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Growth and survival of the mycoparasite *Coniothyrium minitans* on lettuce leaves in contact with soil in the presence or absence of *Sclerotinia sclerotiorum*

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Abstract. Lettuce leaves co-inoculated with *Sclerotinia sclerotiorum* and *Coniothyrium minitans* and controls were placed on, or buried in, soil for a period of two weeks to study development and survival of *C. minitans*. On *S. sclerotiorum*-infected leaves on the soil surface, the number of colonies of *C. minitans* recovered was about 40% of the number of pycnidiospores applied. When buried in the soil there was a reduction to about 2% of the spores applied. When *C. minitans* was applied on healthy lettuce leaves, which were subsequently placed on or in soil, the recovery was about 4%. It is argued that these figures indicate multiplication of *C. minitans* on *S. sclerotiorum*-infected lettuce leaves on the soil, and good survival in all other cases.

Introduction

Coniothyrium minitans is a well-known mycoparasite, notably of *Sclerotinia* spp. [Whipps and Gerlagh, 1992]. It can be recovered easily from sclerotia from soil [e.g., Campbell, 1947; Tribe, 1957], but whether it can compete and survive in other situations, such as on crop residues, is unknown. Although it colonizes aerial tissue infected by *S. sclerotiorum* [Trutmann et al., 1982], colonization of diseased tissue in contact with soil has only been described once on sunflower roots [Huang, 1977]. The work reported here compares the survival of *C. minitans* on lettuce leaves placed on and buried in the soil in the presence or absence of *S. sclerotiorum*.

Materials and methods

Leaves, size about 50–100 cm^2, of two-month-old glasshouse grown lettuce (*Lactuca sativa* L.), cv Hudson, were detached and inoculated on the midrib a quarter of the way from the base of the leaf with a 9 mm diameter

disk of an isolate of *S. sclerotiorum* from lettuce, grown on potato dextrose agar (Oxoid, PDA). The disks were taken from the margin of an actively growing, three-day-old culture. Uninoculated leaves served as a control.

The leaves were placed on moist filter paper in trays and enclosed in plastic bags at 20 °C for two days, when rotting began. Eighteen leaves per treatment were then sprayed to run-off on both sides with 30 ml of a pycnidiospore suspension of *C. minitans* (IPO-isolate C10) containing 3×10^7 spores in total. Controls were sprayed with distilled water. The spore suspensions of the *C. minitans* isolate were obtained by flooding Petri dishes containing two to three-week-old colonies on PDA with 20 ml of water. The colony surface was rubbed with a glass rod to release the pycnidiospores, and suspensions were filtered through two layers of cheesecloth and diluted to 10^6 spores ml^{-1}. No pycnidia were found in the suspensions.

The leaves were placed either side by side in rows on the surface of field soil (Loamy mixed calcareous mesic family of Typic Eutrochrepts; 25% < 2 μm; pH/KCl 7.0; 2.8% organic matter) on benches in a glasshouse or buried by covering with a thin layer (≤ 1 cm) of soil. The treatments were randomized. To prevent spread of the antagonist by water splash during irrigation, the treatments were separated from each other by 40 cm of bare soil. Continuously favourable conditions for white mould were ensured by maintaining temperatures between 15 and 25 °C and watering at least twice daily.

After two weeks the lettuce leaves placed on the soil were scored for the percentage healthy tissue remaining. Sclerotia were rarely observed. The remains of all lettuce leaves were collected, weighed and macerated in a Waring blender at high speed for 30 sec in 100 ml sterile distilled water. Aliquots (0.1 ml) from a dilution series of the leaf suspension made in sterile distilled water were plated out in 5 replicates on a semi-selective medium of PDA containing 0.32 g l^{-1} of a powder containing 5.5% chlortetracyclin hydrochloride (Cyanamid) and 2 ml l^{-1} Triton X-100 (Sigma) (Whipps and Budge, 1990). After 10 days incubation at room temperature the Petri dishes were scored for the presence of *C. minitans*. Other fungi were not considered.

The experiment was performed as a split-plot design with surface placement or burial as the main plots and *S. sclerotiorum* and *C. minitans* treatments randomized within plots. All treatments were in triplicate. Statistical analysis was done using Genstat 5, with logit transformation of percentage recovery data.

Two other experiments with slightly differing conditions and using more isolates of *C. minitans* gave similar results.

Results and discussion

S. sclerotiorum caused rotting of most of the leaves irrespective of whether *C. minitans* was present or not. The fresh weight of the remains of *S. sclerotiorum*-infected leaves was 23–43% of that of the control leaves not inoculated with *S. sclerotiorum* (Table 1).

No *C. minitans* was recovered on dilution plates from the control or the *S. sclerotiorum*-only treatments. Recovery of the mycoparasite from the *S. sclerotiorum*-infected leaves sprayed with *C. minitans* depended on the position of the leaves, with 38% recovery when placed on the soil and 2% when buried. Non-infected leaves inoculated with *C. minitans* gave a recovery of about 4% whether placed on the soil or buried (Table 1). The ANOVA of the percentage (logit transformed) *C. minitans* recovered, showed a significant ($P < 0.05$) effect of the position of the leaves (burial or not) and of the interaction leaf position * *S. sclerotiorum.*

The survival of *C. minitans* is even better than indicated by the simple recovery figures of the last column of Table 1. Since leaves bear the conidia of *C. minitans*, the rotting of the leaves when infected by *S. sclerotiorum*, which left only 23–43% of the surface area, already accounts for a corresponding reduction of the recovery. As viability of conidia of *C. minitans* in spore suspensions is only about 40%, combination of these two factors leads to 9–11% recovery being considered as the theoretical full survival of spores when leaves are inoculated with both *S. sclerotiorum* and *C. minitans*, whereas 40% recovery is expected for full survival on leaves sprayed with *C. minitans* only (Table 1). On the lettuce leaves placed on the soil, and inoculated with *S. sclerotiorum* and *C. minitans*, more than the theoretical 11% of *C. minitans* was recovered. This indicates reproduction of *C. minitans*, notwithstanding the competition of phyllosphere and soil microflora. According to this reasoning, when buried in the soil, the 2.1% recovery represents about 23% real survival (23% of 9% = 2.1%). Most probably the more intensive competition on the leaves in the soil compared with that on the soil surface will have prevented the mycoparasite from multiplying freely on its host. In the absence of *S. sclerotiorum* the survival of the mycoparasite is much less than on *S. sclerotiorum*-infected leaves placed on the soil, but, with about 10% real survival (3.5% and 4.5% as against 40% expected), still better than expected in view of its reputation as a poor saprophytic competitor [Magan and Whipps, 1988; Adams, 1989].

In conclusion, *C. minitans* was able to survive for a period of at least two weeks in contact with leaf and soil microflora. Competition from the soil microflora on leaves exposed on the soil, besides the normal phyllosphere flora, did not suppress its capacity to reproduce. A much higher degree of competition, when *C. minitans* was on buried leaves, prevented reproduction, or, at least, this was outweighed by decomposition. Since lettuce leaves are a very fragile food base for the growth of

Table 1. Leaf weight (fresh), spore viability and theoretical and actual recovery of *C. minitans* from lettuce leaves after two weeks incubation in or on soil in the presence or absence of *S. sclerotiorum*

Treatment			Leaf weight (g)[1]	% of control[2]	% spore viability[3]	Recovery of *C. minitans*			
						Theoretical[4]		Actual[5]	
Position	*S. sclerotiorum*	*C. minitans*				Number expected	(% of applied)	Number recovered[6]	(% of applied)
Leaves placed on soil	–	–	34.4 (± 10.2)	100	–	0		0	
	–	+	26.7 (± 10.2)	100	40	60	(40)	5.3 b	(3.5)
	+	–	6.9 (± 1.5)	23	–	0		0	
	+	+	8.4 (± 1.5)	28	40	17	(11)	56.7 a	(37.8)
Leaves buried in soil	–	–	44.0 (± 9.2)	100	–	0		0	
	–	+	46.1 (± 9.2)	100	40	60	(40)	6.7 b	(4.5)
	+	–	19.3 (± 6.4)	43	–	0		0	
	+	+	10.2 (± 6.4)	23	40	14	(9)	3.2 b	(2.1)

[1] Data in parentheses are s.e. based on combined *C. minitans* treatments within the same *S. sclerotiorum* treatment.

[2] % of control: the average leaf weight of each of the *S. sclerotiorum* treatments expressed as a percentage of the weight of the two *S. sclerotiorum* minus treatments combined.

[3] % of spores of *C. minitans* viable on PDA, based on 5 counts of 100 spores; s.e. 8.2.

[4] Number of *C. minitans* colonies expected on a Petri dish at a dilution of 5×10^3, based on the number of spores originally sprayed on the lettuce leaves (= 150 at this dilution), the percentage of leaf weight recovered, and the vitality of the spores. In parentheses, the number of colonies expected is expressed as the percentage of spores originally applied.

[5] Number of colonies of *C. minitans* actually recovered at this dilution. In parentheses this number is expressed as a percentage of the spores originally applied.

[6] Figures followed by the same letter are not significantly different ($P < 0.05$).

S. sclerotiorum, the situation might be more favourable for the mycoparasite when a more substantial food base for the former is available.

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