RACE VARIABILITY IN LEPTOSPHAERIA MACULANS AND THE IMPLICATIONS FOR RESISTANCE BREEDING IN AUSTRALIA

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SUMMARY

Seedling tests identified 14 distinct races of Leptosphaeria maculans in Australia, three of which are capable of attacking Brassica juncea. Similar mixtures of races were evident in all major rapeseed growing areas with some races demonstrating the ability to dominate other in terms of disease expression. There was no relationship, however, between seedling and adult plant resistance. Thus, selection at the seedling stage is of no use in breeding for adult plant resistance to blackleg.

INTRODUCTION

Blackleg, caused by the fungus Leptosphaeria maculans (Desm.) Ces. & de Not., is a serious disease of rapeseed (Brassica napus L. and B. campestris L.) worldwide. In Australia it has caused substantial yield losses and limited the expansion of the rapeseed industry (Barbetti, 1975; McGee and Emmett, 1977). The availability of blackleg resistant cultivars has led to the recent revival of the industry. However, the resistance in these cultivars is incomplete (Salisbury and Dennis, 1985) and when they are widely grown, it is possible that strains of L. maculans virulent on these cultivars might be selected.

To enable breeding for resistance to blackleg to be carried out more effectively, thus ensuring the survival of the industry, knowledge of the pathogenic variability of the causal organism is essential. Host-pathogen interactions which indicate the existence of different races have been reported in Western Australia (Cargeeg and Thurling, 1980; Melvin, 1982) and limited evidence from overseas (e.g., McGee and Petrie, 1978; Williams and Delwiche, 1979; Newman, 1984; Humpherson-Jones, 1986) has also indicated the existence of different races.

This paper reports seedling studies to identify the race structure of L. maculans in Australia. It also examines the relationship between seedling and adult plant reactions and discusses the implications of these for resistance breeding.

RACE STUDIES

The pathogenicity of isolates on a group of differential cultivars was determined by the rapid cotyledon test of Williams and Delwiche (1979). Eight days after sowing, a drop of pycnidiospore suspension (2.5 x 10^6 spores/ml) was placed on a cotyledon, which was then wounded with a needle and assessed 12 days later on lesion development (0-9 scale). Infected trash was collected from 12 sites representing the major rapeseed growing regions around Australia, with testing eventually concentrated at five sites. The reactions of 110 single ascospore derived isolates of L. maculans were evaluated on 21 seedlings of each of 12 differential Brassica lines; Chisaya, Marnoo, Midas, Niklas, Wesbrook, RX3 (spring B. napus), Jet Neuf, Rafal (winter B. napus), Bunyip, Jumbuck (spring B. campestris), Stoke and Zaria (B. juncea). The response of the differentials was classified as either resistant (0-4.0) or susceptible (4.1-9.0) and the differential-isolate interactions used to classify isolates into races.

Fourteen distinct races, three of which are pathogenic on B. juncea have been identified (Table 1).

All of the isolates tested were highly virulent. This result is in agreement with McGee and Petrie (1978), who tested several isolates from Australia and found them all to be virulent. The detection of only virulent races in Australia contrasts with overseas results which report a high proportion of avirulent isolates (e.g., McGee and Petrie, 1978; Hanacziwskyj and Drysdale, 1984; Humpherson-Jones, 1986). This may explain why blackleg is a greater disease problem in Australia than in most other countries. However, the possibility of selection bias toward virulent isolates, through the collection of ascospores on rapeseed trash, rather than via pycnidiospores from leaf lesions (where avirulent isolates may be present, but do not develop sufficiently to cause stem cankers and survive on trash) is being investigated.

Table 1. Races of Leptosphaeria maculans in Australia

L. maculans Race No.	Resistant differentials
1	Stoke, Zaria
2	Bunyip, Stoke, Zaria
3	Bunyip, Rafal, Stoke, Zaria
4	Bunyip, Chisaya, Jumbuck, RX3, Stoke, Zaria
5	Bunyip, Chisaya, Jumbuck, Stoke, Zaria
6	Bunyip, Rafal
7	Chisaya, Jumbuck, RX3, Stoke, Zaria
8	Chisaya, Stoke, Zaria
9	Chsiaya, RX3, Stoke, Zaria
10	RX3, Stoke, Zaia
11	Rafal, Stoke, Zaria
12	-
13	Jumbuck, Stoke, Zaria
14	Zaria

This study revealed that several races were present at each major site, that a similar range of races occurred at each site (Table 2) and that there were no major differences in the virulence of the pathogen at the major sites tested. Differences in the severity of blackleg infection at these sites was therefore probably due to environmental conditions.

Table 2. Location and frequency of races of Leptosphaeria maculans in Australia

Site	Race and frequency[2] of occurrence
Dooen (Victoria)	1(1)
Galong (New South Wales)	3(1), 9(1)
Gerogery (New South Wales)	1(2)
Millicent[1] (South Australia)	1(7), 2(3), 3(1), 8(1), 9(1), 11(1), 13(1), 14(1)
Mount Barker[1] (Western Australia)	1(10), 2(1), 5(1), 9(2), 10(2), 12(2)
Mundalla (South Australia)	1(2)
Numurkah[1] (Victoria)	1(11), 2(1), 4(1), 10(3), 12(3)
Old Junee[1] (New South Wales)	1(10), 2(1), 6(1), 7(1), 8(1), 10(1)
Penshurst (Victoria)	1(1), 2(1)
Rutherglen (Victoria)	2(1)
Streatham[1] (Victoria)	1(11), 6(1), 7(1), 8(2), 12(2), 14(1)
Wagga (New South Wales)	1(2), 7(1)

[1]Major test site
[2]Figure in brackets denotes number of isolates within each race

Given that in the field, seedlings are attacked by a mixture of races, it was considered important to investigate the effects of inoculation with a mixture of races compared with a single isolate. Thus, the effect of inoculation of three races of L. maculans separately and in combination was studied in a glasshouse seedling test. The races used in the test were 1 (non B. juncea attacking), 6 and 12 (both B. juncea attacking).

When they were inoculated in combination, Race 12 dominated Race 6 in terms of disease reaction, and that Race 1 dominated both Race 6 and Race 12 (Table 3). It is possible that spores of Race 1 germinate and infect more quickly than those of Races 6 and 12 and subsequently preclude infection and disease expression by them. This is being studied in further tests.

Table 3. Effect of inoculation of three races of Leptosphaeria maculans separately and in combination

Differential	Race/mixture reaction						
	1	6	12	1/6	1/12	6/12	1/6/12
Bunyip	S[1]	R	S	S	S	S	S
Chisaya	S	S	S	S	S	S	S
Jet Neuf	S	S	S	S	S	S	S
Jumbuck	S	S	S	S	S	S	S
Marnoo	S	S	S	S	S	S	S
Midas	S	S	S	S	S	S	S
Niklas	S	S	S	S	S	S	S
Rafal	S	R	S	S	S	S	S
RX3	S	S	S	S	S	S	S
Wesbrook	S	S	S	S	S	S	S
Stoke	R	S	S	R	R	S	R
Zaria	R	S	S	R	R	S	R

[1]S = susceptible; R = resistant

ADULT PLANT STUDIES

Classification of isolates of L. maculans into different races has been based entirely on the results of seedling tests. Seedling infection followed by systemic growth of the fungus through the lamina and petiole into the stem is the main pathway of infection leading to cankering of the stem base (Hammond et al., 1985). Because resistance to stem canker in the adult plant is the major component of field resistance, glasshouse and field studies were initiated to determine the relationship between seedling reaction and resistance to cankering in the adult plant. For the adult plant test, 4-week old glasshouse seedlings were inoculated by spraying with a pycnidiospore suspension (2.5×10^6 spores/ml). At maturity, stems were rated internally (% cross section infected) and externally (% circumference cankered). While in some instances the seedling and adult plant reactions of the differential lines against individual races were the same, there were several exceptions. Some differentials were susceptible as seedlings but resistant as adult plants while others were resistant as seedlings but susceptible as adult plants. This suggests that seedling resistance and adult plant resistance may be distinct characters.

To determine the relationship between glasshouse seedling reactions and adult plant field reactions, the differential lines used for seedling race classification were sown at several field locations throughout Australia in 1989 and 1990. In 1989, plant counts were taken at establishment and again at maturity and survival percentage calculated. All surviving plants were rated internally and externally for disease as previously described. As disease ratings were highly correlated with survival percentages, only survival was measured in 1990. Although disease pressure varied between sites and years, affecting survival rate, in general the resistance rankings of the differential lines was similar at all sites (Table 4). The B. juncea differentials were the most resistant followed by the winter B. napus lines, then the spring B. napus lines Wesbrook and RX3, with the remaining spring B. napus and B. campestris lines having the least resistance. A site specific exception to this trend was the relatively low survival of the B. juncea lines at Hamilton. While the B. juncea lines were the most resistant at Mundulla 1989, their survival was lower than expected when compared to the other sites.

The high survival of B. juncea lines in the field could be attributed to the low number of isolates capable of attacking these lines in the seedling stage (Table 4). However, this explanation does not account for the relatively high survival of the winter B. napus

Table 4. Effect of L. maculans on survival of differential lines at six field sites in 1989 and 1990

Differential	% of virulent field isolates[2]	Disease rating[1]										
		Millicent	Streatham		Rutherglen		Lake Bolac		Mundalla		Hamilton	
		1989	1989	1990	1989	1990	1989	1990	1989	1990	1989	1990
Bunyip	86	3	1	1	1	3	1	1	1	3	3	2
Chisaya	92	1	1	1	1	2	1	1	1	2	3	2
Jet Neuf	100	4	1	3	3	3	2	2	2	3	4	3
Jumbuck	95	2	1	1	1	2	1	1	1	2	3	2
Marnoo	100	3	1	1	2	2	1	1	1	1	3	3
Midas	100	2	1	1	1	3	1	1	1	2	4	2
Niklas	100	2	1	1	1	2	1	1	1	2	3	2
Rafal	95	4	1	3	3	3	2	3	2	3	5	3
RX3	87	3	1	2	3	3	2	2	1	3	4	3
Wesbrook	100	4	1	2	2	3	1	2	1	2	4	3
Stoke	10	5	4	4	4	4	5	4	3	4	4	3
Zaria	5	5	4	4	5	4	5	4	3	4	4	2

[1]Disease rating - 1 = <10% survival, 2 = 11-25%, 3 = 26-50%, 4 = 51-75%, 5 = >75%

[2]Seedling classification

differentials (Jet Neuf and Rafal), which were susceptible to most isolates as seedlings. The good field resistance of Jet Neuf, despite its susceptibility in the seedling stage, has been reported previously by Hanacziwskyj and Drysdale (1984). They demonstrated that resistance to blackleg in Jet Neuf increases with age and that it is highly resistant by the fifth leaf stage.

The absence of a relationship between seedling and adult plant resistances has also been observed in studies aimed at characterising B. juncea resistance genes in B. napus lines developed by Roy (1984). Progeny of selections which had good seedling resistance to the B. juncea attacking races as seedlings, largely did not show B. juncea type resistance as adult plants.

IMPLICATIONS FOR BREEDING

Seedling cotyledon tests are effective in identifying resistance to specific isolates of L. maculans and have clearly identified 14 distinct races of the fungus in Australia. The race-specific seedling resistance in highly heritable (Williams and Delwiche, 1979; Ballinger and Salisbury, 1989) with the likelihood that resistance to each race is controlled by a single dominant gene (Delwiche, 1980). However, the utilisation of the seedling cotyledon test in resistance breeding will only be effective if seedling resistance is correlated with adult plant resistance in the field. The results from this study indicate that there is no consistent relationship between seedling and adult plant resistance. One possible reason for this is that many of the results from seedling tests are race specific, being evaluated against single races. In this study, when a mixture of races was used in seedling tests the effect was not cumulative as expected, but one where a single race dominated the reactions of one or more other races in the mixture. In this case, Race 1 (a non-B. juncea attacker which constitutes 53% of the isolates studied) dominated the reactions of two B. juncea attacking races, Races 6 and 12. This may explain the low incidence of these races (8% of isolates studied) detected in the field and consequently, the high field survival rate of B. juncea lines. The potential ability of one race to limit disease expression by other races raises the possibility of cross protection, particularly if resistance to virulent isolates could be induced by infection with avirulent ones.

Given the likely independence of seedling and adult plant resistance, selection at the seedling stage would only be useful if both characters are required in cultivars. A potentially important use for seeding resistance could be the prevention of initial infection by the fungus, thus preventing the systemic growth which results in cankering.

A finding of major concern is the occurrence of races capable of attacking both seedlings and adult plants of the previously highly resistant B. juncea differentials. This may indicate a short life for B. napus cultivars with B. juncea resistance to blackleg.

In contrast to seedling resistance, adult plant resistance appears to be polygenically inherited (Cargeeg and Thurling, 1980; Thompson, 1983) and partly mechanical, with production of lignified tissue preventing fungal growth (Brunin, 1972) and may therefore be non-specific (Thompson, 1983), although cases of clear specific interaction have been noted (Cargeeg and Thurling, 1980). In addition, site specific field reactions, particularly with B. juncea, were identified in this study. The possibility of race specific reactions in adult plants is currently being investigated.

The observed pathogenic variability is only of limited significance if the adult plant resistance is mechanical or non specific. However, it assumes major significance if adult plant resistance is race specific.

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