Virulence of Different Isolates of *Alternaria brassicae* on Winter Oilseed Rape Cultivars

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Thirteen isolates of *Alternaria brassicae* were tested on selected cultivar of winter oilseed rape. All were pathogenic but differed in their virulence. When nine cultivars were inoculated with four single isolates there were significant differences in disease severity which could be attributed both to cultivar and isolate but there was no significant isolate x cultivar interaction. The virulence of isolates was not related consistently to their growth on agar at 25°C.

Introduction

Infection by *Alternaria brassicae* (Berk.) Sacc. has become increasingly common on oilseed rape (*Brassica napus* L. ssp. *Oleifera* [Metzg.] Simsk.) in the U.K. as the acreage sown to this crop has increased. Studies elsewhere (van Schreven, 1953; Changsri, 1960; Morton, 1964; Singh, 1977), have indicated that isolates of *A. brassicae* from other crops, especially brassicas, can infect oilseed rape but there is little published on this aspect from the U.K. This lack of information prompted the present study.

Materials and Methods

Leaves infected with *A. brassicae* from crops listed in Table 1 were obtained from the Southern Region Trial Centre of the National Institute of Agricultural Botany. The fungus was isolated by plating pieces (c. 9 mm^2), cut from the edge of lesions and surface-sterilized for 3-4 min in 10% Chloros (1% available chlorine), onto plates of V8 agar (10 ml V8 Juice, Campbells Soups Ltd., 90 ml distilled water, 1.5 g agar). Subcultures of each isolate were obtained subsequently from single spores produced by cultures. Linear growth of the isolates on V8 agar was determined over 14 days at 25°C. There were six replicates of each isolate and two diameters (at right angles) of each colony were measured.

To prepare inoculum, isolates derived from a single spore were first grown for 2-3 days at 20-25°C in flasks of liquid V8 medium on an orbital incubator (Gallenkamp). The mycelial aggregates formed were distributed over sterile filter paper in plastic petri dishes which were incubated at 15°C in the light. Conidia were then washed off the filter paper cultures and suspensions adjusted to 10^4 spores/ml.

Oilseed rape seedlings were raised in pots (13.5 cm diameter) of John Innes compost No.3 and thinned to three seedlings per pot. Seedlings were kept either for 2 months in a glasshouse (four to six true leaves) or for 3 months outdoors (six to eight leaves). Seedlings in pots and detached leaves were sprayed with conidial suspensions almost to run-off. Inoculated seedlings were kept in humid chambers for 48 h and then placed on the glasshouse bench for a further 5 days when infection was assessed. Seedlings grown outdoors were kept in humid chambers for 24 h at 20°C before inoculation to acclimatize and were kept at 20°C after inoculation for 48 h before placing in the glasshouse. Detached leaves were placed in seed germination trays (41.5 x 26.5 x 10 cm, Leithen Valley Plastics), on wet absorbent tissue with their petioles wrapped in wet cotton wool and were sprayed individually with inoculum. Trays were kept at 20°C for 48 h, the lids were removed, and infection was assessed one day later.

Infection on each leaf was graded on a 0-5 scale: 0, no infection; 1, < 1%; 2, 1-5%; 3, 6-10%; 4, 11-25% and 5, over 25% leaf area infected. An infection index for each plant was then derived by calculating the mean of the grades for its individual leaves.

Experimental

Seedlings and detached leaves of oilseed rape cv. Jet Neuf were inoculated with 13 isolates of *A. brassicae* in three experiments which are summarized in Table 1. All isolates were pathogenic to Jet Neuf but they differed in virulence, isolates from Jet Neuf and the turnip (*Brassica rapa* L. var. *rapa* (L.) Thell.) cultivars Tyfon and Vobra being generally the most virulent.

Four selected isolates, two from oilseed rape, one from swede (*B. napus* L. var. *napobrassica* Peterm.) and one from fodder radish (*Rapharus sativa* L. ssp. *oleifera* (DC.) Metzg.), were tested further on nine oilseed rape cultivars. The isolates were pathogenic to all the cultivars tested (Table 2). An analysis of variance indicated significant differences between cultivars ($P \leq 0.05$), in particular infection on Jet Neuf and Mitre was usually higher than on other cultivars. There were also highly significant differences ($P \leq 0.001$) between isolates, that from Jet Neuf being generally the most virulent.

Finally, the virulence of the 13 isolates, as indicated in Table 1, was compared with their rate of colony growth on V8 agar at 25°C using regression analysis. No consistent and significant relationship between these two parameters was found.

Discussion

The results show that isolates of *A. brassicae* from other common cruciferous crops in the U.K. are capable of infecting oilseed rape and in this respect agree with reports from other countries (van Schreven, 1953; Changsri, 1960; Morton, 1964; Singh, 1977). These crops, therefore, are potential sources of inoculum for the infection of oilseed rape. Isolates varied in their virulence to Jet Neuf, the cultivar most commonly grown in the U.K., indicating that a diversity of strains might exist as suggested by Fazardo & Palo (1934) and Neergaard (1945). Diversity of strains was also indicated when four selected isolates were tested on nine oilseed rape cultivars. These became infected to different extents, a feature found in other studies with this fungus (Husain & Thakur, 1963; Bhander, 1965; Stankora, 1972; Degenhardt, 1973; Petrie, 1973; Rozeij, 1974), but there was no isolate x cultivar interaction to indicate a differential response nor could differences in virulence be related to growth rates on agar.

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Table 1 : Virulence of 13 isolates of *Alternaria brassicae* on winter oilseed rape, cv. Jet Neuf.

Source of isolate	Mean index of disease severity/plant		
	Detached leaves[a]	Seedlings grown in the glasshouse[b]	Seedlings grown outdoors[c]
Oilseed rape (*Brassicae napus* L. ssp. *oleifera* (Metzg.) Sinsk.)			
cv. Elvira	2.7	1.7	2.8
Jet Neuf	3.8	1.7	3.0
Norli	2.6	1.3	2.8
Rafal	2.9	1.4	2.4
Unknown	2.6	1.4	2.0
Forage rape (*B. napus* L. ssp. *oleifera* (Metzg.) Sinsk.)	2.5	1.0	2.1
Swede (*B. napus* L. var. *napobrassica* Petern.)	2.2	1.3	2.5
Turnip rape (*B. rapa* L.) cv. Primax	2.9	1.4	2.2
Turnip (*B. rapa* L. var. *rapa* (L.) Thell.)			
cv. Appin	2.2	1.3	2.0
Tyfon	3.5	1.8	2.6
Vobra	3.1	1.2	3.0
Fodder radish (*Raphanus sativa* L. spp. *oleifera* (DC.) Metzg.)	2.5	1.2	2.5
White mustard (*Sinapsis alba* L.)	2.8	1.2	2.0
S.E. ±	0.30	0.13	0.12

[a]Each figure is based on three replicate plants from which the 2nd, 3rd and 4th true leaves were detached.

[b]Based on 9 plants, each with 4-5 leaves.

[c]Based on 9 plants with 6-7 leaves.

Table 2 : Disease severity induced by four isolates of *Alternaria brassicae* on nine oilseed rape cultivars.

Cultivars	Mean index of disease severity[a] induced by isolates from				Cultivar mean
	Oil seed rape		Swede	Fodder radish	
	cv. Jet Neuf	Rafal			
Jet Neuf	3.0	2.4	2.4	2.4	2.56
Mitre	2.9	2.2	2.5	2.4	2.51
Rafal	2.9	2.5	2.2	2.4	2.48
Primor	2.9	2.2	2.4	2.3	2.45
Elvira	2.7	2.5	2.1	2.5	2.44
Norli	2.7	2.4	2.3	2.2	2.39
Lester	2.7	2.2	2.4	2.1	2.36
Fernando	2.6	2.0	2.0	2.2	2.19
Hercules	2.5	1.9	2.2	2.2	2.16
Isolate mean	2.76	2.26	2.27	2.28	(S.E. = 0.17)

[a]Each figure is based on 9 replicate plants (grown outdoors), each with 6-8 leaves.