STUDIES OF COMPATIBILITY IN AUSTRALIAN ISOLATES OF BIPOLARIS SOROKINIANA

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Summary

Australian and Canadian isolates of B. sorokiniana were used for studies on compatibility and perithecial development. Australian and Canadian isolates belonged to the same two compatibility groups, a and A. When two isolates, one from each compatibility group, were paired, perithecia (*Cochliobolus sativus*) were formed. Both groups occurred in close association at a number of localities in Victoria. Of the 16 Australian isolates collected at random, 8 were of group a and 8 of group A.

I. INTRODUCTION

Tinline, Sallans, and Simmonds (1950) showed that perithecia were formed when monoconidial isolates of *Bipolaris sorokiniana* (Sacc.) Shoemaker were paired in culture. Later Tinline (1951) established that two compatibility groups of the fungus occurred in Canada and that perithecia were produced only between pairs comprising a member from each group. Isolates from the same compatibility group when paired produced only perithecial primordia. Tinline (1951) identified the two compatibility groups as 'a' and 'A' and showed that both groups occurred at single geographic locations.

B. sorokiniana [perfect state *Cochliobolus sativus* (Ito & Kuribayashi) Drechs.] occurs frequently in most cereal-growing areas of Australia.

As no information was available concerning the compatibility of Australian isolates of B. sorokiniana, experiments were conducted to determine whether Canadian and Australian isolates were compatible and whether perithecia could be produced by pairing Australian isolates. The geographical distribution of the compatibility groups of Australian isolates was also examined.

II. MATERIALS AND METHODS

(a) Isolates of B. sorokiniana

The places of origin and compatibility groups of isolates of *B. sorokiniana* used in this study are listed in the following tabulation. The host was unknown for isolates 1 and 2 and 11 and 12.

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Isolate No.	Place of Origin	Compati- bility Group	Isolate No.	Place of Origin	Compati- bility Group
1	Canada	a	11	Canada	Α
2	Canada	a	12	Canada	Α
3	Sea Lake, Vic.	a	13	Cowra, N.S.W.	Α
4	Nullan, Vic.	a	14	Gunnedah, N.S.W.	\mathbf{A}
5	Lallat, Vic.	a	15	Nullan, Vic.	Α
6	Walpeup, Vic.	a	16	Lallat, Vic.	\mathbf{A}
7	Wyperfeld National	a	17	Walpeup, Vic.	\mathbf{A}
•	Park. Vic.		18	Rutherglen, Vic.	\mathbf{A}
8	New South Wales	a	19	Walpeup, Vic.	\mathbf{A}
9	Walpeup, Vic.	a	20	Walpeup, Vic.	\mathbf{A}
10	Walpeup, Vic.	a			

For all other isolates except No. 7 the host was wheat whilst for No. 7 it was Hordeum leporinum:

The four Canadian isolates $(1, J_1-15; 2, R_3-11; 11, J_1-19; and 12, K_3)$ were of monoascosporic origin (Fig. 1). All carried the same white colour marker and were bisexual and self-sterile. The compatibility group of each isolate had been previously determined. All other isolates were multiconidial isolates with tan spores.



Fig. 1.—Genealogy of Canadian monoascosporic isolates (Tinline, personal communication).

(b) Preparation of Conidial Suspensions

Conidial cultures of all isolates were prepared either on sterilized wheat straw (Tinline 1951) or on potato dextrose agar. Agar slant cultures were grown at $23-24^{\circ}$ C for 14 days. The sterilized wheat straw cultures were prepared by adding 0.5 ml of a conidial suspension from a 14-day-old agar slant and incubating at room temperature in darkness for 21 days.

Suspensions of conidia were prepared by adding sterile distilled water to the test cultures, rubbing gently to dislodge the spores, and filtering through 32-mesh wire gauze. The suspensions were then adjusted to approximately equal opacity.

(c) Pairing Isolates

Conidial suspensions from the respective isolates to be paired were mixed and barley kernels were dipped into the mixture and plated on Sachs nutrient agar (Tinline and Dickson 1958). Before dipping, the barley kernels were soaked in tap water (2 hr), surface-sterilized in mercuric chloride (1:1000) for 5 min, and washed in three changes of sterile distilled water. To prevent germination, the kernels were boiled for 2 min in the last change of water.

The plates were incubated in darkness for 7 days at 23–24 $^{\circ}$ C and a further 16 days at 20 $^{\circ}$ C before being examined for perithecia.

III. EXPERIMENTAL AND RESULTS

(a) Comparison of Australian and Canadian Isolates

The four Canadian isolates (Nos. 1, 2, 11, and 12) and three Australian isolates (Nos. 3, 13, and 14) were paired in all possible combinations. Conidial suspensions of isolates 1 and 11 were prepared from agar slants, all others from wheat straw cultures. Barley kernels cv. Montcalm were used as the host substrate.

The Australian isolates represented two distinct compatibility groups and were compatible with the Canadian isolates (Table 1). Both black and white perithecia

TABLE 1

RESULT OF PAIRING SEVEN ISOLATES OF B. SOROKINIANA W, white; B, black; +, perithecia; 0, perithecial primordia; -, no evidence of perithecial development

Isolate No.					w	B	W B		12 W B		13 W B			
14	+		+	+		+	0		0		_			
13	+		+		_	+	0		0	*		_		
12	+	_	+		+	+								
11	+		+		+	+		_						
3	0	-				_								
2	0		0											
1	0	-												

were produced from some pairings and in three pairings involving two of the Australian isolates with two of Canadian origin, only white perithecia occurred.

Isolates 13 and 14, being compatible with isolates 1 and 2, belong to compatibility group A of Tinline (1951) while isolate 3 belongs to compatibility group a.

(b) Further Compatibility Studies with Australian Isolates

Isolates 3, 13, and 14 were paired with each other and with thirteen additional Australian isolates, Nos. 4–10 and 15–20 inclusive. Additional pairings among these latter thirteen isolates were also carried out. The conidial suspensions of all isolates were prepared from wheat straw cultures. Barley kernels cv. Beecher were used as the host substrate.

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Confirmation of the occurrence of compatible isolates of Australian origin was obtained (Table 2). Of the thirteen additional Australian isolates, seven (Nos. 4–10) belonged to group a and six (Nos. 15–20) to group A. Both the perithecial primordia and perithecia produced in these pairings were black.

TABLE 2

RESULT OF SOME PAIRINGS OF 16 ISOLATES OF B. SOROKINIANA

The unmarked squares indicate pairings not tested. +, perithecia; 0, perithecial primordia; -, no evidence of perithecial development

Isolate No.	3	4	5	6	7	8	9	10	13	14	15	16	17	18	19	20
$20 \\ 19 \\ 18 \\ 17 \\ 16 \\ 15 \\ 14 \\ 13 \\ 10 \\ 9 \\ 8 \\ 7 \\ 6 \\ 5 \\ 4$	+++++++	++++	+++ +++	++ + + + + + +	+++	++++	+++	++ + ++			0 0 0		0	0		0
3	-	1														

(c) Distribution of Compatibility Groups within Australia

The compatibility group of each of the 16 Australian isolates is given in the tabulation in Section II. Isolates of the same compatibility group occurred at widely separated locations but isolates from both groups were present in close association at three locations. The isolates belonged equally to the two compatibility groups.

IV. DISCUSSION

Results indicate quite clearly that two compatibility groups exist within Australian isolates of *B. sorokiniana*. It has been possible to refer these isolates to the groups a and A of Tinline (1951).

The occurrence of both black and white perithecia in certain pairings may be explained by the suggestion of Tinline and Dickson (1958) that reciprocal fertilization had occurred, but no attempt was made to determine the reason for the absence of black perithecia in certain pairings which might have been expected to yield both black and white perithecia.

The absence of white perithecia in all pairings of Australian isolates examined suggests that, although the multiconidial isolates used may have contained mixtures of strains, all the strains present carry the character for black perithecial production. Similarly, no perithecia were formed when a single multiconidial isolate was inoculated on to the host substrate. Strains, if present, were of the same compatibility group.

Perithecia of C. sativus were produced by a number of pairings of Australian isolates in all three experiments. This constitutes a first record for the production of perithecia of C. sativus in Australia.

The probability that the compatibility group factors of the fungus segregate in a 1 : 1 ratio in nature (Tinline 1951) is supported by the finding that of the 16 Australian isolates collected at random, 8 were in group A and 8 in group a.

The compatibility of Canadian and Australian isolates and the segregation of the isolates used into two compatibility groups expands the knowledge of the geographical distribution of isolates of each group. It also extends the findings of Tinline (1951) that isolates of the same group occur in widely separated areas and that isolates of each group can be found at single geographic locations, and suggests that only two compatibility groups occur.

Although C. sativus was first described in culture over 40 years ago (Kuribayashi 1929), there is no record of its occurrence in nature. However, if it did occur, it could result in the production of new strains of the fungus which may be of even greater economic importance to the cereal industry than the existing strains. The occurrence of both compatibility groups of the fungus at specific field locations, e.g. Walpeup, at least provides the opportunity for perithecia to be produced. The specific environmental and nutritional requirements for the production of perithecia in culture (Tinline 1951; Shoemaker 1955; Tinline and Dickson 1958) are such that the occurrence of suitable conditions in the field may occur only infrequently in Australia.

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