1–2 cm^2) with abrasive tissue, and inoculating each wounded spot by depositing a mycelial plug (5 mm diameter) cut from a 7-day-old culture of *P. violae* grown at 20°C on carrot juice agar. Plugs were held in place with sterile pins. Inoculated roots were incubated for 48 h in hermetically sealed plastic boxes to create high-humidity conditions. Agar plugs were then removed and roots were transplanted into the pots. Microcosms were watered as needed to adjust soil moisture to the water-holding capacity. Air temperature and soil temperature at a depth of 15 cm were measured with a Hobo H8® at 15-min intervals.

Effect of fungicide application on alloinfections

This experiment was set up in a greenhouse as a randomized block design (four blocks), with fungicide application as one factor with two treatments: fungicide application (T) and untreated control (NT). The experiment was repeated once (rep1 and rep2). Microcosms consisted of rectangular pots (36 cm long, 25 cm wide, 32 cm high), into which 21 carrots were sown in a regular pattern in five rows (5rp/3rp/rp + dp + rp + dp + rp/3rp/5rp; dp = donor plant, rp = receptor plant). Twelve weeks after sowing, two donor roots with two CCS lesions were transplanted into each microcosm. Seven days after transplanting, mefenoxam (35 µL of Santhal TC® in 150 mL of water) was sprayed onto the soil of each microcosm (except controls, which were sprayed with water). Symptoms were scored on the receptor roots at three dates of harvest (S1, S2 and S3 – 21, 33 and 46 days after transplanting, respectively) and AUDPCs were calculated over the whole duration of the experiment.

Effect of distance between donor and receptor plants on alloinfections

This experiment, repeated once (rep1 and rep2), was set up in a growth chamber as a randomized block design (three blocks), with distance between donor and receptor roots as one factor with three treatments: L50 = 50 mm, L70 = 70 mm and L90 = 90 mm. Six seeds, destined to produce the receptor plants, were sown at equidistant points from the centre of circular 10-L pots (28 cm diameter, 18 cm high). Twelve weeks after sowing, a donor root with four CCS lesions was transplanted into the centre of each pot. The receptor roots were harvested weekly from 1 to 4 weeks after transplantation and symptoms scored. AUDPCs were calculated from 1162 dd (S0) to 1550 dd (S4).

Effect of planting density on alloinfections

This experiment, repeated twice (rep1, rep2 and rep3), was set up in a greenhouse as a randomized block design (four blocks), with carrot planting density as one factor
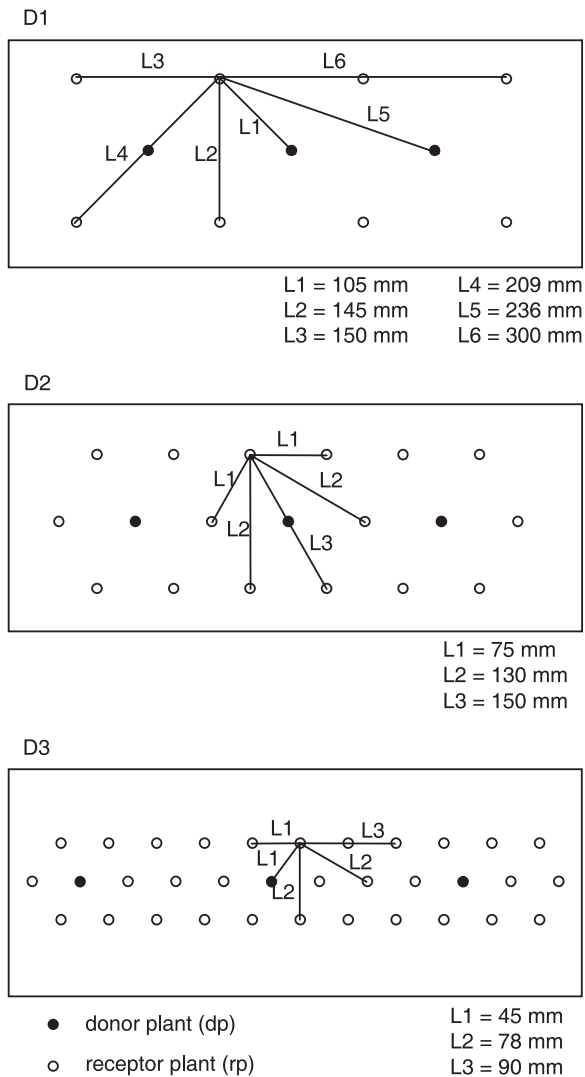
Figure 1 Illustration of three carrot root patterns in experimental microcosms to test the effect of planting density on carrot cavity spot alloinfections by *Pythium violae*.

with three treatments: D1 with eight rp, D2 with 16 rp, and D3 with 31 rp. Three donor plants with two CCS lesions were transplanted into a central row in microcosms consisting of rectangular pots (55 cm long, 25 cm wide, 25 cm high) 12 weeks after sowing receptor plants in a geometric arrangement to obtain planting patterns (Fig. 1) characterized by two variables:
(i) 'mean linear density' (*D*) of the carrot population (D1: 20·0 roots m⁻¹; D2: 34·5 roots m⁻¹; D3: 61·8 roots m⁻¹); this variable, classically used by growers to characterize sowing density, did not permit assessment of the effective distance between roots, which depends on the width of the row and on the sowing pattern;
(ii) 'mean root-to-root distance' (*L*) of the carrot population, assessed by the mean distance between each root and the six nearest roots (D1: 143 mm; D2: 86 mm; D3: 51 mm); *L* was calculated based on the six roots closest to

the donor plant, under the hypothesis that the potential of alloinfections generated by more distant roots was negligible.

Receptor plants were harvested and symptoms scored 5 weeks after transplanting.

Disease assessment, statistical analyses and modelling

Two types of variable were used to quantify the development of the epidemics. Disease measurements at a specific time (*Y*) were evaluated at different sampling dates: disease incidence *i* (proportion of diseased roots), lesion density *d* (number of lesions per root), symptom intensity *si* (diameter of lesions) and total diseased area *tda* (total necrotic area per root) (Suffert, 2006, 2007). Epidemic development over the season was quantified though the computation of areas under disease progress curves (AUDPCs) (Campbell & Madden, 1990), using the formula of Shaner & Finney (1977):

$$\text{AUDPC}_{Y, j_a \rightarrow j_b} = \sum_{j=j_a}^{j_b} \frac{1}{2}(y_j + y_{j+1}) \times (t_{j+1} - t_j) \qquad (2)$$

where y_j is the disease score at the jth evaluation, t_i is the thermal time (accumulated degree days from sowing, base 3·5°C) at the jth evaluation, $n = j_a - j_b$ is the number of intervals of integration ranged from the j_a^{th} to the j_b^{th} evaluation.

The temporal decrease of the time-dependent parameter $a(t)$ in the pathometric relationship between *i* and *tda* during CCS epidemics was established by Suffert (2006):

$$i = b \cdot (1 - e^{-a(t) \cdot tda}) \qquad (3)$$

This suggested changes in epidemiological processes, because the product $a(t) \times b$ reflects the balance between primary and secondary infections (Willocquet & Savary, 2004). Thus, the influence of cropping factors on the fluctuations of $a(t)$ was tested with $b = 100$.

Statistical analyses were performed using the SAS statistical package, version 8·1 (SAS, 2000). Treatment effects on *Y* (*i*, *tda*, *d*, and *si*) were tested with the ANOVA procedure and the Scheffe test (*P* < 0·05). The NPAR1WAY procedure and Wilcoxon-Mann-Whitney test (*P* < 0·1) were used to analyse treatment effects on AUDPC_Y and $a(t)$ because these variables were not normally distributed.

Data from the fungicide experiment in microcosms (rep1, treatments T and NT) were used to measure the dispersal gradient of alloinfections by fitting the power law model, which assumes that the number of contaminations is inversely proportional to some power of the distance from the source (Campbell & Madden, 1990) and corresponds to:

$$Y = \alpha \cdot L^{-\gamma} \qquad (4)$$

where α and γ are parameters and the slope of the gradient γ represents the rate of decrease in *Y* with distance. Symptoms were scored distinguishing the position of each receptor root in the microcosms and its distance from the donor roots. The relationship in Eqn 4 was tested for *Y* = *tda* and the slope of the gradient was assessed using
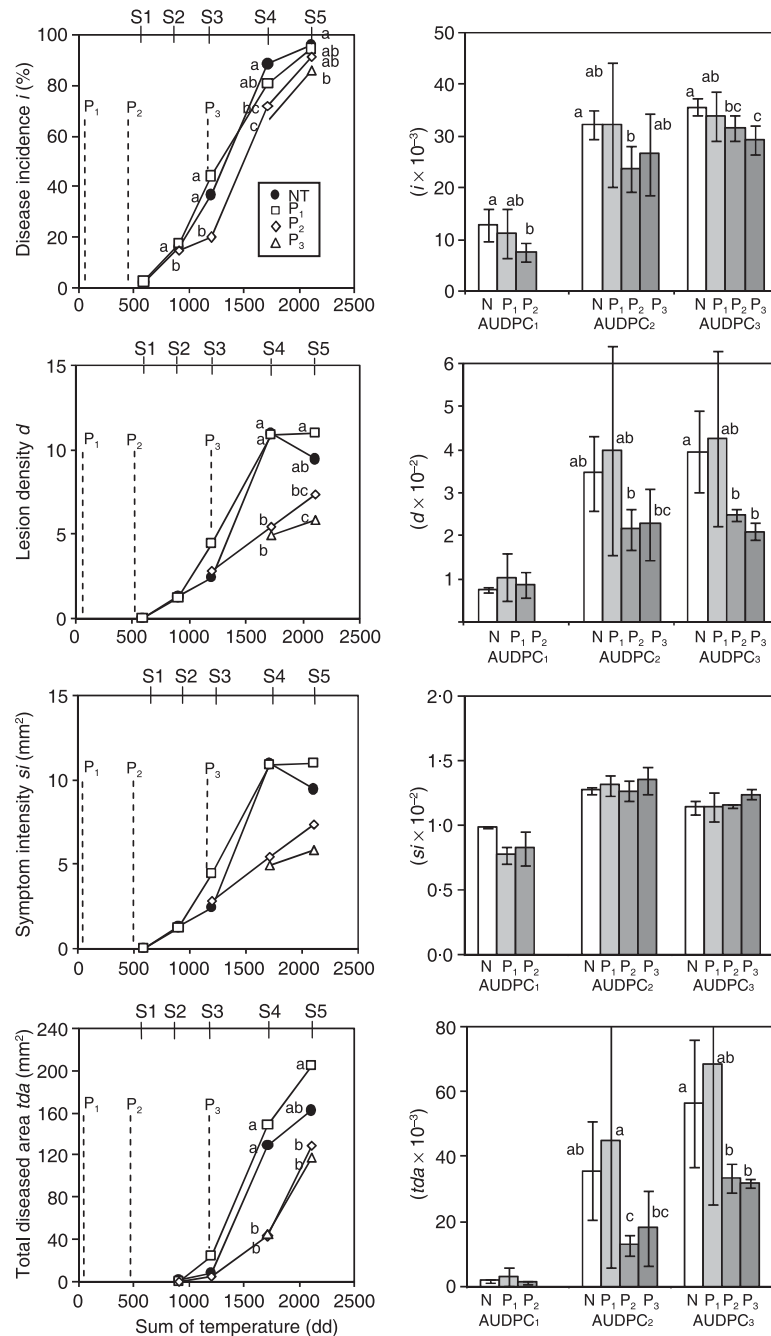
Figure 2 Effect of three dates [at sowing (P_1) or 5 (P_2) or 9 weeks (P_3) after sowing] of fungicide application (mefenoxam) on the dynamics of a carrot cavity spot field epidemic (Le Rheu, France, 2002). Three intervals were distinguished to calculate AUDPCs [$AUDPC_1$ from S0 (sowing) to S3 (1197 degree days (dd) after sowing), $AUDPC_2$ from S3 to S4 (1715 dd), and $AUDPC_3$ from S4 to S5 (2103 dd)]. Different lowercase letters indicate significantly different disease measurements at a specific time (ANOVA, Scheffe test, $P < 0.05$) or AUDPCs (NPAR1WAY, Wilcoxon-Mann-Whitney test, $P < 0.1$). NT = untreated controls.

the average of γ values fitted by the method of minimization of sums of squares at each sampling date.

Results

Effects of fungicide application

Field epidemic
The earliest mefenoxam application (P_1) had no significant impact on either disease variables at a specific time or AUDPCs (Fig. 2). Later applications (P_2 and P_3) significantly reduced i, d and tda during the last stage of the epidemic (between S4 and S5) (Scheffe test, $P < 0.05$), and thus the respective $AUDPC_2$ and $AUDPC_3$ values (Wilcoxon-Mann-Whitney test, $P < 0.1$) (Fig. 2). Neither treatment had any effect on si. The term $a(t)$ was stable between S3 and S5, and decreased with later fungicide application dates (Fig. 3a). However, the only statistically significant differences were between untreated and intermediate- and late-treatment plots (P_2 and P_3).
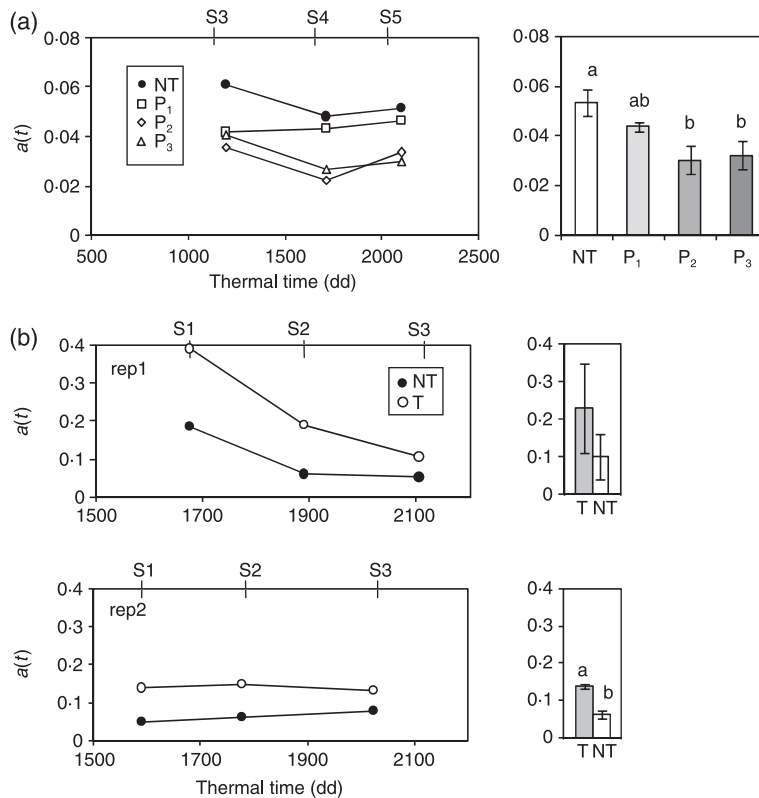
Figure 3 Effect of a fungicide application (mefenoxam) on the time-dependent parameter $a(t)$ in the relationship $i = 100 \cdot (1 - \exp(-a(t) \cdot tda))$ in (a) a carrot cavity spot (CCS) field epidemic (Le Rheu, France, 2002) (NT, untreated controls; P_1, fungicide application at sowing; P_2, 5 weeks after sowing; P_3, 9 weeks after sowing) and (b) a CCS epidemic generated in experimental microcosms by alloinfections of *Pythium violae* only (NT, untreated controls; T, fungicide-treated). Values of $a(t)$ were optimized by the method of minimization of sums of squares ($P < 0.01$). t refers to the thermal time (accumulated degree days from sowing, base 3.5°C). Different lowercase letters indicate significantly different mean values of $a(t)$ between treatments (NPAR1WAY, Wilcoxon-Mann-Whitney test, $P < 0.1$).

Alloinfections

Mefenoxam applications significantly decreased all disease variables in the microcosm experiment (Fig. 4). The term $a(t)$ decreased with time in rep1, but remained stable in rep2 (Fig. 3b). Interestingly, $a(t)$ was significantly larger in treated than in untreated microcosms (Wilcoxon-Mann-Whitney test, $P < 0.1$).

Effects of soil moisture

The summer of 2003 was exceptionally dry, as shown by the critical low point reached in non-irrigated soils (NI) in the middle of summer ($GSM = 6\%$), before the GSM increased following scattered rainfall (Fig. 5). The three irrigation levels (NI, I_1 and I_2) generated significantly different soil moisture contents and different temporal patterns for the increase of i, d, si and tda (Fig. 6). The differences were significant between NI and I_1/I_2 for each disease variable (ANOVA, Scheffe test, $P < 0.05$), notably lesion density d (five to seven lesions per root in I_1/I_2 between S4 and S6, vs. two lesions per root in NI). A the end of the epidemic, disease incidence i reached 90% and tda ranged from 55 to 85 mm² in I_1/I_2, but i barely exceeded 60% and tda was less than 15 mm² in NI. Symptom intensity si was stable over time or slightly decreasing; significant differences were observed between NI, I_1 and I_2 at sampling date S4. As a consequence, soil moisture significantly affected AUDPC$_1$ and AUDPC$_2$ for several disease variables (Wilcoxon-Mann-Whitney test, $P < 0.1$) (Fig. 6). During the first part of the epidemic, differences were high between NI and I_1/I_2 for AUDPC$_1$ of i, d and tda; during the second part, high differences were observed for AUDPC$_2$ of i, d, si and tda. Differences between I_1 and I_2 were observed only for AUDPC$_1$ of d and for AUDPC$_2$ of si. The term $a(t)$ was nearly stable and ranged from 0.07 to 0.08 between S2 and S6 for the three treatments (Fig. 7). After S3, $a(t)$ decreased in I_1/I_2 to 0.04/0.06, but exceeded 0.10 in NI. A linear decreasing relationship existed between $a(t)$ and GSM ($R^2 = 0.560$). On average, $a(t) = 0.099$ for $GSM = 11.5\%$, $a(t) = 0.048$ for $GSM = 17.7\%$, and $a(t) = 0.038$ for $GSM = 20.2\%$.

Effects of planting density

Effect of distance between donor and receptor plants on alloinfections

A negative relationship between the distance between donor and receptor roots and the intensity of alloinfections was identified (Fig. 8). However, this effect was heterogeneous for i, d, si and tda and the variance was high, especially in L7. Significant differences were found for i and tda at sampling date S4 in rep1 and rep2 (Scheffe test, $P < 0.05$). Effects were homogeneous and significant on AUDPCs in rep2, and particularly high for d (344 in L5, 269 in L7, and 89 in L9) and tda (5735 in L5, 2463 in L7, and 385 in L9).

The estimated slope γ of the dispersal gradient of alloinfections ranged from 1.33 to 2.23 in the microcosm experiment. The average value ($\gamma = 1.72$) was used to fit the power law model (Eqn 4) in both treatments T
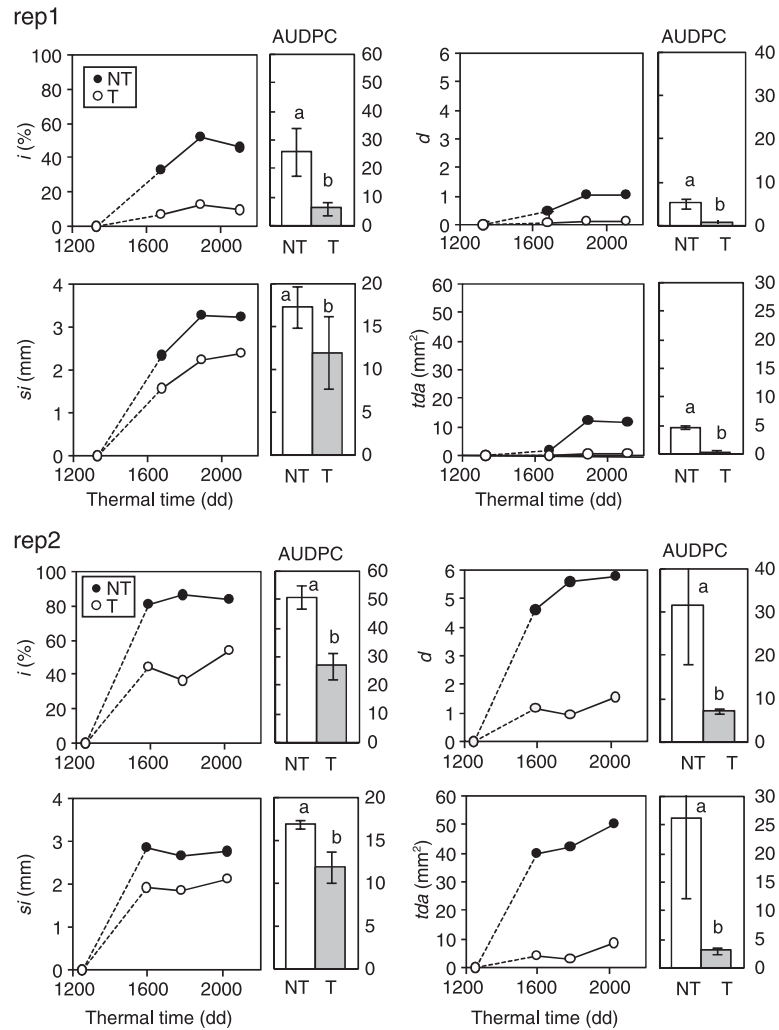
Figure 4 Effect of a fungicide application (mefenoxam) on the dynamics of carrot cavity spot alloinfections generated by *Pythium violae* in experimental microcosms. Three samples were taken 21, 33 and 46 days after transplantation of the donor root. Different lowercase letters indicate AUDPCs significantly different between treatments (NPAR1WAY, Wilcoxon-Mann-Whitney test, *P* < 0·1). *i* is disease incidence, *d* lesion density, *si* symptom intensity and *tda* total diseased area. T, treated; NT, untreated controls.
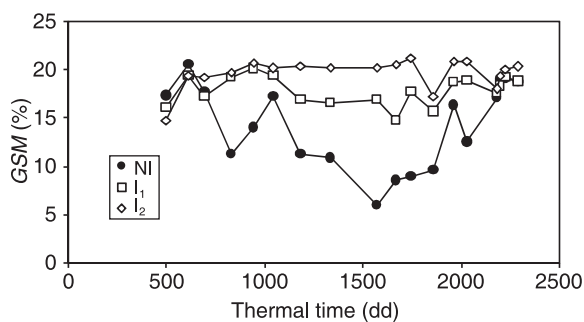
Figure 5 Monitoring of gravimetric soil moisture (*GSM*) generated by three irrigation levels in the field (Le Rheu, France, 2003). NI, non-irrigated control; I_1, medium irrigation level (about 200 L m^{-2} over 5 months); I_2, high irrigation level (about 600 L m^{-2} over 5 months).

and NT (Fig. 9). Terms α_T and α_{NT} increased with time and seemed to reach maxima in S2 ($16·6 \times 10^{-3}$ and $9·8 \times 10^{-2}$, respectively) and S3 ($15·7 \times 10^{-3}$ and $8·5 \times 10^{-2}$, respectively).

Effect of planting density on alloinfections

Disease severity, as measured by both *d* and *tda*, significantly increased with root density in rep 1 (Fig. 10), although differences were not significant in rep2 and rep3. The relationship $i = 100 \cdot (1 - \exp(-a(t) \cdot tda))$ was not significantly affected by planting density (data not shown).

Discussion

The occurrence of secondary infections in CCS was formally demonstrated through experiments (Suffert & Montfort, 2007) and modelling (Suffert, 2006, 2007). The current study assessed the relative importance of primary vs. secondary infections in the development of an epidemic, distinguishing autoinfection from alloinfection. Since the interactions between the main pathogenic *Pythium* species during the infection phase are non-significant (Suffert & Guibert, 2007), the epidemiological processes described here can probably be generalized whatever the composition and temporal fluctuations
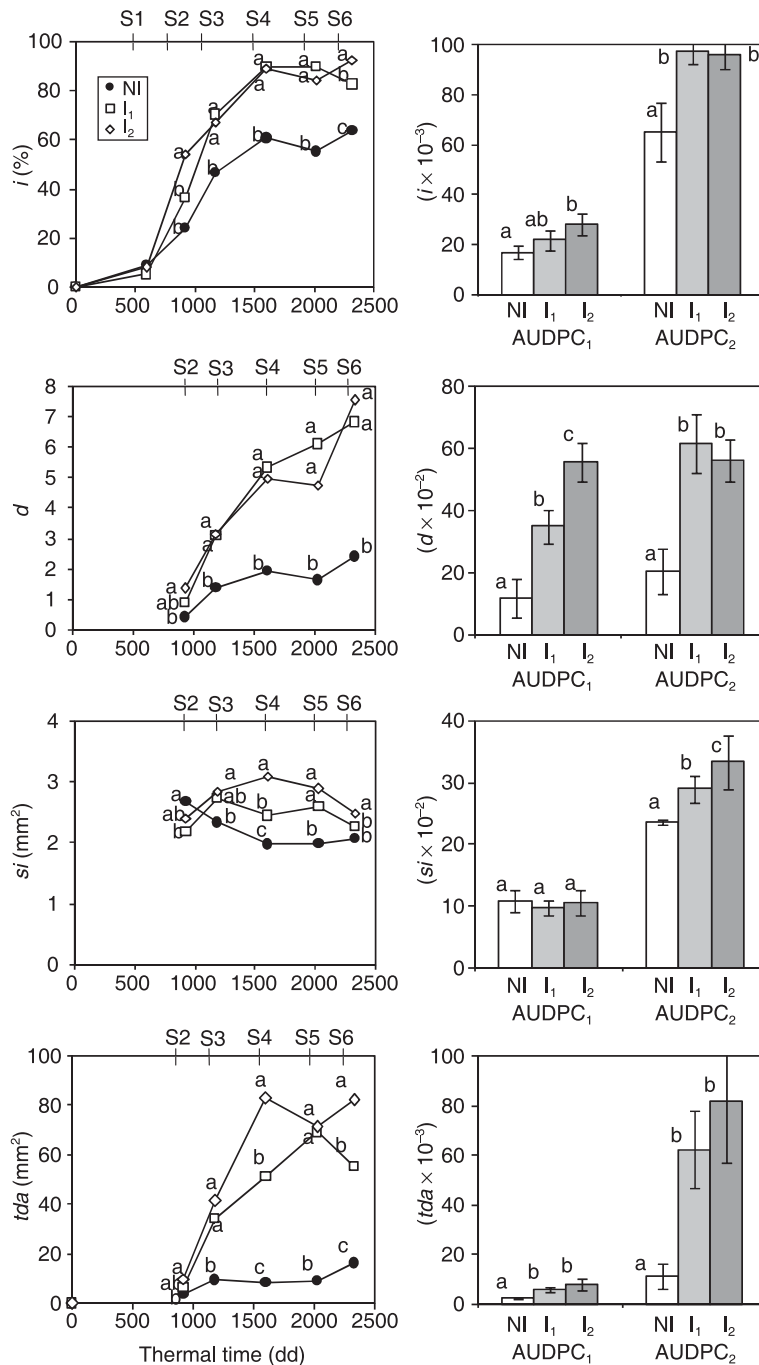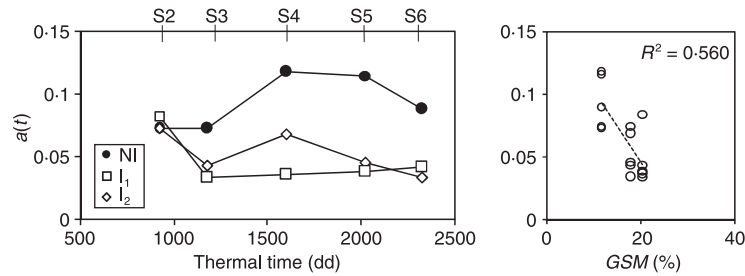
Figure 6 Effect of three soil moisture contents generated by different irrigation levels [NI, non-irrigated control; I_1, medium irrigation level (about 200 L m^{-2} over 5 months); I_2, high irrigation level (about 600 L m^{-2} over 5 months)] on the dynamics of a carrot cavity spot epidemic in the field (Le Rheu, France, 2003). Two intervals were distinguished to calculate AUDPCs [AUDPC$_1$ from S0 (sowing) to S3 (1181 degree days after sowing), and AUDPC$_2$ from S3 to S6 (2329 dd)]. Different lowercase letters indicate disease measurements at a specific time (ANOVA, Scheffe test, $P < 0.05$) and AUDPCs (NPAR1WAY, Wilcoxon-Mann-Whitney test, $P < 0.1$) significantly different between treatments. i is disease incidence, d lesion density, si symptom intensity and tda total diseased area.

in relative species abundance within the *Pythium* complex.

The fungicide field experiment showed that intermediate and late applications of mefenoxam were more effective on a CCS epidemic than an application immediately after sowing, suggesting that the fungicide primarily controlled secondary infections. This was confirmed by the microcosm experiment, in which mefenoxam applied late at a high dose (4 L ha^{-1}) was effective on alloinfections. However, this is not consistent with some field experi-

ments showing that sprays applied at similar or later timings are generally less effective (Hiltunen & White, 2002), nor with the fact that the product is registered for use in the early stages of a CCS epidemic (applications on plants having two or four leaves), which both imply that mefenoxam should reduce primary infections. There is conflicting evidence in the literature about the performance of early sprays (Lyshol *et al.*, 1984; Gladders & McPherson, 1986; McDonald, 1994), which might be caused in part by differences in soil types and soil moisture. If this is

Figure 7 Effect of three soil moisture contents generated by different irrigation levels [NI, non-irrigated control; I_1, medium irrigation level (about 200 L m^{-2} over 5 months); I_2, high irrigation level (about 600 L m^{-2} over 5 months)] on the time-dependent parameter $a(t)$ in the relationship $i = 100 \cdot (1 - \exp(-a(t) \cdot tda))$ in a carrot cavity spot field epidemic (Le Rheu, France, 2003). Values of $a(t)$ were optimized by the method of minimization of sums of squares ($P < 0.01$). t refers to thermal time (accumulated degree days from sowing, base 3·5°C). Mean GSM (gravimetric soil moisture) was calculated from 15 July to 30 September 2003. Different lowercase letters indicate mean values of $a(t)$ significantly different between treatments (NPAR1WAY, Wilcoxon-Mann-Whitney test, $P < 0.1$).

the case, the erratic performance of early sprays could result from a deficit in controlling secondary infections (probably generated by motile zoospores) when the fungicide is applied to wet soils or during high rainfall, resulting in leaching and hence reduced activity. Because transplanted roots are not necessarily a mimic of natural infection, the results should be validated in commercial fields.

The higher levels of disease in irrigated than in non-irrigated plots, consistent with past reports (White, 1988; Vivoda *et al.*, 1991; McDonald, 1994), point to a highly significant effect of these secondary infections in fuelling CCS epidemics. The data on increased disease severity and symptom intensity in moist treatments, confirming the observations of Benard & Punja (1995), point to the fact that soil moisture probably also favours lesion expansion, and thus has a pleiotropic effect on most epidemiological processes identified by Suffert (2006). Low soil moisture may reduce the number of primary infections during the earlier stages of the epidemic, preventing the pathogen from reaching the host, probably by impeding the growth of mycelium and strongly decreasing the spread of zoospores. The limiting effect of low soil moisture on CCS would then be less evident in non-zoosporic species, such as *P. violae*, than in zoosporic members of the CCS *Pythium* complex. A deficit of soil moisture also favours the healing of CCS lesions and reduces the number of lesions having a water-soaked appearance as a result of the maceration of superficial tissues (Campion *et al.*, 1997). This reduces the potential for secondary infections, which increases with increasing lesion size (*si*) and increasing total diseased area (*tda*).

The steep gradient of alloinfections generated in microcosms (rep1) illustrated that root-to-root contaminations can largely affect the progression of a CCS epidemic: the narrower the plant spacing, the more efficient the spread of the pathogen, the less time needed for infection, and the higher the number of infected plants. During the first 10 days after transplanting the donor roots, the maximal daily air temperature oscillated around 25°C in rep1 (ranged from 21·5°C to 27·5°C), although it was stable

around 20°C and never exceeded 22°C in rep2 and rep3. The optimum temperature for *P. violae* was found to be 19°C by Suffert & Guibert (2007), while other authors showed it was about 15°C (Montfort & Rouxel, 1988) or ranged from 20°C to 25°C (Van der Plaats-Niterink, 1981; Schrandt *et al.*, 1994). Thus, the conditions of development of *P. violae* were limiting in rep1, but optimal in rep2 and rep3. The mean root-to-root distance has a strong impact when edaphic conditions (soil moisture and temperature) limit the movement of *Pythium* propagules to host tissue; however, when conditions are optimal, the mycelium can grow from a CCS lesion through the sandy soil and easily reach the adjacent roots, whatever the planting density (up to a mean root-to-root distance of about 10 cm). The latency period for alloinfections ranged from 1 to 2 weeks, and the minimum root-to-root distance for alloinfection after 5 weeks exceeded 90 mm. Although White (1988) suggested that high plant densities may facilitate root-to-root spread and lead to greater levels of CCS, Vivoda *et al.* (1991) established no significant differences in the incidence of CCS among three plant densities. Such discrepancy is probably caused by differences in environmental conditions (edaphic and cropping factors), illustrated here by the heterogeneous results between rep1, rep2 and rep3.

Plant density generally increases the development of several soilborne plant pathogens; however, most authors failed to take the planting geometry into account besides actual density, resulting in the erroneous conclusion that disease incidence is inversely proportional to root spacing (Gibson, 1956; Scott, 1956). Clark & Ewans (1954) established a non-linear relationship between mean planting density (*D*) and mean root-to-root distance (*L*) when roots are uniformly distributed:

$$L = 0.5 \cdot D^{-0.5} \tag{5}$$

However, two crops with a constant *D* can differ in *L*, because the relationship also depends on the width of the row and on the geometry of the planting pattern. This was indirectly suggested by Crowe & Hall (1980) and Firman & Allen (1995), who showed that the rate of root-to-root
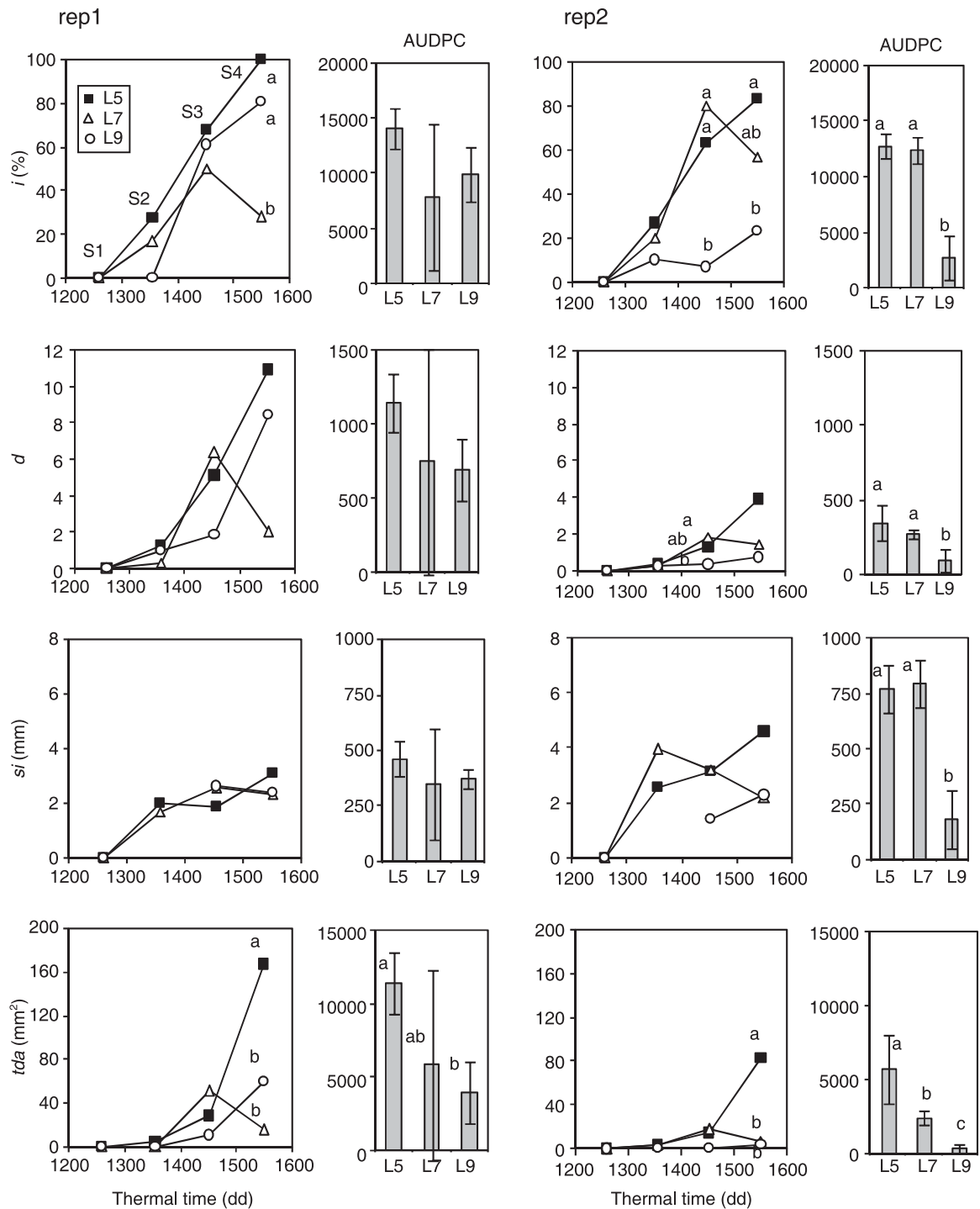
Figure 8 Effect of the distance between donor and receptor plants (L5, 50 mm; L7, 70 mm; or L9, 90 mm) on the dynamics of carrot cavity spot alloinfections generated by *Pythium violae* in experimental microcosms. Samples were taken 1, 2, 3 and 4 weeks after transplantation of the donor roots (S1, S2, S3 and S4, respectively). Different lowercase letters indicate disease measurements at a specific time (ANOVA, Scheffe test, *P* < 0·05) or respective AUDPCs (NPAR1WAY, Wilcoxon-Mann-Whitney test, *P* < 0·1) significantly different between treatments. *i* is disease incidence, *d* lesion density, *si* symptom intensity and *tda* total diseased area.
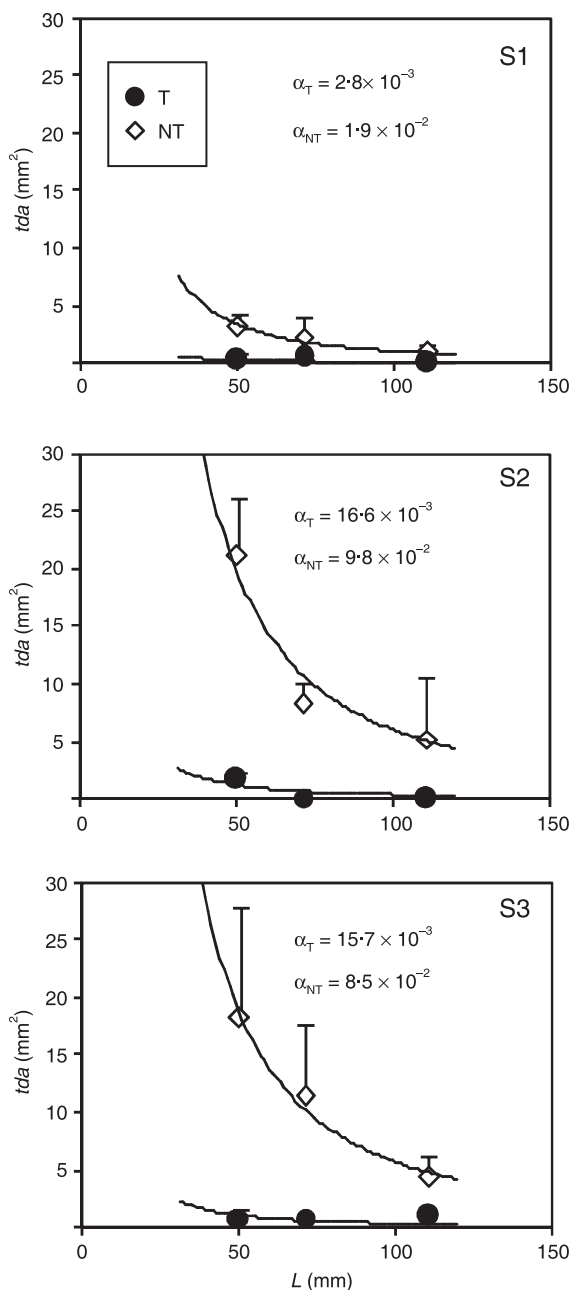
Figure 9 Dispersal gradient of carrot cavity spot alloinfections generated by *Pythium violae* in experimental microcosms. The power law model given by $tda = \alpha \cdot L^{-\gamma}$ was fitted with $\gamma = 1 \cdot 72$; *tda* is the total diseased area and *L* the distance between the receptor root and the nearest donor root. Three samples were taken 21, 33 and 46 days after transplantation of the donor root (S1, S2 and S3, respectively). T, plots treated with mefenoxam; NT, untreated control plots.

contamination is intrinsically influenced by both distance between roots and spatial arrangement. Because carrots are grown to meet a particular specification of root size, quality and yield, reducing seed density in commercial crops is not a realistic prospect; wider spacing would increase root size. However, it may be possible to devise planting patterns that increase the mean root-to-root distance at a constant mean linear density, and then decrease the potential for alloinfections.

An integrated disease management (IDM) system based on the epidemiology of CCS and a combination of cultural methods, such as optimizing fungicide application, date of harvest vs. soil moisture contents, and host density vs. planting pattern, seems to be a promising and sustainable way to control CCS. Synergistic effects of methods should be identified and applied, especially when they act on different stages of the epidemic. Of course, the current discussion should not understate the importance of primary infections. Even small lesions make roots unmarketable and crop losses ensue when the percentage of roots affected becomes too great to be sorted on the grading line. If secondary infections arising from primary ones are considered, the primary lesions become even more important. Quantification of soil inoculum during the life of the crop is thus a fundamental factor to include in an efficient IDM system. For a polycyclic disease such as CCS, the most effective strategies must combine methods that complement each other and reduce rates of primary infection, secondary infection and lesion expansion. Mefenoxam may be able to reduce alloinfections when sprayed during the cropping season, and thus slow the progression of CCS in time and space down. However, the failure of metalaxyl or mefenoxam to control CCS, associated with its reduced persistence in soil (Davison & McKay, 1999), has reduced the efficacy of fungicides on farms, so that targeting key infection processes and optimizing dates of applications has become more important. A deficit of soil moisture can reduce both primary and secondary infections, but this is difficult to control because of natural rainfall, and can also affect markedly plant growth. Growers can only reduce soil moisture by methods such as drainage, soil ridging or raised beds. However, an awareness of the soil moisture content during the storage of carrots in the field in winter is essential; the choice of harvest date is an indirect means to manage soil moisture effects in the development of CCS. Therefore, control through a sowing pattern that reduced the mean root-to-root distance may have significant advantages. Unlike decreasing inoculum density, which can only affect the number of primary infections, reducing plant spacing may decrease the rate of transmission through space.
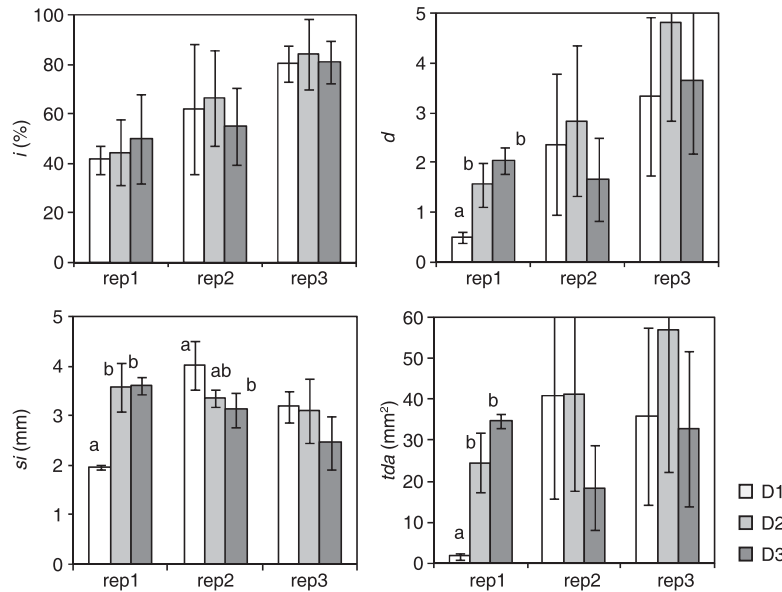
Acknowledgements

Figure 10 Effect of planting density on the dynamics of carrot cavity spot alloinfections generated by *Pythium violae* in experimental microcosms. D1 = eight receptor roots, D2 = 16 receptor roots, and D3 = 31 receptor roots. The experiment was carried out three times. Different lowercase letters indicate disease measurements at a specific time significantly different between treatments (ANOVA, Scheffe test, $P < 0.05$). *i* is disease incidence, *d* lesion density, *si* symptom intensity and *tda* total diseased area.

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