Can sclerotium size of Sclerotinia sclerotiorum be used as a predictor of susceptibility to Coniothyrium minitans ?

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Can sclerotium size of *Sclerotinia sclerotiorum* be used as a predictor of susceptibility to *Coniothyrium minitans*?

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Abstract: In previous work, we observed wide levels of diversity among strains of *Sclerotinia sclerotiorum* in the susceptibility of their sclerotia to colonization by the mycoparasite *Coniothyrium minitans*. Here, we investigated a possible relationship between the level of *in vitro* susceptibility of the strains and the morphological traits of their sclerotia, with the aim of providing a simple predictive tool for field assessment. We focused on the average thickness of whole sclerotia and on that of the melanised cortical tissue that the mycoparasite needs to penetrate to colonize the medullar tissue. Significant differences were found among strains, with ranges of 0.80-1.72 mm and 51-84 µm, respectively, for sclerotium size ($P < 0.001$) and for cortex thickness ($P = 0.05$). However, sclerotium size was not correlated to any of the susceptibility indices examined for the strains (P values > 0.26; $R^2 < 0.06$). Cortex thickness was significantly correlated ($P = 0.019$; $R^2 = 0.32$) to the average frequency of detection of *C. minitans* in inoculated sclerotia but not to other susceptibility indices. These results suggest that other factors (possibly related to the biochemical composition of the tissues) play a determinant role in susceptibility of sclerotia to *C. minitans*. As a high level of intra-strain variability was observed (both in terms of morphological traits and susceptibility of sclerotia), work is under way to characterize individually larger numbers of sclerotia for selected strains.

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

Introduction

Coniothyrium minitans is known to colonize and destroy sclerotia of *Sclerotinia sclerotiorum* and biocontrol products based on this mycoparasite are widely used by farmers to reduce the amount of soilborne inoculum in the field. In previous work, we observed differences in the susceptibility of sclerotia among strains of *S. sclerotiorum* (Nicot *et al.*, 2016), providing more evidence for the interest of questioniong the frequently accepted view that biocontrol is likely to be more durable than chemical control (Bardin *et al.*, 2015). The behaviour of *S. sclerotiorum* strains could not explain regional differences in control efficacy reported by growers in the North (better control) and in the South of France. Instead, a wide level of diversity was found among strains of *S. sclerotiorum* regardless of their geographic area and host of isolation, suggesting that local differences in control efficiency could also occur depending on the susceptibility of strains prevalent in a given field.

This further raised interest in providing growers with simple tools that could help predict the susceptibility of strains in a field without resorting to the lengthy and costly *in vitro* test used in our previous work (Nicot *et al.*, 2016). Based on the mode of action of the mycoparasite, the present study was initiated to test the hypothesis that the susceptibility of sclerotia could be related to their morphological traits.

Material and methods

Strains of S. sclerotiorum *and production of sclerotia*

A selection of 24 strains previously caracterized for their susceptibility to *C. minitans* (Nicot *et al.*, 2016) were used in this study. They were stored at -20 °C. To produce sclerotia, each strain was grown for 3 weeks on PDA medium at 22 °C. The sclerotia were then collected on the Petri dishes and examined for their morphological traits.

Quantification of morphological traits of sclerotia

Thin cross-sections were excized from the center of sclerotia and examined under the microscope at low magnification. We recorded the thickness of the cortical melanised tissue that needs to be penetrated by *C. minitans* to colonize the sclerotia after its spores germinate on the outside surface (Figure 1). As a measure of the effort needed for the mycoparasite to fully invade the medullar tissue, we also recorded the thickest part of the sclerotium (examplified by red arrows in Figure 1).

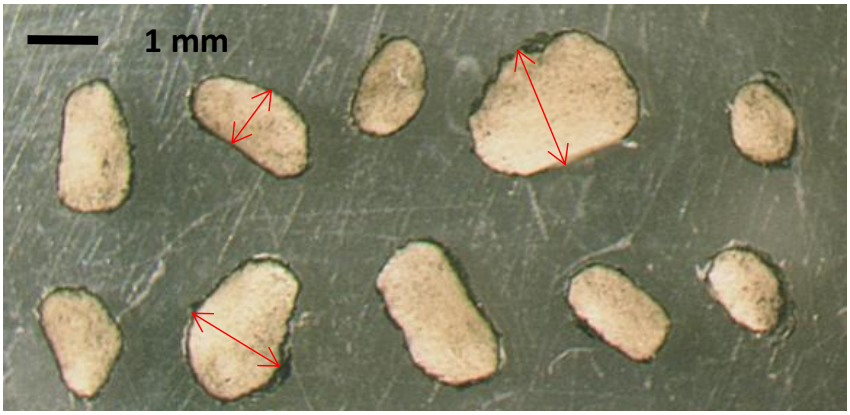

Figure 1. Variability in the thickness and shape of *S. sclerotiorum* sclerotia, as seen from cross-sections. The red arrows illustrate the thickness measurements recorded for sclerotia.

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

The susceptibility of *S. sclerotiorum* sclerotia to the colonization by *C. minitans* was assessed with a standardized *in vitro* test as reported before (Nicot *et al.*, 2016). For each strain under study, this consisted of incubating batches of sclerotia for three weeks in sterile sand in the presence of *C. minitans*, after which the sclerotia were surface disinfested, cut in halves and plated on PDA medium to allow for both fungi to develop. We recorded the presence and measured the growth of *C. minitans* and of *S. sclerotiorum* after 3 and 7 days of culture on the PDA medium. To account for possible intrinsic differences in growth rate among strains of *S. sclerotiorum*, regardless of the effect of *C. minitans* on sclerotia, similar observations were

carried out on batches of sclerotia incubated without inoculum of *C. minitans*, and we computed an index of growth inhibition for each strain of *S. sclerotiorum* 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. Similarly, the production of daughter sclerotia was quantified on the PDA plates both for inoculated and non-inoculated sclerotia and we computed an index of inhibition of the production of daughter sclerotia. The susceptibility of a strain was thus assessed on the basis of four indices.

Results and discussion

Morphological traits of S. sclerotiorum *sclerotia*

The sizes and shapes of sclerotia varied greatly for a given strain of *S. sclerotiorum* (illustrated for strain SS48 in Figure 1), but significant differences were found between strains in terms of the thickness of the sclerotia ($P < 0.0001$; Figure 2). The strains also differed in the average thickness of the melanised cortical part of the sclerotia ($P = 0.05$; Figure 3).

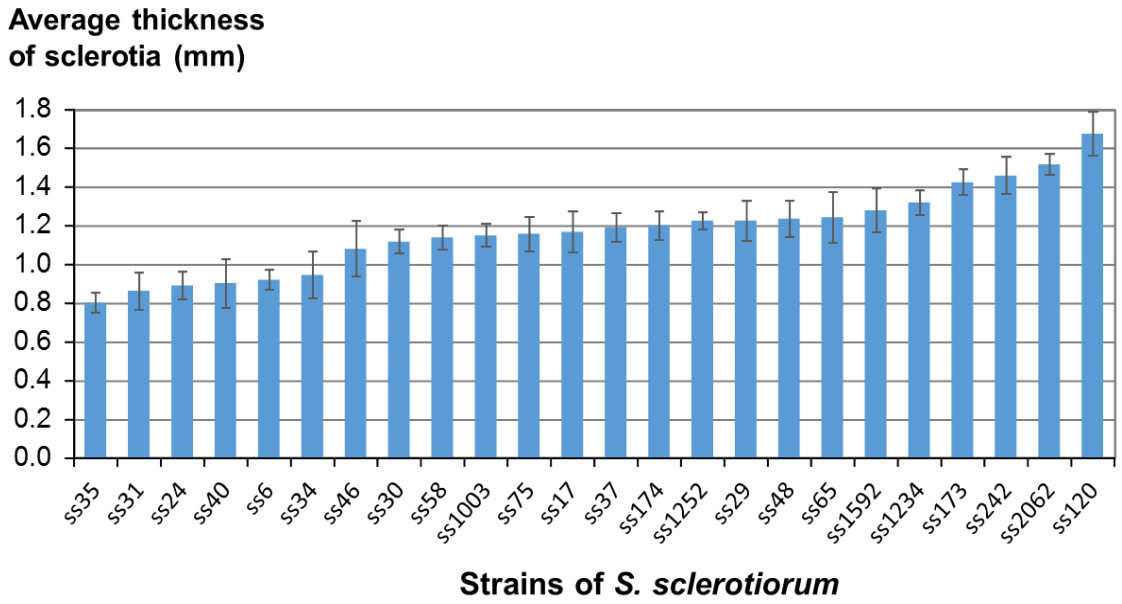

Figure 2. Variability in the size of sclerotia among strains of *S. sclerotiorum*. Error bars indicate the standard error of the mean.

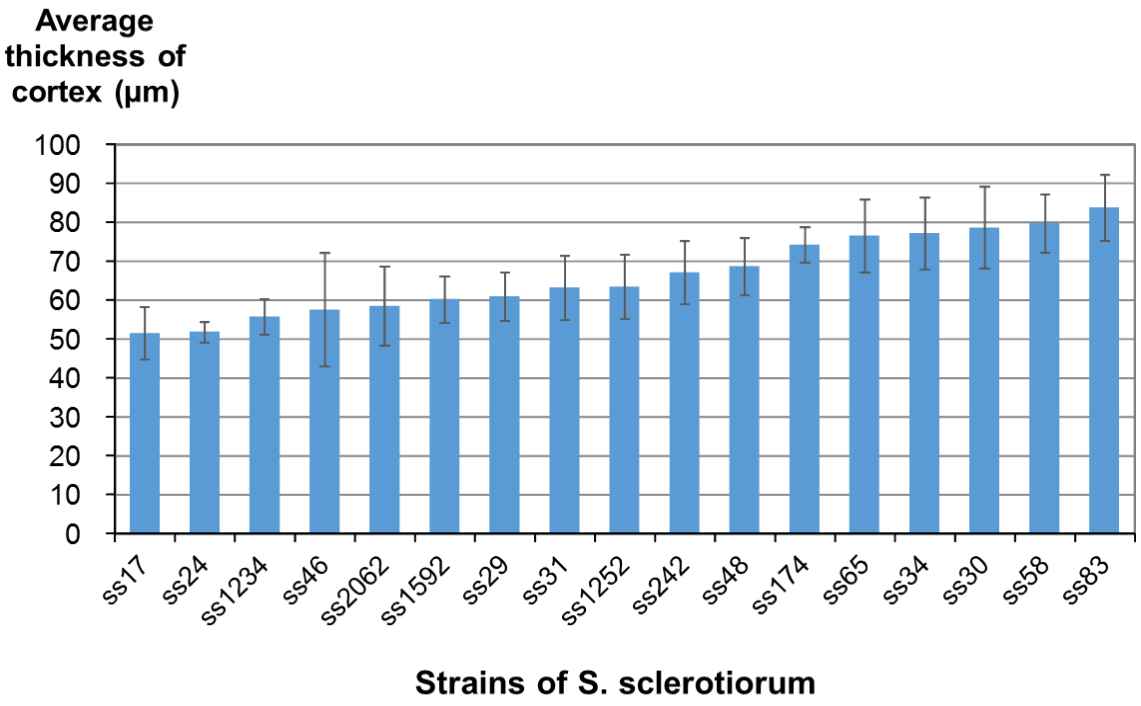

Figure 3. Variability in the thickness of the melanised cortical part of the sclerotia among strains of *S. sclerotiorum*. Error bars indicate the standard error of the mean.

***Relationship between the morphology of sclerotia and their susceptibility to* C. minitans**

Correlation analysis between the average thickness of the sclerotia of a strain of *S. sclerotiorum* and its susceptibility revealed no significant relationship for any of the four susceptibility indices examined (Figure 4).

A significant negative correlation was observed between the average thickness of the melanised cortical part of the sclerotia and the frequency of presence of *C. minitans* in inoculated sclerotia ($P = 0.019$; $R^2 = 0.32$), but not with the three other susceptibility indices examined (P values between 0.115 and 0.303; R^2 between 7 and 15%). The equation of the significant linear regression was

$$Y = - 1.934\ X + 186.21$$

where Y was the average frequency of presence of *C. minitans* (in %) evaluated after 7 days of culture on PDA medium of *S. sclerotiorum* sclerotia previously inoculated and incubated with *C. minitans* for 3 weeks, and X was the average thickness of the cortical part of the sclerotia (in µm).

All the correlations reported here were established on average values (both for the predictor variables and for the indices of susceptibilities) for all the strains examined. This may have resulted in a low level of precision, as a high level of intra-strain variability was observed. The lack of correlation observed in many cases, and the low R^2 obtained in case of statistically significant correlations, could be in part a result of this lack of precision. They also suggest that other possibly strain-dependent factors (for example the biochemical composition of the cortex or medulla of the sclerotia) could play a significant role in the susceptibility of sclerotia to *C. minitans*.

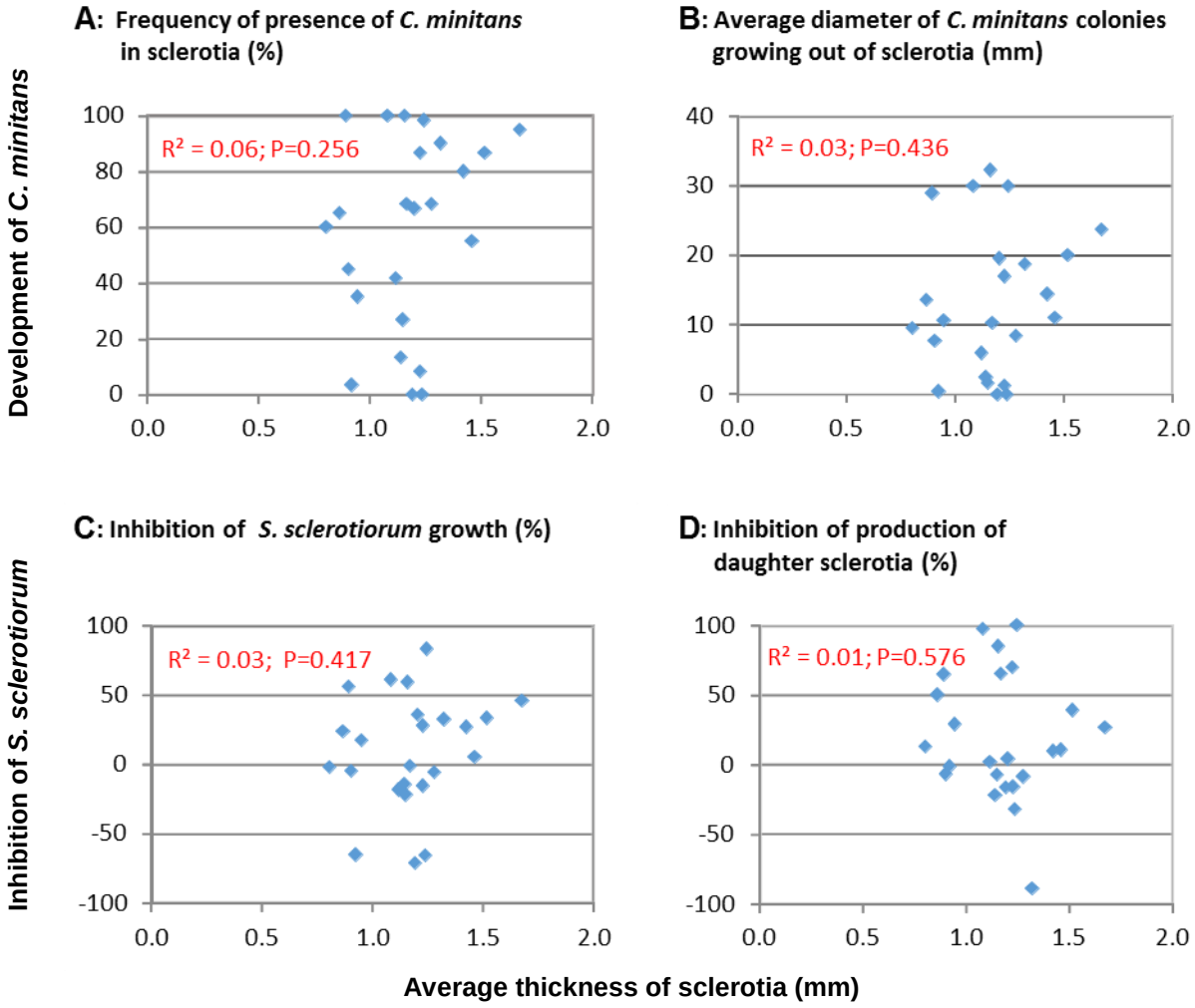

Figure 4. Relationship between the average thickness of the sclerotia of *S. sclerotiorum* strains and their susceptibility to *C. minitans* assessed with four criteria: A and B: the frequency of development and colony diameter on PDA of *C. minitans* growing out of inoculated sclerotia; C and D: the inhibition of mycelial growth by *S. sclerotiorum* and production of daughter sclerotia. Each data point represents a strain of *S. sclerotiorum.*

Conclusions and perspectives

In the present study, we seek to identify a predictor of the susceptibility of *S. sclerotiorum* strains to *C. minitans* that could be easily implemented to provide growers with information relevant to strains present as soilborne inoculum in individual fields. The preliminary results presented here showed that the thickness of the cortical part of the sclerotia, rather than the thickness of the whole sclerotia, may offer such a potential. The lack of correlation observed in many cases, and the low R^2 obtained in case of statistically significant correlations, could be in part a result of the lack of precision resulting from using average values for the different strains. In an effort to gain precision, work is in progress to evaluate a possible relationship on the basis of new data recorded for large numbers of individual sclerotia.

Our results also suggest that other, possibly strain-dependent, factors may play a substantial role in the susceptibility of sclerotia to *C. minitans*. Such factors (for example the biochemical composition of the cortex or medulla of the sclerotia), may also need to be examined in future work.

Acknowledgements

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