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Are there regional differences in the susceptibility of *Sclerotinia sclerotiorum* strains to *Coniothyrium minitans*?

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Abstract: In an attempt to understand regional differences in the efficacy of biocontrol against *Sclerotinia sclerotiorum* in France, strains of the pathogen were collected from different locations and tested for their susceptibility to the biocontrol fungus *Coniothyrium minitans*. Based on results obtained for the first 22 strains examined, wide and highly significant differences were observed, suggesting that the efficacy of this biocontrol method could vary locally depending on the frequency of susceptible *vs* less susceptible strains of *S. sclerotiorum*. However, the differences in susceptibility observed so far for strains from the North and the South of France cannot explain global regional differences in the efficacy of this biocontrol method. If confirmed by ongoing work on additional strains of *S. sclerotiorum*, these results will point to other hypotheses examined in the framework of national project "ScleroLeg".

Key words: *Sclerotinia sclerotiorum*, *Coniothyrium minitans*, biological control

Introduction

Since the registration of *Coniothyrium minitans* strain CON/M/91-08 in France (commercial product Contans®), biological control against *Sclerotinia sclerotiorum* has increasingly been used on various crops, with overall good results. However, returns from growers suggest regional differences in the efficacy of biocontrol, with better results in the North than in the South of France in open fields. Several possible environmental and agronomic hypotheses could be proposed to explain this situation, including differences in the pedoclimatic conditions of the farms (temperature, composition, structure, chemical and microbial properties of the soil, for example). Another possible hypothesis could be the existence of differences in the susceptibility to *C. minitans* among strains of *S. sclerotiorum*. Although little information is available, a few reports have pointed to the possibility that plant pathogens could possess or develop reduced susceptibility to biocontrol agents (Bardin *et al*., 2015).

The present study was initiated in the framework of national project "ScleroLeg" (https://www.picleg.fr/Les-Projets-en-cours/Scleroleg) to compare the susceptibility to *C. minitans* of strains of *S. sclerotiorum* collected from different different regions of France.

Material and methods

Strains of* S. sclerotiorum *and production of sclerotia

Strains were collected by project partners as mature sclerotia taken from diseased plants in commercial fields from several regions of France. The sclerotia received in the laboratory were systematically surface-sterilized and subjected to single-hypha culturing. The strains were then stored at -20 °C. Prior to its use in tests with *C. minitans*, each strain was grown for 3 weeks on PDA medium at 22 °C. The sclerotia were then collected on the Petri dishes and used immediately as described below.

Inoculum of* C. minitans *and inoculation of sclerotia

The inoculum of *C. minitans* consisted of spore suspensions adjusted to a concentration of 10^8 spores/ml. For each strain of *S. sclerotiorum*, 4 batches of 20 sclerotia were prepared in sterile tubes and mixted with 2 ml of either *C. minitans* inoculum (3 inoculated batches) or sterile water (one control batch). Each batch of sclerotia was then mixted into 150 g of sterile sand and incubated in the dark at 22 °C.

***Assessing the susceptibility of* S. sclerotiorum *to* C. minitans**

After 3 weeks of incubation, the sclerotia were disinfested for 3 minutes in sodium hypochlorite and rinsed in sterile water to remove *C. minitans* from their surface. Each sclerotium was then cut in half and the two fragments were placed on PDA medium and incubated for one week at 22 °C. To assess the susceptibility of *S. sclerotorium* to sclerotial colonisation by *C. minitans*, we examined each half sclerotium for presence and growth of *C. minitans* and of *S. sclerotium* after 3 and 7 days of incubation. For each strain 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.

To account for possible intrinsic differences in growth rate among strains of *S. sclerotiorum*, regardless of the effect of *C. minitans* on sclerotia, we computed an "index of growth reduction" as:

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

where $D_{inoculated}$ was the diameter of the *S. sclerotiorum* colonies after 3 days of incubation of half sclerotia inoculated with *C. minitans* and $D_{control}$ was that for non-inoculated control sclerotia.

Results and discussion

Among strains of *S. sclerotiorum* collected by partners of the ScleroLeg project, over 70 are to be tested for their susceptibility to sclerotium colonization by *C. minitans*. To date, results have been obtained and analysed for only a subsample of these strains (10 from the North and 12 from the South of France). They will be presented below.

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

Development of *C. minitans* colonies on the PDA medium was never observed from non-inoculated sclerotia of *S. sclerotiorum*. The mycoparasite developed from most 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 average diameter of the *C. minitans* colonies after 7 days varied widely depending on the strains of *S. sclerotiorum* (Figure 1), presumably reflecting differences in the amounts of *C. minitans* biomass present in the half-sclerotia at the time they were deposited on PDA. The effect of the *S. sclerotiorum* strain on this diameter was highly significant ($P < 0.001$).

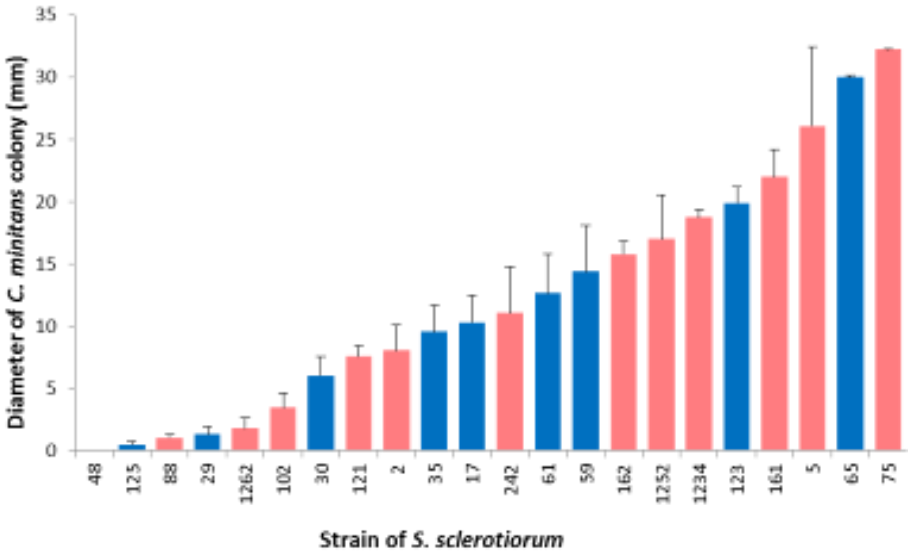

Figure 1. Development of *Coniothyrium minitans* from half-sclerotia of 22 strains of *Sclerotinia sclerotiorum* (from the North: ■ and from the south: ■ of France) 7 days after their deposition on PDA medium. Each data point represents the average of 60 observations; error bars are the standard error of the mean.

Germination of* S. sclerotiorum *sclerotia on PDA medium

Development of *S. sclerotiorum* colonies was observed from almost all half-sclerotia plated on the PDA medium, wether they had been inoculated or not with *C. minitans* before their incubation in sand. The diameter of *S. sclerotiorum* colonies after 3 days on PDA medium varied widely wether the sclerotia had been inoculated with *C. minitans* (Figure 2A) or not (data not shown). In both cases, highly significant differences were found among strains ($P < 0.001$). The comparison of colony diameter for inoculated and non-inoculated control sclerotia (using the "inhibition" index described above) showed that for many strains of *S. sclerotiorum*, mycelial growth was reduced in the presence of *C. minitans*, presumably reflecting the desctruction of biomass by the mycoparasite in inoculated sclerotia (Figure 2B). For certain strains of *S. sclerotiorum*, however, inoculation of sclerotia with *C. minitans* did not impact mycelial growth negatively and surprisingly, a strong stimulation (negative values of the "inhibition" index) was even observed in some cases (Figure 2B). The differences among strains were highly significant ($P < 0.001$).

***Regional differences among strains of* S. sclerotiorum**

According to our initial hypothesis, strains from the South of France would be less susceptible to *C. minitans*, lowering the efficacy of biocontrol compared to the situation in Northern France. However, both groups of strains showed wide differences in their susceptibility to *C. minitans* (Figures 1 and 2). Furthermore, strains for the North were among those for which

the recovery of *C. minitans* from inoculated sclerotia was the lowest (for example N° 48, 125 and 29) and that of *S. sclerotiorum* the least reduced. On average, no significant differences were found between the groups of North and South strains for either susceptibility criteria ($P > 0.05$).

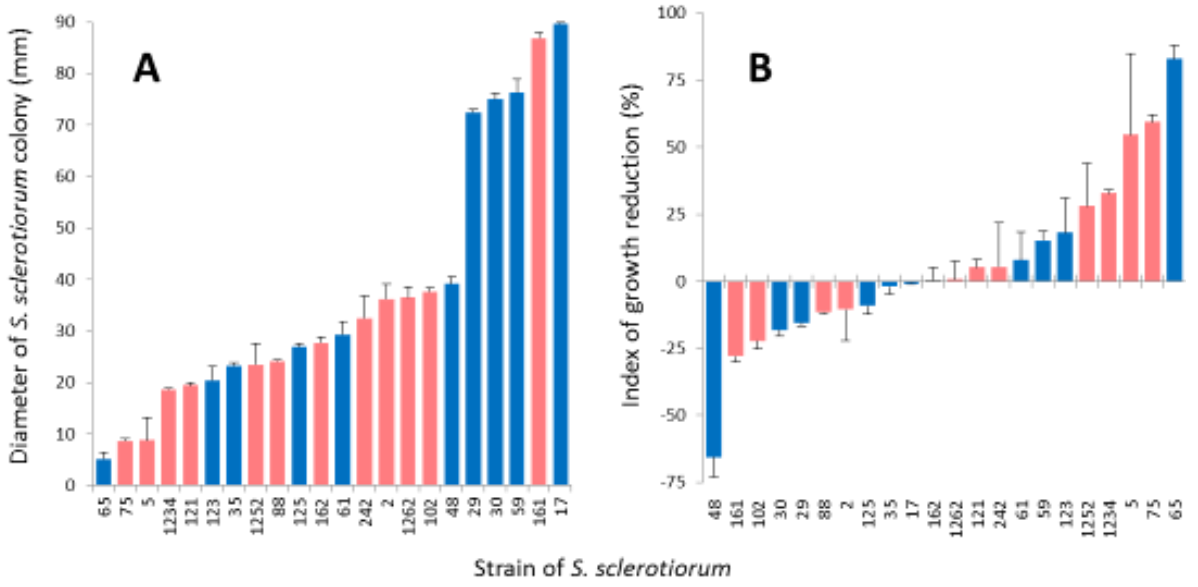

Figure 2. Development of 22 strains of *S. sclerotiorum* (from the North: ■ and from the south: ■ of France) from half-sclerotia 3 days after their deposition on PDA medium. A. Sclerotia inoculated with *C. minitans*; B. Comparison of growth from inoculated and non-inoculated sclerotia. Each data point represents the average for 60 half-sclerotia; error bars are the standard error of the mean.

Conclusions and perspectives

In the present study, the impact of *C. minitans* on sclerotia of *S. sclerotiorum* was assessed through (i) the extent of mycelial growth of *C. minitans* from inoculated sclerotia of *S. sclerotiorum* and (ii) the comparison of mycelial growth of *S. sclerotiorum* from inoculated and non-inoculated sclerotia. Both of these criteria revealed wide and highly significant differences in susceptibility to *C. minitans* among 22 strains of *S. sclerotiorum* examined so far. This finding suggests that the efficacy of this biocontrol method might vary locally depending on the frequency of susceptible *vs* less susceptible strains of *S. sclerotiorum*. However, differences in susceptibility observed so far for strains from the North and the South of France cannot explain reported regional differences in the efficacy of Contans®.

The present data will be complemented shortly with the assessment of ca 50 additional strains. If this consolidates the present results, more focus will need to be put on other hypotheses evaluated in the ScleroLeg project to explain North-South differences in biocontrol efficacy.

Acknowledgements

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