A Taxonomic Study of *Sclerotinia sclerotiorum* and Related Species: Mycelial Interactions

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(Received 26 November 1974; revised 23 January 1975)

SUMMARY

Selected isolates of *Sclerotinia sclerotiorum* (Lib.) de Bary were grown together on agar, and the mycelial reactions in the regions of association studied. Three types of reaction were observed: (i) free intermingling of mycelia without antagonism and with occasional hyphal anastomoses; (ii) a white, later pigmented, incompatibility zone associated with excessive branching of hyphal tips at the margins of one or both of the colonies; (iii) a brown incompatibility zone of variable intensity, accompanied by early lysis of the tips of the peripheral hyphae of one of the colonies. Associated with reaction types (ii) and (iii) were interactions in which the hyphae of one isolate attached themselves to and grew around those of another isolate; this resulted in the lysis of the 'attacked' hyphae. The grouping of the selected isolates by means of reaction types and 'predatory' interactions gives support for the separation of *S. sclerotiorum* into three distinct species, *S. minor*, *S. trifoliorum* and *S. sclerotiorum*.

INTRODUCTION

In the past there has been much controversy regarding the taxonomy of *Sclerotinia sclerotiorum* (Lib.) de Bary [= *Whetzelinia sclerotiorum* (Lib.); Korf & Dumont, 1972], mainly because of the inadequacy of morphological criteria. Some workers (Jagger, 1920; Keay, 1939; Williams & Western, 1965) recognized *S. sclerotiorum*, *S. trifoliorum* and *S. minor* as separate species, but Purdy (1955) concluded that there was insufficient evidence to justify this separation and included them all in the one species, *S. sclerotiorum*. In recent work we have used ontogenetic (Willetts & Wong, 1971) and electrophoretic (Wong & Willetts, 1973) techniques to study the taxonomy of this group of fungi. Our findings have provided further evidence for the existence of three distinct species.

Reinhardt (1892) found, from co-inoculation experiments, that *S. trifoliorum* was incompatible with *S. tuberosa* and that the hyphae of the former appeared to attack those of *S. tuberosa*. He concluded that this antagonism between the mycelia indicates specific differences. Björling (1951) found that mycelia of different isolates of *S. trifoliorum* were compatible, but Loveless (1951) observed a pigmented 'incompatibility line' between *S. trifoliorum* isolates from pasture legumes and others from *Vicia faba*. He used this as a criterion to separate *S. trifoliorum* var. *fabae* from *S. trifoliorum*. We sought further information on the taxonomy of this group of fungi by studying the mycelial interactions of selected isolates.

Table 1. *Details of isolates of Sclerotinia spp.*

Isolate reference code	Host from which isolated	Size of sclerotium in culture	Name given at time of isolation
SM-P	Potato	Small (0.3–2.0 mm diam.)	<i>Sclerotinia minor</i>
SM-T	Tomato		
SM-S1	Sunflower		
SM-S2	Sunflower		
ST-L1	Lucerne	Large (1.0–8.0 mm diam.)	<i>S. trifoliorum</i>
ST-L2	Lucerne		
ST-B1	French bean		
ST-B2	French bean		
SS-CL1	Cauliflower	Large (1.0–8.0 mm diam.)	<i>S. sclerotiorum</i>
SS-CB	Cabbage		
SS-CP	Capsicum		
SS-SU	Sunflower		

METHODS

Details of the 12 isolates of *Sclerotinia* used are given in Table 1. These were cultured either directly on 10 ml of potato–dextrose agar (PDA) in plastic Petri dishes (9 cm diam) or on boiled cellophane discs placed over the medium. Each dish was inoculated with three isolates placed at equal distances around the edge. All possible combinations of the 12 isolates were studied, with eight replications for each combination on agar and four on cellophane. The cultures were incubated at 20 °C under fluorescent light of about 160 lx and were examined daily over a period of 3 weeks. The experiment was repeated.

RESULTS

The following three types of reaction were observed when hyphae of different isolates approached each other on agar cultures (Table 2).

(i) Mycelia intermingled freely and no antagonism between the hyphae was apparent (Fig. 1*a, b, c, d*). Hyphal fusions (anastomoses) were observed between different mycelia, particularly between the hyphae of the small-sclerotium producing isolates.

(ii) A white zone, which later became pigmented, developed between mycelia and could be seen without magnification (Fig. 1*c, e*). Microscopic examination showed that this white zone was due to excessive branching of the hyphal tips of one or both colonies, but that it was more frequent at the periphery of only one of the colonies (Fig. 2*a*) (cf. Fig. 2*b*). Eventually the hyphal tips at the margins of both of the interacting mycelia lysed to give an unevenly pigmented zone along the region of association. The hyphae of the different isolates were sometimes in physical contact, especially in regions where mycelial growth was less abundant, as when the fungi were grown on cellophane discs placed over agar; the hyphal tips of one colony then attached themselves to and grew around the hyphae of the others (Fig. 2*c, d*). This 'predatory' action eventually resulted in the lysis of the hyphae of the less aggressive isolate.

(iii) A brown zone developed rapidly between colonies (Fig. 1*d, e*); the intensity of this varied from very light to dark brown, depending on the isolates used. Upon microscopic examination it was found that the tips of the peripheral hyphae of one of the colonies readily lysed as the margins of the two mycelia intermingled. Lysis could also occur before the

Table 2. *Types of mycelial interactions occurring between Sclerotinia isolates*

Cultures were incubated on PDA at 20 °C and 160 lx for 8 days.

Isolates	SM-S1, SM-S2, SM-P, SM-T	ST-L1, ST-L2	ST-B1, ST-B2, SS-CL1, SS-CB, SS-CP, SS-SU
SM-S1 } SM-S2 } SM-P } SM-T }	Reaction type (i) (mycelia intermingle freely without any in- compatible reactions)	Reaction type (ii) (formation of a white zone); ST-L1 and ST-L2 'predatory' on SM iso- lates when hyphal con- tacts occur	Reaction type (iii) (rapid formation of a brown zone – intensity of zone variable); SM isolates 'predatory' on SS, ST-B1 and ST-B2 isolates when hyphal contacts occur
ST-L1 } ST-L2 }	Reaction type (ii)	Reaction type (i)	Reaction type (ii); ST-L1 and ST-L2 isolates 'predatory' on ST-B1, ST-B2 and SS isolates when hyphal contacts occur
ST-B1 } ST-B2 } SS-CL1 } SS-CB } SS-CP } SS-SU }	Reaction type (iii)	Reaction type (ii)	Reaction type (i)

different mycelia made physical contact with one another. A 'predatory' type of interaction similar to that described in (ii) was also frequently observed (Fig. 2e).

DISCUSSION

The type of reaction which occurs between mycelia of fungal isolates is probably an indication of their genetic relationship. Intermingling of hyphae without incompatibility and accompanied by anastomoses suggests a very close relationship. The development of a white zone due to excessive hyphal branching (reaction type ii) was probably induced by accumulation of substances in the substrate ahead of the opposing colonies. Eventual lysis of the hyphal tips occurred in both colonies and was probably the result of the adverse effects of the accumulating substances on the metabolism of the hyphae. This reaction indicated possible genetic incompatibility between the isolates. Reaction type (iii) also indicated metabolic and thus possible genetic differences between the mycelia involved. The 'predatory' growth of one hypha around another was obviously an antagonistic reaction, as it invariably resulted in the death of the hypha which was 'attacked'. *Sclerotinia trifoliorum* was always the 'predator' when grown with the other two species; when *S. minor* and *S. sclerotiorum* were grown together, the former was usually the 'predator' when physical contact was achieved.

The mycelial interactions observed during this investigation can be used to separate the 12 selected isolates into three groups. Group 1 comprises isolates SM-P, SM-T, SM-S1 and SM-S2, all of which are small-sclerotium forms obtained from miscellaneous crops. Group 2 comprises isolates ST-L1 and ST-L2, which produce large sclerotia and were originally obtained from forage legumes (lucerne). Group 3 comprises large-sclerotium forming isolates ST-B1 and ST-B2 obtained from vegetable legumes (beans), and SS-CL1, SS-CB, SS-CP and SS-SU from miscellaneous crops. This grouping agrees, without exception, with that which we obtained from the use of ontogenetic and electrophoretic criteria (Willetts & Wong, 1971; Wong & Willetts, 1973). Further support is therefore given for the separation of *S. sclerotiorum* into three species, *S. minor* (exemplified by the isolates included above in group 1), *S. trifoliorum* (group 2) and *S. sclerotiorum* (group 3).

Fig. 1. Reactions between *Sclerotinia* isolates at regions of association on agar-plate cultures. (a), (b) Reaction between compatible isolates showing no antagonism: (a) between ss isolates; (b) between sm isolates. (c) Reaction between ST-L1 and ST-L2, showing no antagonism, and reaction of ST-L1 and ST-L2 with ST-B1, resulting in the formation of a white reaction zone. (d) Reaction between ST-B1 and ss-CB, showing no antagonism, and reaction of ST-B1 and ss-CB with SM-P, resulting in the formation of a brown reaction zone. (e) Reaction of ss-SU and SM-T with ST-L1, resulting in the formation of a white reaction zone, and reaction between ss-SU and SM-T, resulting in the formation of a brown reaction zone.

Fig. 2. Microscopic observations of excessive hyphal branchings and 'predatory' interactions at regions of association between *Sclerotinia* isolates. (a) Excessive hyphal branching reaction of ST-L1 when approaching the mycelial front of SM-T in agar. (b) Normal hyphal tips and branchings of ST-L1 at mycelial front. (c), (d), (e) 'Predatory' action of hyphal tips of one isolate on the hyphae of another: (c) 'predation' of SM-T by ST-L1; (d) 'predation' of SS-SU by ST-L1; (e) 'predation' of SS-SU by SM-T.

Co-inoculation techniques could have practical application in specific identifications when standard, known species are available. It is always necessary to make careful microscopic observations of the periphery of colonies as weak interactions could be overlooked or misinterpreted in a macroscopic examination. However, final identification, may require an electrophoretic analysis of protein extracts from the fungi.

We thank Drs R. J. W. Byrde, M. Marsden and A. E. Ashford for their critical reading of the manuscript, and the Reserve Bank of Australia for financial support.

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