Electrophoretic Studies of Australasian, North American and European Isolates of *Sclerotinia sclerotiorum* and Related Species

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SUMMARY

Forty-seven isolates of *Sclerotinia* species, collected from a variety of crops growing in Australia, New Zealand, North America and Europe, have been classified into three distinct groups on the electrophoretic patterns for soluble proteins, arylesterase, acid phosphatase, tetrazolium oxidase, glucose-6-phosphate dehydrogenase (NADP-linked) and reduced nicotinamide adenine dinucleotide phosphate dehydrogenase. There were only small intra-group differences. The electrophoretic patterns of an isolate of *Whetzelinia* (= *Sclerotinia*) *tuberosa* were characteristically different from those of the other isolates. These results support the findings from previous studies when ontogenetic, electrophoretic and mycelial-interaction criteria were used to group a smaller number of isolates from New South Wales, Australia. It is concluded that *S. sclerotiorum*, *S. trifoliorum* and *S. minor* are three distinct species.

INTRODUCTION

Our ontogenetic (Willetts & Wong, 1971), electrophoretic, and mycelial-interaction (Wong & Willetts, 1973, 1975) studies of *Sclerotinia sclerotiorum* (Lib.) de Bary [= *Whetzelinia sclerotiorum* (Lib.) Korf & Dumont, 1972] and related species, have consistently suggested that this group of fungi comprises three distinct species, *S. minor*, *S. trifoliorum* and *S. sclerotiorum*, although Purdy (1955), who used morphological criteria, included the three in the one species *S. sclerotiorum*. In earlier work we used fifteen isolates of *Sclerotinia* obtained from various crops growing in New South Wales, Australia. Analyses of isolates from a wider variety of crops, including more forage and vegetable legumes from other parts of Australia and the northern hemisphere, were needed before our conclusions from the New South Wales material could be generally applied to these fungi. This paper describes polyacrylamide gel electrophoretic studies on isolates collected from crops growing in four Australian states, and from New Zealand, North America and Europe.

METHODS

Forty-eight isolates of *Sclerotinia* were obtained from the Australian states of New South Wales, Victoria, Western Australia and Tasmania, and from New Zealand, the U.S.A., Canada, England and Switzerland (Table 1).

The isolates were grown on Oxoid's modified Czapek Dox agar, supplemented with L-asparagine (2 g/l) and thiamine hydrochloride (2 mg/l). The techniques used for culturing the fungi, protein extractions and 18-slot horizontal flat-bed polyacrylamide gel electrophoresis have been published (Wong & Willetts, 1973).

Table 1. *Isolates of Sclerotinia spp.*

Isolate Code	Size of sclerotium*	Host from which isolated	Place of origin†	Year isolated	Source
S1	M	Sunflower	N.S.W.	1950	Isolates used by Wong & Willetts (1973)
S2	M	Tomato	N.S.W.	1944	
S3	M	Potato	N.S.W.	1971	
S4	M	Sunflower	N.S.W.	1971	
S6	L	Lucerne	N.S.W.	1969	
S7	L	Lucerne	N.S.W.	1970	
S8	L	French bean	N.S.W.	1970	
S9	L	French bean	N.S.W.	1969	
S10	L	Cauliflower	N.S.W.	1969	
S11	L	Cabbage	N.S.W.	1969	
S12	L	Sunflower	N.S.W.	1969	
S13	L	Capsicum	N.S.W.	1970	
S14	L	Anemone	N.S.W.	1970	
S15	L	Stock	N.S.W.	1969	
S16	L	Cauliflower	N.S.W.	1969	
S17	L	Soybean	N.S.W.	1972	D. Letham (isolate U)
S18	L	Crimson clover	N.S.W.	1971	D. Letham (isolate N)
S19	L	Soybean	N.S.W.	1973	V. Patchanee
S20	L	Rape	Vic.	1973	P. Merriman
S21	M	Tomato	Vic.	1973	P. Merriman
S22	M	Unknown	N.S.W.	1973	V. Patchanee
S23	M	Soybean	N.S.W.	1973	R. Keogh
S24	L	Soybean	N.S.W.	1973	R. Keogh
S25	M	Suckling clover	W.A.	1961	G. C. MacNish (C278)
S26	M	New Zealand blue lupin	W.A.	1971	G. C. MacNish (WA1469)
S27	L	Lupin	W.A.	1971	G. C. MacNish
S28	M	White lupin	W.A.	1972	G. C. MacNish
S29	M	Subterranean clover	Vic.	1973	R. Price
S31	L	Lettuce	Vic.	1974	P. Merriman
S32	L	French bean	N.S.W.	1973	City market
S33/1-9	L	White clover	Tas.	1973	P. J. Sampson
S34	L	Tomato	N.Z.	—	B. T. Hawthorne
S35	M	Tomato	N.Z.	—	B. T. Hawthorne
S36	LL	Bush bean	U.S.A.	—	R. P. Korf (Lorbeer 73-6)
S37	L	Anemone	Switz.	1972	R. P. Korf
S38	M	Lettuce	U.S.A.	—	R. P. Korf
S41	M	Unknown	Can.	1936	M. E. Elliott (599)
S42	L	<i>Centaurea</i> sp.	Can.	1971	M. E. Elliott (841)
S43	L	Lettuce	Eng.	1970	C. Booth (IMI148268)
S44	L	Clover	Eng.	1970	C. Booth (IMI147202)

* M, small (0.3 to 2 mm); L, large (1 to 8 mm); LL, large (2 to 15 mm).

† N.S.W., New South Wales, Australia; Vic., Victoria, Australia; W.A., Western Australia; Tas., Tasmania, Australia; N.Z., New Zealand; U.S.A., United States of America; Switz., Switzerland; Can., Canada; Eng., England.

Soluble proteins and five enzymes were studied. Proteins were stained with Coomassie blue (Chrambach *et al.* 1967); reduced nicotinamide adenine dinucleotide phosphate (NADPH) dehydrogenase (EC. 1.6.99.1) was stained in an incubation medium containing 50 ml 0.05 M-tris-glycine buffer, pH 8.3, 20 mg nitroblue tetrazolium, and 25 mg NADPH; tetrazolium oxidase was detected by the method of Vrba & Zampa (1973), and arylesterase (EC. 3.1.1.2), acid phosphatase (EC. 3.1.3.2) and NADP-linked glucose-6-phosphate dehydrogenase (EC. 1.1.1.49) by the methods outlined by Cole, Blondin & Temple (1968).

The electrophoretic patterns of the soluble proteins and five selected enzymes were repeated at least three times for each isolate.

Fig. 1. Representative electrophoretic patterns of soluble proteins and enzymes of *Sclerotinia* isolates: (a) Soluble proteins; (b) NADPH dehydrogenase; (c) glucose-6-phosphate dehydrogenase (NADP-linked); (d) arylesterase; (e) acid phosphatase; (f) tetrazolium oxidase. The intensities of the bands are shown by the degree of shading. Stippled areas are sites of enzyme activity not resolved into discrete bands.

Table 2. *Grouping of Sclerotinia isolates based on electrophoretic patterns of general proteins and five enzymes*

Group	Isolates	Size range of sclerotia (on PDA)	Host-range
1	s1, s2, s3, s4, s21, s22, s23, s25, s26, s28, s29, s35, s38, s41	Small (0.3–2 mm)	Sunflower, tomato, potato, lettuce, soybean, clover, lupin, unknown hosts
2	s6, s7, s33/1–9, s44	Large (1–8 mm)	Lucerne, clover
3	s8, s9, s10, s11, s12, s13, s14, s15, s16, s17, s18, s19, s20, s24, s27, s31, s32, s34, s36, s42, s43	Large (1–8 mm)	Cauliflower, cabbage, sunflower, capsicum, anemone, lettuce, stock, tomato, <i>Centaurea</i> sp., rape, French bean, soybean, bush bean, clover, lupin
4	s37	Large (2–15 mm)	Anemone

RESULTS

Representative electrophoretic patterns for soluble proteins and the five enzymes of fourteen isolates selected from a wide geographical area and a variety of hosts are given as interpretative drawings (Fig. 1). With the exception of isolate s37, which was supplied by R. P. Korf as *Whetzelinia tuberosa*, the isolates form three distinct groups (Table 2).

Group 1. The fourteen isolates of this group were obtained from a wide host-range and produced small sclerotia. Their protein patterns were almost identical and each had a cathodic band which was absent from the patterns of the other isolates. Some intra-group variations occurred with arylesterase, glucose-6-phosphate dehydrogenase and tetrazolium oxidase. The variations were greatest with arylesterase patterns, which could not be used on their own to group a number of isolates. Some isolates showed an additional but minor band for glucose-6-phosphate dehydrogenase and tetrazolium oxidase, but otherwise the patterns were very similar. The patterns for NADPH dehydrogenase and acid phosphatase were very uniform.

Group 2. Twelve large-sclerotium producing isolates from lucerne and clover were included in this group. The protein and enzyme patterns were similar; variations in the protein patterns were mainly confined to the minor bands. There were some qualitative differences in the electrophoretic patterns for arylesterase and NADPH dehydrogenase, but the differences were relatively minor.

Group 3. Twenty-one large-sclerotium forming isolates from vegetable and forage legumes and non-legumes were included in this group. Some intra-group differences in protein patterns were observed in the region of the slow-moving bands. At least two bands for acid phosphatase were detected for most of the isolates, with an additional slow-moving one for some. Most isolates had two bands of glucose-6-phosphate dehydrogenase, but a few had an additional fast-moving one. The NADPH dehydrogenase and the tetrazolium oxidase patterns were very uniform.

The isolate from anemone sent to us by R. P. Korf as *W. tuberosa* (s37) produced electrophoretic patterns for tetrazolium oxidase which were similar in some respects to those produced by group 3 isolates. All other patterns for s37 were markedly different from the corresponding ones of the other isolates.

DISCUSSION

These results indicate that, apart from the one culture of *W. tuberosa*, the isolates analysed can be classified into three distinct groups. The intergroup electrophoretic differences were consistent and there were no apparent exceptions. As the isolates were obtained from a wide geographical area and a variety of host plants, they constitute a reasonably representative world and crop sample. The grouping agrees with our previous findings when other criteria were used (Willetts & Wong, 1971; Wong & Willetts, 1975). We suggest that the isolates in groups 1, 2 and 3 (Table 2) correspond to three distinct species, *S. minor*, *S. trifoliorum* and *S. sclerotiorum* respectively.

The relatively small amount of variability of intra-group electrophoretic patterns suggests that there are no further major taxonomic sub-divisions within the three species. Keay (1939) and Loveless (1951) observed differences between isolates of *Sclerotinia* obtained from forage legumes and from beans. They concluded that the fungus parasitizing forage legumes is *S. trifoliorum*, while that on beans is a distinct subspecies, *S. trifoliorum* var. *fabae*. The electrophoretic patterns and mycelial interactions of bean isolates are the same as those produced by isolates of *S. sclerotiorum*. It appears that the host range of *S. trifoliorum* is confined to forage legumes, while *S. minor* and *S. sclerotiorum* have wide host ranges which include both vegetable and forage legumes. Thus, the use of host association is not a reliable taxonomic criterion for the three species.

There has been some controversy regarding the presence of *S. trifoliorum* in Australia. Isolate s44 was obtained from the Commonwealth Mycological Institute as a culture of *S. trifoliorum*. Electrophoretically, s44 is very similar to isolates s6 and s7 from New South Wales and to s33/1-9 from Tasmania. These results provide evidence that *S. trifoliorum* parasitizes crops in Australia.

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REFERENCES

- CHRAMBACH, A., REISFELD, R. A., WYCKOFF, M. & ZACCARI, J. (1967). A procedure for rapid and sensitive staining of protein fractionated by polyacrylamide gel electrophoresis. *Analytical Biochemistry* **20**, 150-154.
- COLE, F. E., BLONDIN, J. & TEMPLE, L. (1968). Enzyme patterns during a synchronous growth cycle of *Chlorella pyrenoidosa*. *Cell and Tissue Kinetics* **1**, 281-288.
- KEAY, M. A. (1939). A study of certain species of the genus *Sclerotinia*. *Annals of Applied Biology* **26**, 227-246.
- KORF, R. P. & DUMONT, K. P. (1972). *Whetzelinia*, a new generic name for *Sclerotinia sclerotiorum* and *S. tuberosa*. *Mycologia* **64**, 248-251.
- LOVELESS, A. R. (1951). The confirmation of the variety *fabae* Keay of *Sclerotinia trifoliorum* Eriksson. *Annals of Applied Biology* **39**, 252-275.
- PURDY, L. H. (1955). A broader concept of the species *Sclerotinia sclerotiorum* based on variability. *Phytopathology* **45**, 421-427.
- VRBA, M. & ZAMPA, P. (1973). Interspecies variation of tetrazolium oxidase electrophoretic patterns in mycobacteria. *Folia microbiologica (Praha)* **18**, 32-39.
- WILLETTS, H. J. & WONG, A.-L. (1971). Ontogenetic diversity of sclerotia of *Sclerotinia sclerotiorum* and related species. *Transactions of the British Mycological Society* **57**, 515-524.
- WONG, A.-L. & WILLETTS, H. J. (1973). Electrophoretic studies of soluble proteins and enzymes of *Sclerotinia* species. *Transactions of the British Mycological Society* **61**, 167-178.
- WONG, A.-L. & WILLETTS, H. J. (1975). A taxonomic study of *Sclerotinia sclerotiorum* and related species: mycelial interactions. *Journal of General Microbiology* **88**, 339-344.