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Original article

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Abstract

The mycoparasitic fungus *Paraphaeosphaeria minitans* (formerly *Coniothyrium minitans*), is increasingly used by farmers to reduce soilborne inoculum of *Sclerotinia sclerotiorum*. In France, its field efficacy tends to be higher in the North than in the South, leading to the hypothesis of possible regional differences in the susceptibility of the pathogen to the biocontrol agent. A standardized assay was developed and four quantitative criteria were used to assess

the susceptibility to *P. minitans* of 75 *S. sclerotiorum* isolates collected from different regions of France. There was no significant difference between the group of isolates from the North and that from the South, but wide differences were observed among isolates, with consistent responses for all quantitative criteria. This study suggests that biocontrol efficacy might vary locally depending on the frequency of less susceptible isolates and it brings to attention the possibility that selection pressure could lead to a gradual increase in the frequency of less susceptible isolates, as this biocontrol method becomes widely adopted by farmers. To our knowledge, this is the first report of the variable susceptibility of *S. sclerotiorum* to a commercialized strain of *P. minitans* and the first detailed characterization of a large group of isolates from France for traits related to fitness, such as mycelial growth and production of sclerotia.

Introduction

The ascomycete *Sclerotinia sclerotiorum* has a broad host range and constitutes a threat to a variety of economically important crops, including oilseed rape, sunflower, soybean, and many vegetables (Bolton et al. 2006). The pathogen does not produce asexual conidia, but it can generate large numbers of sclerotia on diseased plants. These resting structures allow its long term survival and contribute to its dissemination (Coley-Smith and Cooke 1971). Under conducive conditions they germinate and produce either mycelium (myceliogenic germination), which can play a role in epidemics on certain crops, or apothecia (carpogenic germination) that may release large numbers of ascospores that are dispersed by air currents and are highly infective on most aerial plant parts (Clarkson et al. 2003; Bolton et al. 2006). Disease management in many crops is focused on the protection of the aerial plant parts against infection by ascospores, but due to their importance in the disease cycles, sclerotia are also a target for a variety of control methods (Kora et al. 2008; Derbyshire and Denton-Giles 2016; Clarkson et al. 2014; Shrestha et al. 2016; Lehner et al. 2017; Willbur et al. 2018).

The potential of using biocontrol agents to reduce soilborne populations of sclerotia or suppress their germination capacity has long been recognized (Campbell 1947; Steadman 1979; Adams 1990). Microorganisms reported to colonize sclerotia and show antagonistic properties against species of *Sclerotinia* include bacteria, predominantly in the genus *Bacillus* (Adams and Ayers 1979; Duncan et al. 2006; Chitrampalam et al. 2008; Wu et al. 2008; Zeng et al. 2012b; Gao et al. 2014; Kamal et al. 2015). However, much research has been focused on species of mycoparasitic fungi, including *Coniothirium minitans* (Whipps and Budge 1990), *Clonostachys rosea* (Rabeendran et al. 2006), *Dictyosporium elegans* (McCredie and Sivasithamparam 1985), several species of *Gliocladium* and *Trichoderma* (McCredie and Sivasithamparam 1985; Whipps and Budge 1990; Budge et al. 1995; Huang and Erickson 2000; Carpenter et al. 2005; Rabeendran et al. 2006; Chitrampalam et al. 2008; Geraldine et al. 2013), *Paecilomyces lilacinus*

(Whipps and Budge 1990), *Talaromyces flavus* (McLaren et al. 1996; Huang and Erickson 2000) and *Trichothetium roseum* (Huang and Erickson 2000). Among those, the widely distributed fungus *C. minitans* has received much attention world-wide and various isolates of this species have shown high biocontrol potential in laboratory or in field conditions (Trutmann et al. 1980; Sandys-Winsch et al. 1993; Gerlagh et al. 1999; Huang and Erickson 2000; Budge and Whipps 2001; Jones et al. 2003; Rabeendran et al. 2006; Chitrampalam et al. 2010; Yang et al. 2011; Zeng et al. 2012b). The taxonomy of this fungus was reexamined in recent years, leading to its assignment first to the species *Paraconiothyrium minitans* (W.A. Campb.) Verkley, and finally to *Paraphaeosphaeria minitans* (W.A. Campb.) Verkley, Göker & Stielow, comb. nov. (Verkley et al. 2014), which is the one adopted in our study.

One strain (CON/M 91-08) of *P. minitans* has been developed into a commercial product (De Vrije et al. 2001) and is now deployed in many countries. Field studies with the commercial product have shown successful reduction in the density of soilborne sclerotia for different species of *Sclerotinia* and disease suppression on various crops (von Tiedemann et al. 2001; Partridge et al. 2006; Chitrampalam et al. 2008; Öhberg and Bång 2010; Zeng et al. 2012a; Elsheshtawi et al. 2017). Variable results have also been reported in certain cases (Jones et al. 2004; Chitrampalam et al. 2010; Van Beneden et al. 2010; Bitsadze et al. 2015). In France, *P. minitans* has been increasingly used against *S. sclerotiorum* on various arable and vegetable crops since the first registration of the commercial product Contans® in 2001. Feedback from farmers are largely positive, but also suggest possible regional differences in efficacy, with a tendency for better control levels reported in the North than in the South of the country in open field conditions.

A wide range of abiotic and biotic factors may influence the efficacy of biocontrol and logically, variability in field efficacy of biocontrol is often attributed to environmental variability (Nicot et al. 2011). A seldom-considered hypothesis could also be that some

variability in biocontrol efficacy might result from differences in the susceptibility to biological control agents among the naturally occurring populations of the pathogens. A few reports have pointed to the possibility that plant pathogens could possess or develop reduced susceptibility to biocontrol agents (Bardin et al. 2015). Although information is lacking about the strain of *P. minitans* used in the commercial product Contans®, variability was reported for the pathogenicity of two *P. minitans* strains from the UK to different isolates of *S. sclerotiorum* and *S. trifoliorum* (Turner and Tribe 1976). In this study sclerotia were inoculated, placed at the surface of wet sand in trays and incubated for several weeks in humid conditions. The sclerotia were then periodically assessed visually for symptoms of infection by *P. minitans* and the numbers of symptom-showing sclerotia were recorded. Interestingly, the authors also observed the development of fungus gnat larvae on some of the sclerotia, and the possible role of these insects in the biocontrol of *S. sclerotiorum* was later formally investigated (Anas and Reeleder 1988; Gracia-Garza et al. 1997). Although the infection study of Turner and Tribe did not allow for statistical analyses, and the experimental conditions did not exclude a possible interference of different biotic factors with mycoparasitism, their results suggest that intrinsic differences in susceptibility to *P. minitans* may exist among isolates of *S. sclerotiorum*.

In order to better understand the regional differences in the field efficacy of biocontrol against *S. sclerotiorum* in France, the objectives our study were (i) to evaluate and quantify the variability in susceptibility to *P. minitans* among a large sample of *S. sclerotiorum* isolates collected from different regions of France and (ii) to test the hypothesis that isolates in the North may be more susceptible than those in the South of France. For this, we used a standardized quantitative *in vitro* assay developed in preliminary study to assess the susceptibility of *S. sclerotiorum* to *P. minitans* under axenic conditions (Nicot et al. 2016).

Materials and Methods

Isolates of *S. sclerotiorum* and production of sclerotia.

A total of 75 isolates of *S. sclerotiorum* were used in the present study. They belong to a core collection of isolates characterized for their genetic diversity in earlier work (Leyronas et al. 2018). They originated from sclerotia collected on diseased plants in commercial fields from several regions of France (Table 1). Upon reception in our laboratory the sclerotia were systematically surface-disinfested with 5% sodium hypochlorite (three minutes, followed with three rinses in sterile water), plated on Potato Dextrose Agar medium (PDA) and incubated in a growth chamber at 21°C under fluorescent light (162 µmol s^{-1} m^{-2}; 10 hour photoperiod) to stimulate myceliogenic germination. The resulting colonies were subjected to single-hyphal tip isolation as described by Lehner et al. (2016). The isolates were then stored at -20°C.

Prior to a test with *P. minitans* fresh sclerotia were produced for each isolate in 90-mm diameter Petri plates containing 17 mL of PDA. The plates were incubated for three weeks in a growth chamber at 21°C under fluorescent light (162 µmol s^{-1} m^{-2}; 10-hour photoperiod). The sclerotia were then collected and used immediately as described below.

Inoculum of *P. minitans* and inoculation of *S. sclerotiorum* sclerotia

One strain of *P. minitans* was used throughout this study. It was isolated by dilution plating from a preparation of the commercial product Contans®. The isolate was then monospored as described for *B. cinerea* (Leyronas et al. 2012) and stored as a concentrated spore suspension (10^8 spores mL^{-1}) at -20°C in 20% glycerol until use. Fresh inoculum was produced for each test with *S. sclerotiorum* by depositing 2 µL aliquots of stored spore suspension in the center of Petri plates containing PDA. The plates were incubated at 21°C under fluorescent light as described above. After three weeks, spores were collected from the colonies in sterile water and filtered aseptically through 30-µm mesh sterile filters to remove

mycelial fragments. The final inoculum of *P. minitans* consisted of spore suspensions adjusted to a concentration of 10^8 spores mL^{-1}.

For each isolate of *S. sclerotiorum*, four batches of 20 sclerotia were prepared in sterile tubes and mixed with 2 mL of either *P. minitans* inoculum (three inoculated batches) or sterile water (one control batch). Each batch of sclerotia was then mixed into 150 g of sterile sand in a 180 mL sample jar and incubated in the dark at 21°C.

Quantifying the effect of *P. minitans* on sclerotia of *S. sclerotiorum*

After three weeks of incubation, the sclerotia were recovered from the sand and disinfested in 5% sodium hypochlorite (for three minutes, followed by three rinses in sterile water) to remove *P. minitans* from their surface. Each sclerotium was then cut in half and the two fragments were plated on PDA, with the cut surface against the medium to facilitate the growth of *S. sclerotiorum* and *P. minitans* from the medulla of the sclerotia. For each isolate of *S. sclerotiorum*, a total of 80 half-sclerotia were plated on PDA, 60 from the batches of inoculated sclerotia and 20 from non-inoculated control sclerotia. All plates were incubated for one week at 21°C under fluorescent light as described above.

To assess the impact of internal colonization of sclerotia by *P. minitans* and quantify the susceptibility of *S. sclerotiorum*, the growth of *P. minitans* and of *S. sclerotium* from each half sclerotium was recorded after three and seven days of incubation. The number of daughter sclerotia produced by *S. sclerotiorum* on the Petri plates after seven days of incubation was also recorded.

To account for possible underlying differences in mycelial growth rates among isolates of *S. sclerotiorum*, a relative index was computed to compare their development from inoculated and non-inoculated sclerotia and thus quantify a reduction that would result

specifically from the effect of *P. minitans*. This index of reduction of mycelial growth was computed as:

$$IRm = 100*(D_{control} - D_{inoculated})/D_{control},$$

where $D_{inoculated}$ was the diameter of the *S. sclerotiorum* colonies on PDA after 3 days of incubation of sclerotia inoculated with *P. minitans* and $D_{control}$ was that for non-inoculated control sclerotia. Similarly, an index of reduction of the production of daughter sclerotia by *S. sclerotiorum* was computed as:

$$IRs = 100*(N_{control} - N_{inoculated})/N_{control},$$

where $N_{inoculated}$ was the number of daughter sclerotia produced on PDA after 7 days of incubation of sclerotia inoculated with *P. minitans* and $N_{control}$ was that for non-inoculated control sclerotia.

Statistical analyses

All statistical analyses were carried out with Statistica (Dell). The "Non-parametric Tests" module (Kruskal-Wallis tests) was used to evaluate differences of susceptibility to *P. minitans* among isolates of *S. sclerotiorum* as characterized by the different criteria described above. Wilcoxon-Mann-Whitney tests were used to evaluate the specific effect of *P. minitans* on each individual isolate by comparing colony diameter and production of daughter sclerotia from sclerotia of *S. sclerotiorum* previously incubated with the mycoparasite and from control non-inoculated sclerotia. These tests were also used to compare the susceptibility of the group of *S. sclerotiorum* isolates from the North to that of isolates from the South of France.

Finally, polynomial regression analyses were implemented with the "General Linear Model" module to examine possible relations between the various criteria used to characterize the susceptibility of the 75 isolates of *S. sclerotiorum*.

Results

Growth of *P. minitans* from sclerotia of *S. sclerotiorum*

Development of *P. minitans* colonies on PDA was never observed from non-inoculated sclerotia of *S. sclerotiorum*. Colonies of *P. minitans* developed from many but not all inoculated sclerotia, suggesting that for some of them, the extent of internal colonization by the mycoparasite was not sufficient to allow detectable growth within 7 days after the half-sclerotia were deposited on PDA. The frequency of detection of growth of *P. minitans* from sclerotia (Figure 1) differed significantly between isolates of *S. sclerotiorum* ($P = 0.002$) despite substantial variability among the three independent batches of sclerotia examined for each isolate, as shown by the size of the error bars. Similarly, the extent of *P. minitans* mycelial growth (Figure 2) from the colonies differed significantly between isolates of *S. sclerotiorum* ($P < 0.001$), with a much smaller level of variability among the 60 half sclerotia examined for each isolate. Polynomial regression analysis showed that these two types of assessment of the extent of sclerotial colonization by *P. minitans* were significantly associated (Figure 3), with the best fit obtained as $y = 0.0024x^2 + 0.0188x + 0.9284$ ($R^2 = 0.8435$; $P < 0.001$), where y was the colony diameter (in mm) and x the frequency of detection (in %) of *P. minitans* from *S. sclerotiorum* sclerotia.

For both of these criteria used to assess the susceptibility of *S. sclerotiorum* to *P. minitans*, there was no significant difference between the group of isolates from the North and those from the South of France (Table 2).

Mycelial growth of *S. sclerotiorum* from sclerotia

Mycelial growth of *S. sclerotiorum* on PDA medium was observed for all non-inoculated sclerotia and for many but not all sclerotia previously incubated for three weeks with *P. minitans* in sterile sand. For 29 of the 75 isolates examined, mycelial development occurred

from 100% of the inoculated sclerotia, while for the other isolates, mycelial growth was absent for up to 80% of the sclerotia (Figure 4), suggesting that the mycoparasite had compromised their viability. There was no significant difference between isolates from the North and from the South of France (Table 2). Based on polynomial regression analyses, the likelihood for the sclerotia of an isolate to show no mycelial germination was significantly related (Figure 5), both to the observed frequency of development of *P. minitans* on PDA medium from those sclerotia ($Y = 0.0085x^2 - 0.4447x + 5.4202$; $R^2 = 0.5547$, $P < 0.001$) and to the diameter of resulting *P. minitans* colonies ($Y = 0.0561x^2 + 0.2479x - 0.052$; $R^2 = 0.8054$, $P < 0.001$).

The diameter of *S. sclerotiorum* colonies three days after the plating of sclerotia on PDA medium varied widely among isolates, both for non-inoculated sclerotia and for those previously incubated for three weeks with *P. minitans* in sterile sand (data not shown; $P < 0.001$ for both). The IRm index, computed to assess the reduction of mycelial growth from inoculated sclerotia relative to non-inoculated control sclerotia also varied significantly among isolates ($P < 0.001$, Figure 6). For 46 of the 75 *S. sclerotiorum* isolates, IRm had a positive value, reflecting the fact that colony diameter from inoculated sclerotia was lower than that for control non-inoculated sclerotia, with statistically significant differences for 17 of those isolates ($P < 0.05$; Wilcoxon-Mann-Whitney test). However, IRm had negative values for 29 isolates, for which the diameter of the *S. sclerotiorum* colony was greater for sclerotia inoculated with *P. minitans* than for control non-inoculated sclerotia (Figure 6). For those isolates, the mycoparasite appeared to have stimulated, rather than inhibited, mycelial germination and subsequent growth of *S. sclerotiorum* on PDA medium. The differences were statistically significant for 19 of those isolates ($P < 0.05$; Wilcoxon-Mann-Whitney test). There was no significant difference between isolates from the North and from the South of France (Table 2). Polynomial regression analyses showed statistically significant relations ($P < 0.05$) between the Index of Reduction of mycelial growth and several predictors such as the frequency of

development of *P. minitans* from sclerotia ($y = 0.0043x^2 + 0.3403x - 29.144$; $R^2 = 0.421$; Figure 7-A), the diameter of resulting *P. minitans* colonies ($y = -0.0239x^2 + 3.6866x - 31.056$; $R^2 = 0.542$; Figure 7-B). In contrast, the diameter of *S. sclerotiorum* colonies growing from control non-inoculated sclerotia was a poor predictor of IRm ($R^2 = 0.094$; Figure 7-C).

Production of daughter sclerotia by *S. sclerotiorum*

The index IRs, computed to assess the reduction in production of daughter sclerotia by colonies growing from inoculated sclerotia relative to non-inoculated control sclerotia varied significantly among isolates ($P < 0.001$, Figure 8). For 49 of the 75 *S. sclerotiorum* isolates, IRs had a positive value, reflecting the fact that production of daughter sclerotia on colonies from inoculated sclerotia was lower than that for control non-inoculated sclerotia. IRs also showed negative values for some of the isolates, for which the production of daughter sclerotia by the *S. sclerotiorum* colonies growing from sclerotia inoculated with *P. minitans* was enhanced compared to control non-inoculated sclerotia (Figure 8). There was no significant difference between isolates from the North and from the South of France (Table 2).

Polynomial regression analyses showed statistically significant relationship ($P < 0.05$) between the Index of reduction of production of daughter sclerotia and several predictors such as the frequency of development of *P. minitans* from sclerotia ($y = 0.0056x^2 + 0.0932x - 8.9736$; $R^2 = 0.331$), the diameter of resulting *P. minitans* colonies ($y = -0.0295x^2 + 2.278x - 12.353$; $R^2 = 0.580$). In contrast, colony diameter and the production of daughter sclerotia by *S. sclerotiorum* colonies growing from control non-inoculated sclerotia were poor predictors of IRs (R^2 of 0.031 and 0.002, respectively; $P > 0.05$ for both predictors).

Discussion

Using a standardized assay in controlled axenic conditions, the present study showed wide differences in susceptibility to *P. minitans* among 75 isolates of *S. sclerotiorum*, with consistent responses for four criteria used to assess the impact of the mycoparasite. To our knowledge, this is the first study documenting the diversity in susceptibility of *S. sclerotiorum* to a commercialized strain of *P. minitans* and the first detailed characterization of a large group of isolates from France for traits related to fitness, such as mycelial growth and production of sclerotia.

A similarly high level of diversity was found among isolates collected from the North or from the South of France, and no significant regional difference was observed, suggesting that other hypotheses should be considered to explain the reported tendency for better field efficacy of biocontrol in the North. Many environmental factors, both abiotic and biotic, are known to influence the survival of sclerotia in soil (Bell et al. 1998; Duncan et al. 2006; Wu et al. 2008; Ćosić et al. 2012). Natural predation of sclerotia by various soil invertebrate species as well as degradation by soilborne microorganisms have been documented (Coley-Smith and Cooke 1971; Anas 1987; Gracia-Garza et al. 1997). Strains of *P. minitans* naturally present in soil, even if their density is likely to be considerably lower than that of the commercial biocontrol strain after soil treatments (Zeng et al. 2012b), could also play a role. Many environmental factors may also influence the biology and mycoparasitic activity of *P. minitans*, and differences in farming practices, including for field application of the biocontrol product, could thus lead to contrasted efficacy of the treatments (De Vrije et al. 2001; Partridge et al. 2006; Jones et al. 2011; Yang et al. 2011).

To avoid possible interference with some of these factors and focus on investigating intrinsic differences in susceptibility among isolates of *S. sclerotiorum*, the *in vitro* assay developed for the present study was highly standardized and the mycoparasitic activity of *P. minitans* on the sclerotia was limited to a three-week incubation period. Therefore, our

observations are unlikely to represent fully the behavior of the *S. sclerotiorum* isolates in a field situation. For example, sclerotia found free of infection by *P. minitans* after a three-week incubation period might eventually become parasitized over a longer time lap. Furthermore, in field conditions the numerous possible interfering factors evoked above might not affect identically the outcome of mycoparasitism for all isolates of *S. sclerotiorum*. Much additional research is needed to understand the mechanisms responsible for the differences of susceptibility revealed in our study and how those mechanisms could be affected by various factors operating in the field.

Even in a simplified experimental context as in the present study, our results highlight that mycoparasitism is clearly the complex outcome of multiple phenomena which occurred both during the three-week incubation period of sclerotia in the sample jars and after the sclerotia were plated on the PDA medium. This is exemplified by the unexpected negative values of the indices IRm (Figure 6) observed for certain isolates. Our starting hypothesis was that the extent of mycoparasitism in a sclerotium would lead to correspondingly reduced biomass of *S. sclerotiorum*, and that this in turn would result in slower germination and less vigorous mycelial growth from inoculated, compared to non-inoculated sclerotia. Things were clearly more complex. Although regression analysis showed that IRm was significantly related to other criteria used to characterize the susceptibility of *S. sclerotiorum* isolates, mycelial growth from inoculated sclerotia of the least susceptible isolates was stimulated (rather than showing reduced inhibition) relative to control non-inoculated sclerotia. Among possible explanations, one could be that two opposite phenomena occurred simultaneously, causing the observed outcome for these least susceptible isolates: (i) the penetration of the hard, melanized cortex of the sclerotia may have been slower or reduced, thus leading to reduced parasitism of the medullar part, and (ii) simultaneously the partial degradation of the cortex may be a stimulating factor for sclerotial germination, facilitating quicker growth of mycelium out of the

sclerotia when they were plated on PDA. Further work will be needed to test formally this and possibly other hypotheses. The same hypotheses may be examined to explain the negative IRs values (Figure 8), as sclerotial production by *S. sclerotiorum* on agar media is mostly initiated when the growing margin of a mycelial colony reaches the rim of the Petri plate. In addition to a possible link between production of daughter sclerotia and the kinetics of mycelial growth on PDA medium, a role of mycoparasitism on mycelia of *S. sclerotiorum* may also need to be further examined, as it is known to occur *in vitro* (Huang et al. 2011) as well as *in planta* (Trutmann et al. 1982), with possible consequences for production of daughter sclerotia in the latter case.

In conclusion, regardless of the possible mechanisms involved, the findings of the present study constitute a clear documentation of differences in susceptibility to a biocontrol agent among isolates of a plant pathogen, adding a contribution to a still very limited knowledge (Bardin et al. 2015). Differences in susceptibility of *S. sclerotiorum* to *P. minitans* cannot explain differences in the efficacy of Contans® in the North and the South of France. Nevertheless, the wide range of variability observed among isolates of *S. sclerotiorum*, regardless of their geographic origin, suggests that biocontrol efficacy might vary locally depending on the frequency of less susceptible isolates in specific fields. These results also bring to attention the possibility that selection pressure could lead to a gradual increase in the frequency of less susceptible isolates of the pathogen, as this biocontrol method becomes widely adopted by farmers.

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Table 1: Code number and origin of the 75 isolates of *Sclerotinia sclerotiorum* characterized in the present study.

Isolate	Host	Region (district)[1]	Year of sampling		Isolate	Host	Region (district)[1]	Year
2	Lettuce	S (84)	2012		83	Melon	S (17)	2014
3	Lettuce	S (84)	2012		86	Melon	S (17)	2014
4	Lettuce	S (84)	2012		88	Melon	S (17)	2014
5	Lettuce	S (84)	2012		90	Melon	S (17)	2014
6	Lettuce	S (84)	2013		93	Melon	S (17)	2014
7	Lettuce	S (66)	2013		95	Melon	S (17)	2014
10	Green bean	N (29)	2011		102	Melon	S (17)	2014
17	Green bean	N (29)	2011		103	Melon	S (17)	2014
23	Green bean	N (29)	2012		106	Melon	S (17)	2014
24	Green bean	N (29)	2012		109	Melon	S (17)	2014
25	Green bean	N (29)	2012		120	Rapeseed	N (21)	2014
28	Green bean	N (29)	2012		121	Rapeseed	S (47)	2014
29	Green bean	N (56)	2012		123	Rapeseed	N (77)	2014
30	Green bean	N (29)	2012		125	Rapeseed	N (78)	2014
31	Green bean	N (29)	2012		138	Rapeseed	N (77)	2014
33	Green bean	N (29)	2012		161	Melon	S (17)	2014
34	Green bean	N (29)	2012		162	Melon	S (17)	2014
35	Green bean	N (29)	2012		170	Melon	S (81)	2014
36	Green bean	N (29)	2013		172	Melon	S (82)	2014
37	Green bean	N (29)	2013		173	Melon	S (82)	2014
40	Green bean	S (40)	2012		174	Melon	S (82)	2014
41	Green bean	S (40)	2012		176	Melon	S (17)	2014
45	Melon	N (86)	2012		242	Melon	S (17)	2014
46	Rapeseed	N (57)	2003		736	Witloof chicory	N (62)	2001
48	Rapeseed	N (18)	2007		739	Witloof chicory	N (62)	2013
51	Rapeseed	N (21)	2007		864	Carrot	S (33)	2014

58	Witloof chicory	N (60)	2012		866	Carrot	S (33)	2014
59	Witloof chicory	N (80)	2012		918	Carrot	S (33)	2014
60	Witloof chicory	N (59)	2013		977	Carrot	S (33)	2014
61	Witloof chicory	N (62)	2012		983	Carrot	S (33)	2014
64	Witloof chicory	N (62)	2001		1003	Carrot	S (33)	2014
65	Witloof chicory	N (80)	2011		1234	Carrot	S (33)	2015
66	Witloof chicory	N (59)	2012		1252	Carrot	S (33)	2015
70	Melon	S (17)	2014		1261	Carrot	S (33)	2015
73	Melon	S (82)	2014		1262	Carrot	S (33)	2015
75	Melon	S (82)	2014		1592	Melon	S (17)	2015
79	Melon	S (17)	2014		1869	Melon	S (17)	2015
					2062	Carrot	N (50)	2015

[1] The numbers between brackets refer to the official codes of French districts (ISO 3166; https://www.iso.org/obp/ui/#iso:code:3166:FR), located in the northern (N) or the southern (S) part of the country

Table 2: Differences in the susceptibility of *Sclerotinia sclerotiorum* isolates to *P. minitans* based on their geographic origin in the North or the South of France

Criteria used to estimate susceptibility to *P. minitans*	Geographic origin of *S. sclerotiorum* isolates		*P* value (Wilcoxon-Mann Whitney test)
	North (32 isolates)	South (43 isolates)	
Frequency of recovery of *P. minitans* from inoculated sclerotia (%)	54.12	62.09	0.21
Diameter of *P. minitans* colonies from inoculated sclerotia (mm)	12.27	12.43	0.86
Frequency of absence of *S. sclerotiorum* from inoculated sclerotia (%)	15.29	14.09	0.41
Reduction of *S. sclerotiorum* mycelial growth from inoculated sclerotia relative to non-inoculated control sclerotia (%)	9.91	2.47	0.32
Reduction in production of daughter sclerotia by colonies growing from inoculated sclerotia relative to non-inoculated control sclerotia (%)	24.31	18.04	0.51

Figures

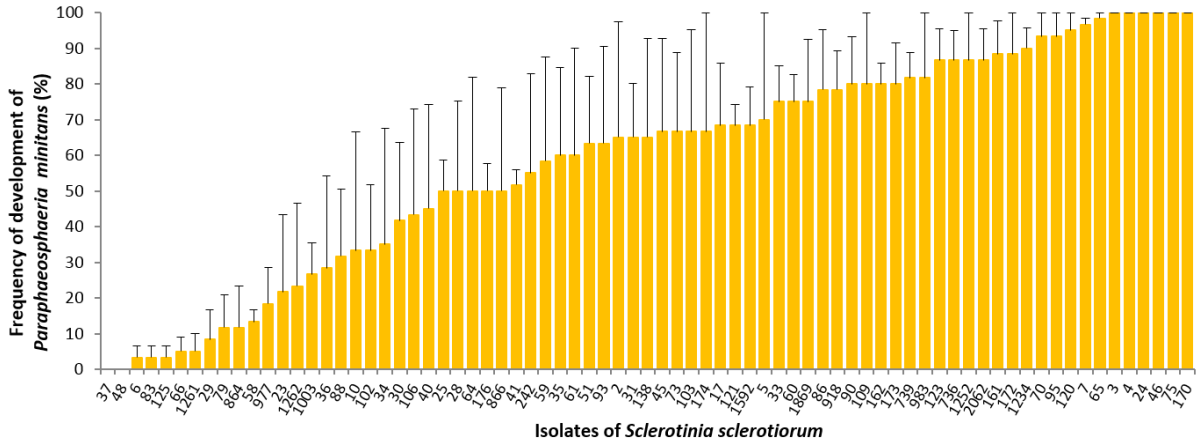

Figure 1: Frequency of development of *Paraphaeosphaeria minitans* from inoculated sclerotia of 75 *Sclerotinia sclerotiorum* isolates incubated for seven days on PDA medium. For each isolate, the data represent averages for three replicate batches of 20 half sclerotia ± standard error of the mean.

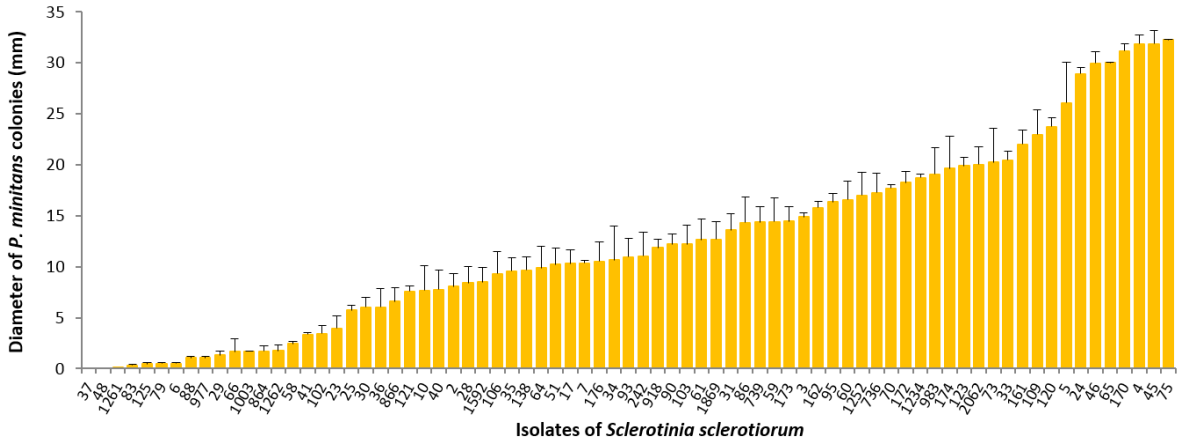

Figure 2: Diameter of *Paraphaeosphaeria minitans* colonies developed from inoculated sclerotia of 75 *Sclerotinia sclerotiorum* isolates incubated for seven days on PDA medium. Data represent averages for 60 half sclerotia ± standard error of the mean.

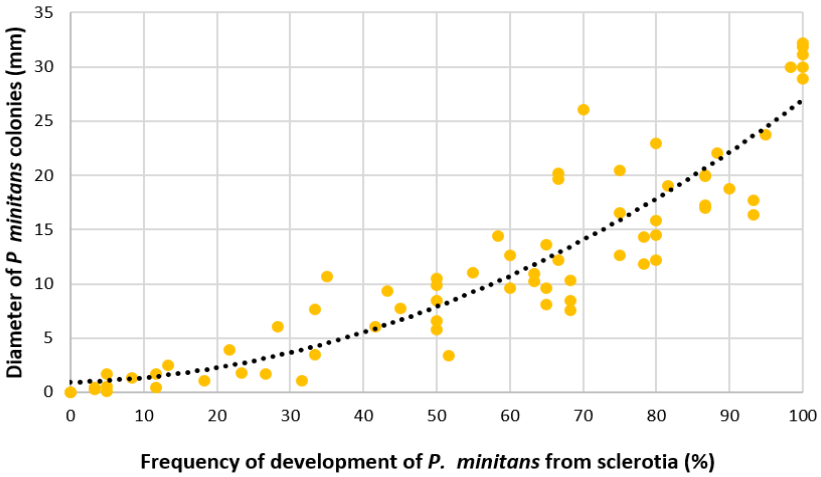

Figure 3: Relation between the frequency of development (%) and the diameter of *Paraphaeosphaeria minitans* colonies (mm) developed from inoculated sclerotia of 75 *Sclerotinia sclerotiorum* isolates after seven days of incubation on PDA medium. The dotted line shows the best fit obtained from polynomial regression analysis.

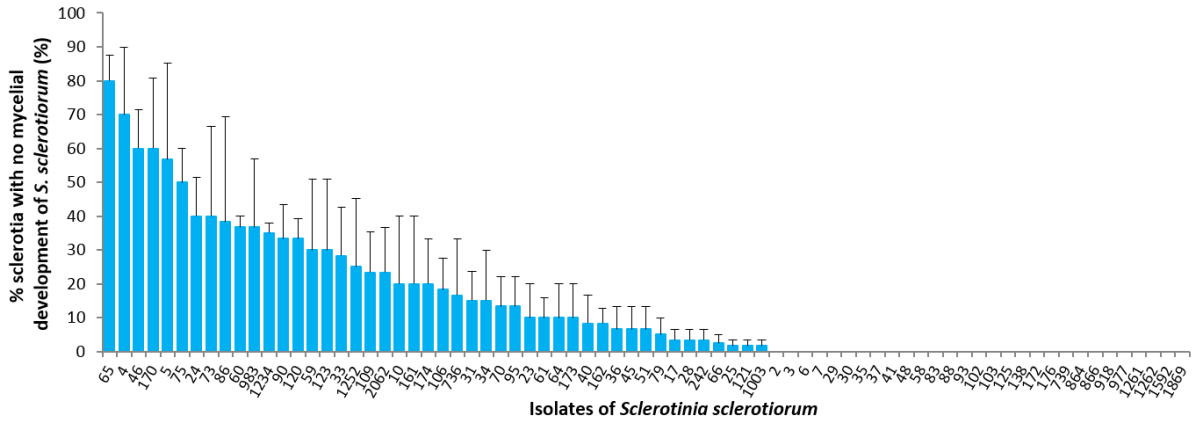

Figure 4: Frequency of absence of mycelial development of *Sclerotinia sclerotiorum* on PDA medium from sclerotia previously inoculated with *Paraphaeosphaeria minitans*. For each isolate, the data represent averages for three replicate batches of 20 half sclerotia ± standard error of the mean.

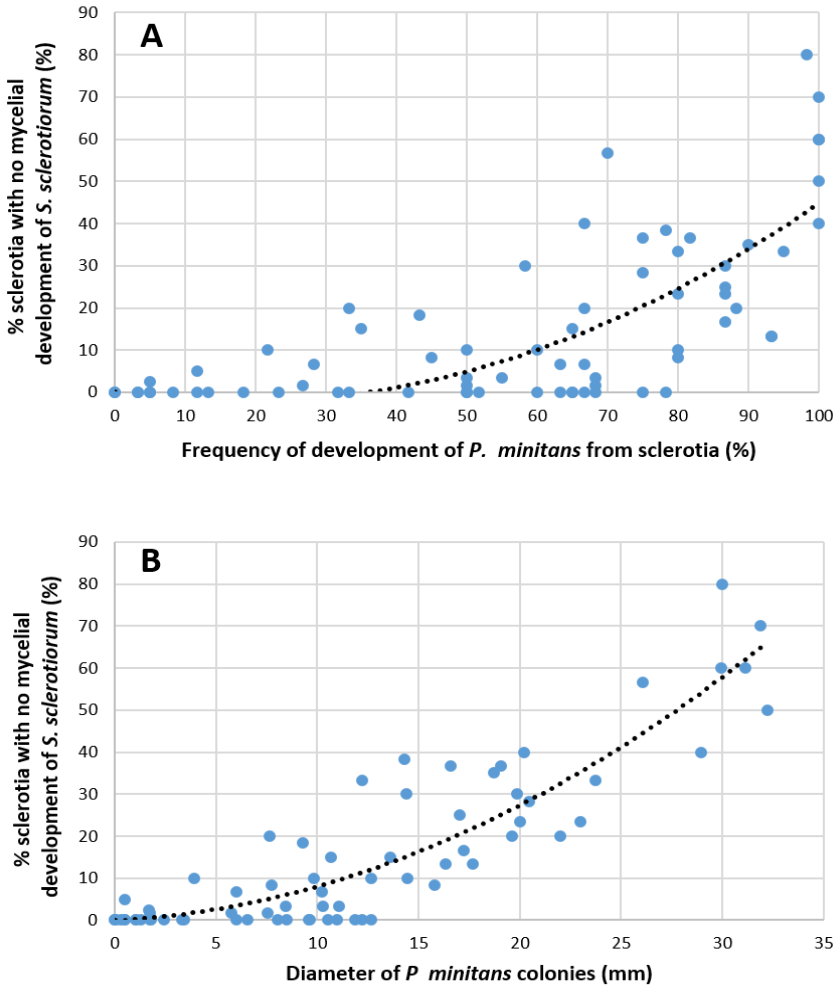

Figure 5: Relationship between the frequency of absence of *Sclerotinia sclerotiorum* development (%) and (A) the frequency of development of *Paraphaeosphaeria minitans* (%), or (B) the diameter of *P. minitans* colonies (mm) developed from inoculated sclerotia of 75 *S. sclerotiorum* isolates after seven days of incubation on PDA medium. The dotted lines show the best fits obtained from polynomial regression analysis.

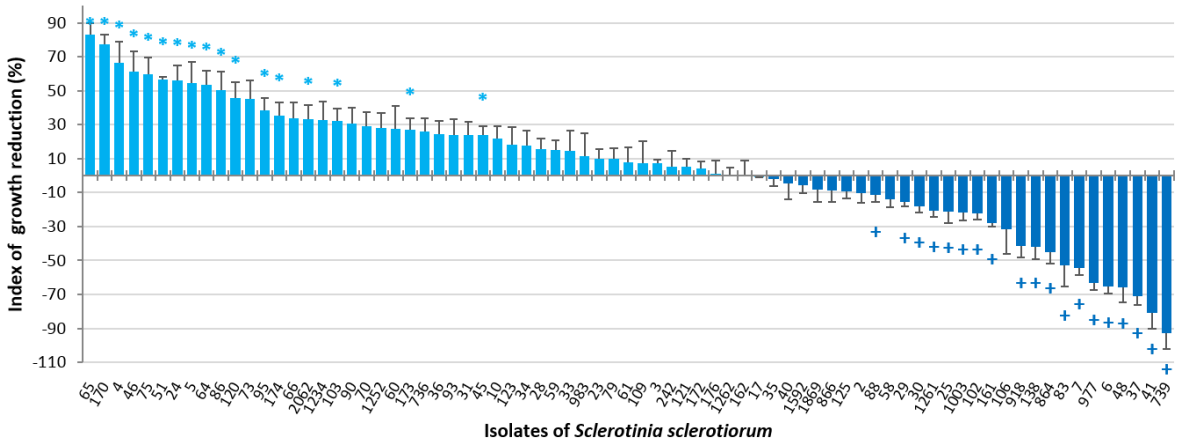

Figure 6: Reduction of mycelial growth of *Sclerotinia sclerotiorum* on PDA medium from sclerotia previously incubated with *Paraphaeosphaeria minitans*, relative to that from control non-inoculated sclerotia. Error bars indicate the standard error of the mean. For the 75 isolates of *S. sclerotiorum*, results of Wilcoxon-Mann-Whitney tests are shown to indicate significant reduction (***;** light blue color) or increase (+; dark blue color) of colony diameter from inoculated sclerotia compared to non-inoculated controls ($P = 0.05$).

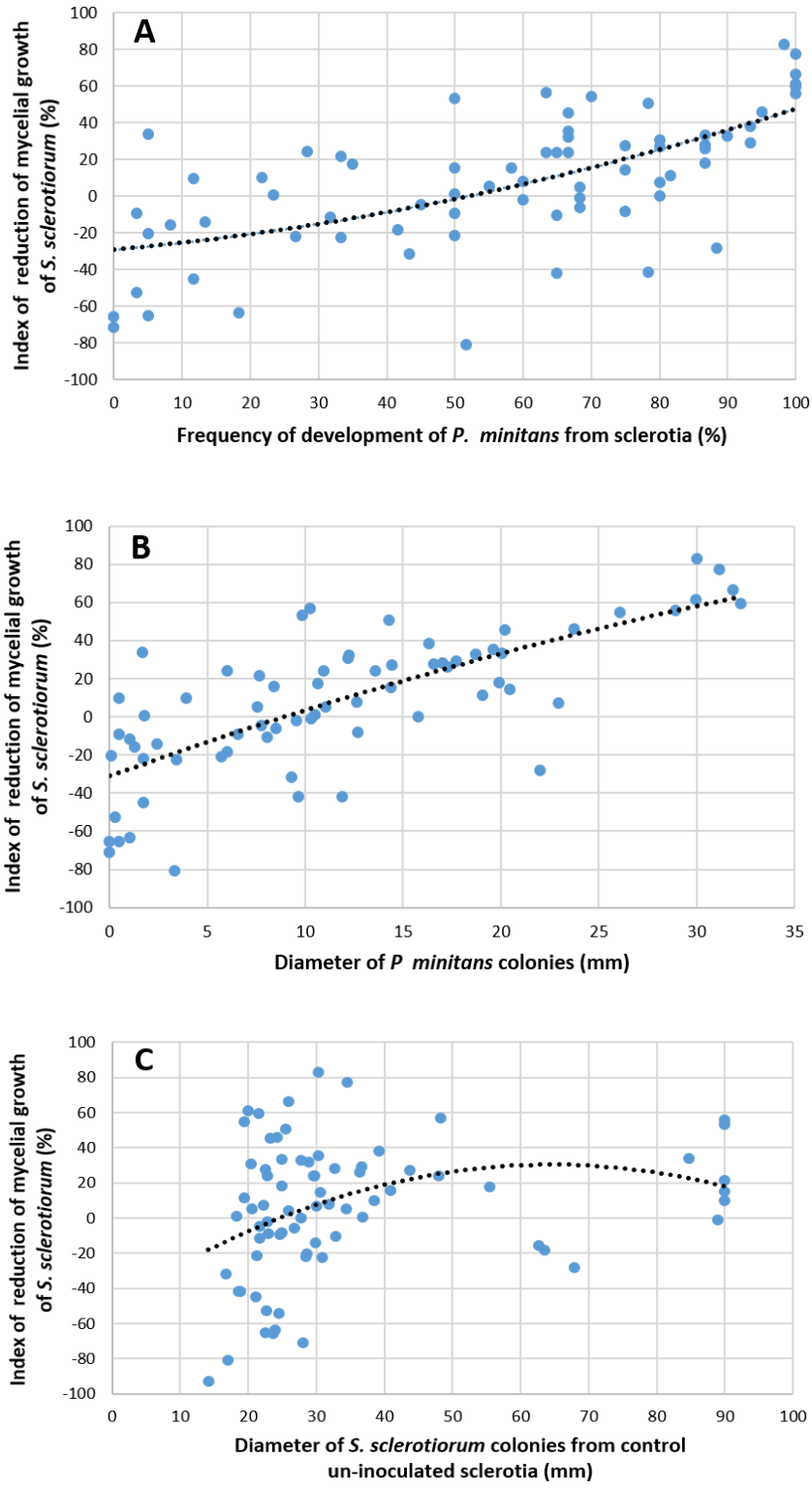

Figure 7: Relation between the reduction of mycelial growth of *Sclerotinia sclerotiorum* (%) and (A): the frequency of development of *Paraphaeosphaeria minitans* (%), (B): the diameter of *P. minitans* colonies (mm) developed from inoculated sclerotia of 75 *S. sclerotiorum* isolates

after seven days of incubation on PDA medium, or (C): the diameter of *S. sclerotiorum* colonies from non-inoculated sclerotia. The dotted lines show the best fits obtained from polynomial regression analysis.

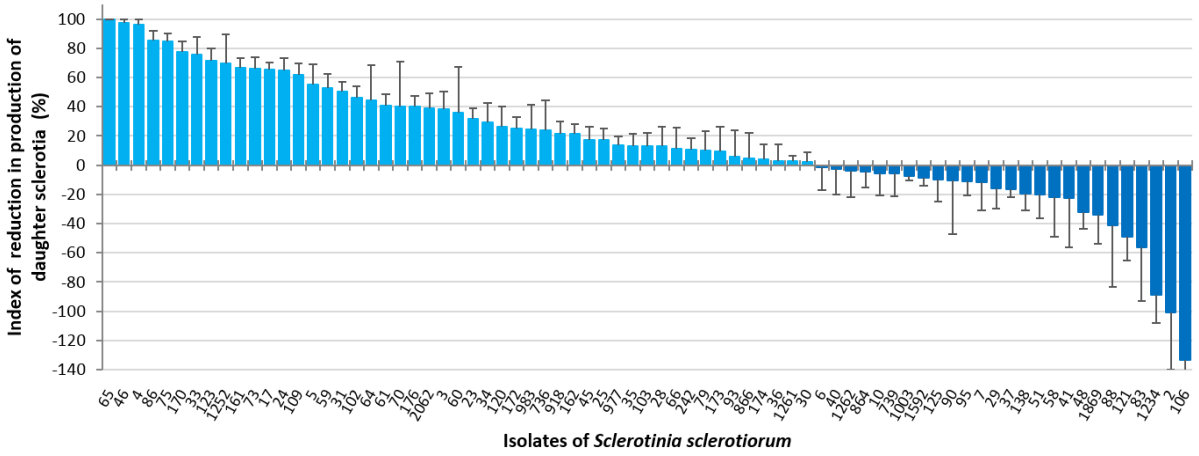

Figure 8: Reduction in production of daughter sclerotia by colonies of *Sclerotinia sclerotiorum* on PDA medium from sclerotia previously incubated with *Paraphaeosphaeria minitans*, relative to that from control non-inoculated sclerotia. Error bars indicate the standard error of the mean.