Heterothallism in *Pyrenopeziza brassicae*: potential for variation in the fungus causing light leaf spot of oilseed rape and other brassicas

T.W. ILOTT and D. S. INGRAM
Botany School, Downing Street, Cambridge, U.K.

C. J. RAWLINSON
Rothamsted Experimental Station, Harpenden, Herts., U.K.

SUMMARY

The control of sexual reproduction in *Pyrenopeziza brassicae* has been investigated by pairing single-spore isolates in conidial suspensions and culturing these on nutrient agar. The results demonstrate that *P. brassicae* is heterothallic, with two mating types. Experiments in which ascospore progeny of fertile pairings were crossed with the parental isolates support this, and suggest that the mating types may be determined by two alleles at a single locus, as in many other Ascomycete fungi.

INTRODUCTION

The sexual stage of *Pyrenopeziza brassicae* has been reported only twice in the field, by Staunton and Kavanagh in Ireland (1966) and by Cheah, Hartill and Corbin in New Zealand (1980). However, apothecia may have been overlooked in the past and may be an important source of phenotypic variation.

From limited studies of the sexual stage of *P. brassicae*, Maddock and Ingram (1981) suggested that most isolates were self-sterile, that genetic blocks at various stages of development could prevent the formation of mature apothecia and that complementation between two isolates could enable sexual development to be completed (as in *Glomerella cingulata*, Wheeler, 1954). We now report the results of more detailed studies using a larger number of isolates.

METHODS

Single-conidial isolates of *P. brassicae*, mostly from rape but also from other brassica hosts, were cultured alone or paired by

placing approximately 0.04 ml of a conidial suspension of each isolate (concentration 1-2.5 x 10^5 conidia ml^{-1}) on 3% malt agar in each of four Petri dishes, which were then incubated in the dark at 15°C and 20°C. The presence of mature apothecia, or the stage of development of immature apothecia, was recorded after incubation for six weeks.

RESULTS AND DISCUSSION

Most of *c.* 500 isolates cultured singly on malt agar produced immature apothecia similar to those described by Rawlinson *et al.* (1978) and Maddock and Ingram (1981), but none was homothallic. A selection of 15 isolates paired in all possible combinations showed that these could be divided into two distinct groups (Table 1), and that fertility was achieved only when isolates were paired with members of a different group. Moreover, mature apothecia usually only formed when recently (< 3 months in culture at 15°C on 3% malt agar) isolated cultures were paired (e.g. isolates CRA, CRB, PG3 or PG4). It is possible that older isolates (>18 months old) had lost some capacity for sexual reproduction during storage on agar.

In a further experiment, 19 recent isolates were paired in all possible combinations. Again isolates fell into two groups; sexual development was completed in 80% of all inter-group pairs, but intra-group pairs formed only immature apothecia which developed no further than those produced by isolates grown alone (Table 1).

These results indicate that *P. brassicae* is heterothallic, with two mating types. These have been designated P_1 and P_2. Not all inter-group pairs were fertile, but such sterility is known to occur in similar experiments with other heterothallic Ascomycetes.

Table 1. *Production of mature apothecia by* Pyrenopeziza brassicae *in pairing experiments.*

Experiment 1

		Type P_1					
	Isolate	CRB	34A	34B	PG4	13A	1
Type P_2	CRA	+	+	+	+	+	+
	PG3	+	-	+	+	+	-
	51A	+	-	-	-	+	-
	51B	+	-	-	+	-	-
	57A	+	-	-	+	-	-
	64S	+	-	-	-	-	+

Isolates 39A, 39B, and 60A were infertile in all pairings.

Experiment 2

		Type P_1											
	Isolate	JD 18	JD 16	JD 26	JH 9	JE 8	JH 1	JD 15	JE 17	JE 13	P 87	JH 14	JD 25
Type P_2	JD19	+	+	-	+	+	-	NT	+	NT	-	-	-
	JH11	+	+	+	+	+	+	+	+	+	+	-	+
	JH5	+	+	+	+	+	+	+	+	+	+	+	+
	JE5	+	+	+	-	+	+	+	+	+	-	+	-
	CK13	+	+	-	-	+	-	+	+	+	+	+	+
	JH3	+	+	-	-	+	-	+	+	+	+	+	+

Isolate CK5 was infertile in all pairings.

\+ Cross producing mature apothecia
\- No mature apothecia produced
NT Cross not tested
Pairings between isolates in the same group were always infertile.

The optimum conditions for sexual reproduction may not have been achieved in these sterile pairs and factors other than mating type may influence the production of apothecia, e.g. genes determining nutritional requirements for sexual reproduction. The results in Table 1 would not be expected if a system of genetic blocks controlled sexual reproduction. If this were the case many more than two groups of isolates would be likely to occur and some pairings might be expected to form more advanced (but not mature) apothecia than those found on isolates grown singly.

Segregation of the two mating types among ascospore progeny of three fertile pairings was investigated by crossing single ascospores from each pair with an isolate of each mating type. The results (Table 2) show a 1:1 ratio of mating type amongst progeny of JD21 x JH3 and JH4 x JD16, but approximately one quarter of the progeny of cross CRB x 51B showed no mating ability. Thus the two mating types are apparently determined by two alleles at one locus, as is common in the Ascomycetes (Whitehouse, 1949; Fincham *et al.*, 1979) but the expression of mating type P_1 may be suppressable. Factors controlling the development of immature apothecia were inherited independently of those determining mating type, but may have exerted an effect on its expression in the progeny of cross CRB x 51B.

Isolate IMI 249942, apparently homothallic in Maddock and Ingram's (1981) studies, was not self-fertile in this investigation and behaved as mating type P_2 only. Hickman *et al.* (1955) also reported apparently self-fertile isolates. However, a homothallic form would be unusual for a heterothallic Ascomycete although it

might be explained by imperfect repression of apothecial production. Isolate IMI 249942 may have lost its ability to reproduce sexually alone due to storage.

Table 2. *Mating types of progeny of fertile pairings between isolates of* Pyrenopeziza brassicae.

(a) Progeny of pairing JH4 (P_2) x JD16 (P_1)

Type P_1: 47 isolates

Type P_2: 48 isolates

Infertile in this experiment: 2 isolates

(b) Progeny of pairing JH3 (P_2) x JD21 (P_1)

Type P_1: 36 isolates

Type P_2: 45 isolates

Infertile in this experiment: 7 isolates

(c) Progeny of pairing CRB (P_1) x 51B (P_2)

Type P_1, immature apothecia similar to CRB: 22 progeny

Type P_2, immature apothecia similar to CRB: 29 progeny

Type P_2, immature apothecia similar to 51B: 16 progeny

Infertile in this experiment, immature apothecia similar to 51B: 26 progeny.

Thirty-four further isolates from several brassica hosts and widely different geographical locations in England were tested for mating type; 22 were type P_1, 10 type P_2 and 2 infertile. Both mating types were widely distributed and not confined to one host or geographical origin. Thus apothecia might be expected to occur widely in nature, unless the fungus is less able to reproduce sexually on the host than in culture, or host plant debris is removed or destroyed before reproduction is completed. More detailed searches in crops are needed to reveal the true frequency of natural sexual reproduction in *P. brassicae* . If apothecia are common, then there is clearly great potential in this fungus for variation in virulence, fungicide tolerance or other agriculturally significant characters of importance to plant breeders and pathologists.

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