Pseudomonas blight and *Nematospora* seed rot of Indian mustard

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Abstract

The necrotic lesions on leaves and stems of *Brassica juncea* (L.) (Czern and Coss) caused by *Pseudomonas syringae* pv. *maculicola* were described by Oram *et al.* at ARAB V in Perth in 1985. That paper also outlined the development of a phytotron method for screening seedlings and the segregation of disease reactions observed in F2 families. This paper describes subsequent research, and briefly reports the discovery and control of a seed rot caused by a yeast morphologically similar to *Nematospora sinecauda*, a species not previously reported in Australia.

The reactions of five resistant and five susceptible mustard accessions to natural infections in the field at Wagga and Canberra were broadly similar to those following inoculation with five isolates of Pseudomonas in phytotron tests. One isolate was much more injurious to both the resistant and susceptible groups, and two other isolates were not injurious to one otherwise susceptible accession. Subsequent experiments were done with the isolate that gave phytotron rankings closest to those in the field. A 1:2:1 ratio of leaf damage scores was observed in 207 F2 seedlings from nine crosses between resistant and susceptible parents, suggesting that disease reaction was controlled by co-dominant alleles at one locus. Parental reactions were sometimes anomalous, indicating environmental effects that produced escapes. Tests on F3 families showed that 3/18 families derived from resistant F2 phenotypes were actually susceptible, whereas all 18 susceptible F2 phenotypes bred true. The level of resistance in F3 families was a high as in the parents, indicating that few, if any, modifying genes affected the disease reactions.

Selection for resistance, higher yield, low erucic acid oil and a predominance of propenyl glucosinolate in meal lead to the release of five cultivars for cold-pressed oil production by the Yandilla Mustard Oil Enterprise between 1989 and 2001, over which time yield increased 2.4% annually. However, Pseudomonas infections had dropped to low levels by 1986. These results will be discussed.

Keywords: resistance, breeding, new cultivars, higher yield, cold-pressed oil.

Introduction

Indian mustard is a condiment and oilseed crop which potentially can yield more reliably than canola in the hotter and drier regions of Australia, particularly those with some mid-late spring rainfall (Oram *et al.* 1997; unpublished data). Oilseed types from India, and condiment types from Europe, Canada and China, all of which had high erucic acid oil and high glucosinolate meal, were imported and assessed as alternative wheatbelt crops in the 1960s and 1970s. Small breeding programs and associated research projects were started in Western Australia, Vic and ACT in the 1970s, leading to the discovery of the low erucic acid oil trait (Kirk and Oram 1981). In field trials at Canberra and Wagga Wagga in 1979–1981, south Asian accessions, but not north Asian, European or Canadian accessions, were severely injured by necrotic lesions on the leaves and stems. During winter and early spring, successive leaves developed large, spreading lesions with brown edges and almost white centres. In spring, stems developed sunken, longitudinal red-brown streaks, which damaged flowers and pods over much or all of each plant, thus reducing yield considerably. The causal organism was identified as the seed-borne bacterium, *Pseudomonas syringae* pv. *maculicola* (McCullock 1911) Young, Dye and Wilkie 1978, at the Plant Research Institute, Victorian Department of Agriculture, Burnley, in 1980 (unpublished information). *Pseudomonas* blight was rare in years of drought or mild, wet winters.

Pseudomonas blight

Developing a phytotron test

Two isolates of the bacterium (No. 30557 and 33362) were obtained from Dr P Fahy, NSW Agriculture, Rydalmere, and three from plants grown in a glasshouse (ex IB 683) or field plots at Canberra. The isolates were grown on King's Medium B (King *et al.* 1954), and checked for motility and blue fluorescence under UV light before use for plant

inoculations, because both these traits are unstable. However, with these precautions, pathogenicity remained unchanged: the extent of leaf damage caused by isolate 33362 on IB 1506 (susceptible) and Domo (resistant) remained very similar after several sub-culturings, storage in a lyophilised state for five years, and reconstitution.

Because field injury was most severe if some frosts were followed by a mild, wet spell, the first step in developing a phytotron test was to vary the temperature of the frost treatment applied to three-week old seedlings for 16 hours on three successive nights. The seedlings were inoculated by spraying them with a suspension of bacteria, frosted, and then grown at 21/16ºC day/night temperatures for five days in a humid chamber, followed by one day uncovered to allow the lesions to dry out. The percentage of the area of the first two leaves on each seedling killed by the bacteria was rated on each seedling to the nearest 10% against a set of standard drawings. Figure 32 shows the mean injury scores for the five resistant and the five susceptible cultivars listed in Table 67 Subsequent tests were done with -7ºC frost treatments to maximise the difference between resistant and susceptible genotypes.

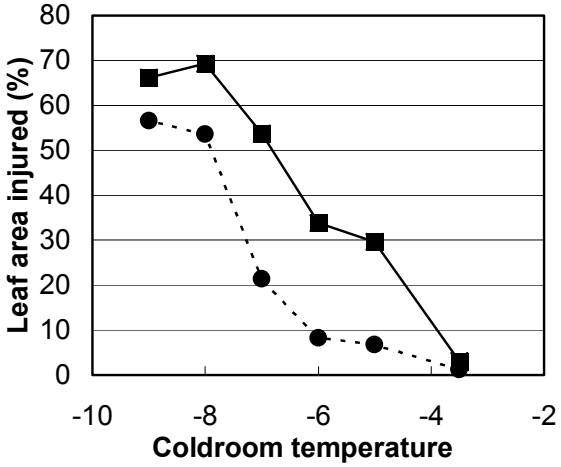

Figure 32: Mean percentages of dead leaf area on seedlings of five susceptible cultivars (■) and five resistant cultivars (●) caused by isolate 33362 of Pseudomonas after frosting treatments on 3 nights at a range of temperatures. The l.s.d. between resistant and susceptible means at the temperatures -5°C, -6°C, -7°C and -8°C was 10.9% (P=0.05). Error variances were homogeneous at these temperatures.

Table 67: Percentage of the area of the first and second seedling leaves of 10 Indian mustard cultivars injured by inoculation with five isolates of *Pseudomonas syringae* pv. *maculicola*.

	Injury scores for five *Pseudomonas* isolates					
Mustard accessions and origins	30557	33362	Ex IB 683	GES 27/7	GES 24/6	Means
Susceptible cultivars						
IB 683 India	41.7	82.3	36.0	58.7	74.3	58.6ab
IB 712 India	37.0	67.0	59.0	83.3	91.0	67.5a
IB 1506 India	71.7	62.3	61.7	56.7	86.3	67.7a
IB 1637 India	44.3	40.0	21.7	14.7	49.7	34.1c
Rai 14 Bangladesh	51.0	42.0	33.7	39.7	72.3	47.7b
Means	49.1bc	58.7b	42.4c	50.6bc	74.7a	55.1
Resistant cultivars						
Domo Canada	11.3	14.0	9.0	12.7	30.3	15.5d
Skorospelka USSR	26.7	23.0	16.3	26.7	53.0	29.1c
Neosypajuscajasia USSR	37.0	28.0	18.7	25.7	48.0	31.5c
Stoke UK	14.3	16.3	8.0	15.3	55.0	21.8c
Lethbridge 22A Canada	17.6	11.3	23.0	21.0	67.0	28.0c
Means	21.4d	18.5d	15.0d	20.3d	50.7bc	25.1
Overall means	35.3b	38.6b	28.7c	35.5b	62.7a	40.1

Isolate means over each set of five cultivars, or over all 10 cultivars, or the means for each cultivar, that are followed by the same letter(s) are not significantly different at P = 0.05.

Host – pathogen interactions

The standard phytotron test was used to determine the effects of the five isolates of Pseudomonas listed in the previous section on the five susceptible and five resistant cultivars shown in Table 67. In general the resistant/susceptible classifications of the cultivars in the field were similar to those in the phytrotron tests. However, isolate GES 24/6 caused significantly more injury than any other isolate on the susceptible cultivars, and overcame the resistance of the five field-resistant cultivars to a greater or lesser extent: cv. Domo was

the cultivar least affected by this and the other isolates. Also, the isolates ex IB 683 and GES 27/7 were avirulent on cv. IB1637, a more specific host-pathogen interaction. The cultivars Domo and IB 1506 were selected for later phytotron experiments in which a single representative of the resistant and susceptible classes was required. Isolate 33362 was used as the representative bacterial strain in subsequent experiments, being among the most injurious on the five susceptible cultivars and among the least injurious on the five resistant cultivars (Table 67).

The temperatures at which the leaves of resistant and susceptible seedlings froze during cooling were determined by placing thermocouples on the leaf surfaces, and noting the temperature at which there was a sharp rise in temperature as the leaves gave up their latent heat during freezing. In twelve tests, the temperatures at which leaves froze ranged from -1.6ºC to -3.0ºC, and averaged 2.3ºC for both the resistant and susceptible cultivars, so the temperature of freezing is not a component of the resistant and susceptible disease reactions.

Genetics of resistance

A trimodal distribution of leaf injury scores was observed within the nine F2 families derived from crosses between one of the resistant cultivars Domo, Lethbridge 22 A, Stoke, Skorospelka and Neosypajuscajasia and one of the susceptible Indian cultivars IB 1506, CPI 81792, CPI 81796 and CPI 81799. Figure 33 shows the combined distribution of all 207 F2 seedlings, and for seedlings of the resistant (Domo) and susceptible (IB 1506) cultivars. A log transformation, log10 (× +20), was necessary to normalise, as far as possible, the percentage injury values.

The distribution for Domo seedlings was unimodal and positively skewed, whereas that for IB 1506 was unimodal and negatively skewed. The F2 distribution was trimodal, with some overlap between the classes. Dividing the ambiguous values between the adjacent classes according to their relative sizes gave a ratio of 55.5 seedlings with low injury, 96 with moderate injury and 55.5 with high injury. These values fitted the 1:2:1 ratio expected for the segregation of codominant alleles at a single locus (χ^2=0.59, P=0.7–0.8). The alleles for resistance and susceptibility are designated PsmR and PsmS, respectively. The segregations in the 9 families were not heterogeneous (χ^2=24.2, P=0.05–0.1).

Yield of resistant and susceptible selections

The extended tails of the distributions for the two parents shows that some F2 seedlings are likely to be misclassified. Of 18 F3 families raised from apparently resistant F2 seedlings, three proved to be wholly or partially susceptible, whereas all 18 phenotypically susceptible F2 seedlings proved to be at least partially susceptible. The 15 resistant and 21 susceptible or mixed families, and three resistant and three susceptible parents, were tested for yield in five m^2 plots in six by seven rectangular lattice trials with two replicates as F3s at Canberra in 1986 and as F4s at Wagga Wagga in 1987. Herbicide and fertiliser treatments were similar to those used on farms. On average, the resistant families yielded more than the susceptible families in both years/locations/generations (Table 68).

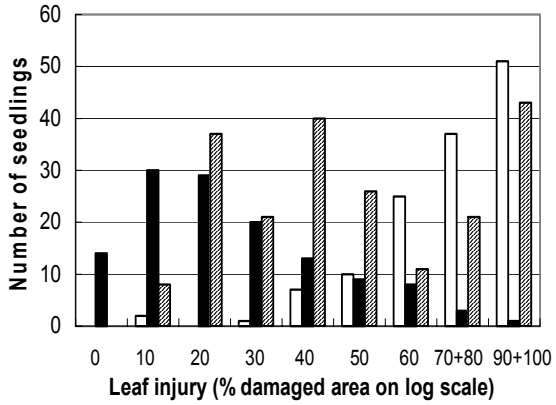

Figure 33: Frequencies of seedlings on a log scale in the resistant (Domo,■) and susceptible (IB 1506,□) parents, and the F2 families from 9 resistant × susceptible crosses (hatched columns) with various degrees of leaf injury following frosting and inoculation with Pseudomonas isolate 33362.

Conclusions from phytotron tests

On average over four other F2 families derived from a resistant, low erucic acid, Chinese and a susceptible Chinese or Indian accession, the percentages of 72 seedlings scored as having 0, 10 or 20% of damaged leaf area that gave rise to uniformly resistant F3 families was 84.6 ± 10.0, 57.1 ± 8.4 and 59.5 ± 5.7, respectively. Thus, the observed F3 segregations are compatible with the two codominant allele hypothesis. In field tests, the F3 families that were uniformly resistant in phytotron tests were as resistant as their resistant parents. Therefore, despite the environmental variation in the phytotron, which probably is due to variable air movements in the cold-room, and some heterogeneity in the pathogenicity of field populations of Pseudomonas, the phytotron test proved to be highly effective as a tool for breeding families that are resistant in the field.

After 1986, damage fell to low levels in field plots, even on cultivars that previously had been severely injured. Some of the decrease probably was due to a decrease in inoculum levels because of the predominance of resistant entries in the trials, but there also may have been an increase in competing phylloplane species when mustard had been grown for a longer period and on larger areas at the trial sites.

Table 68: Yield (g/m²) at two sites of Indian mustard families resistant, or wholly or partially susceptible, to *Pseudomonas* blight.

Parents: Resistant cultivars and susceptible Indian lines	Disease reaction group	No. families per group	Canberra 1986 (F_3)	Wagga 1987 (F_4)	Mean
Domo × Line RH 7384 A	Resistant	2	221*	133*	177*
	Susceptible	4	213*	97*	155*
Domo × Line R 743	Resistant	3	220ab	101bcde	161ab
	Susceptible	3	194bc	137a	166ab
Line RH 7514 × Domo	Resistant	1	220*	126*	165*
	Susceptible	5	197*	142*	170*
Line RH 7514 × Skorospelka	Resistant	3	205abc	121abc	163ab
	Susceptible	3	159d	97cde	128c
Line R 743 × Skorospelka	Resistant	3	176cd	131ab	154b
	Susceptible	3	175cd	83de	129c
Line RH 7384 A × Skorospelka	Resistant	3	229a	139a	184a
	Susceptible	3	194bc	106bcd	150bc
Parents	Resistant	3	196bc	79e	
	Susceptible	3	102e	114bcd	108
All crosses	Resistant	15	210	132	171
	Susceptible	21	191	110	150
L.s.d. for means of all crosses	$P = 0.05$		15.7	10.9	9.6
	$P = 0.01$		-	14.8	12.9

Means of sets of three families within each column followed by the same letter(s) are not significantly different at $P = 0.05$.
* the differences between means of the resistant and more susceptible classes were not significant at either site or on average over sites in either of the crosses in which the disease reaction groups contained more or less than three families, except for the first cross at Wagga

Breeding new cultivars

Resistant, low erucic acid, high propenyl glucosinolate families that yielded well in replicated five or 10 m² plots at Canberra and Wagga were intercrossed and re-selected on a pedigree basis, to produce five new cultivars for the production of cold-pressed mustard seed oil by the Yandilla Mustard Oil Enterprise at Wallendbeen, NSW. The pedigrees of these cultivars are shown in Figure 34.

Seed yields of the five cultivars in eight-row, five m² plots at Wallendbeen, NSW, on average over the years 1997–2000, rose from 1.28 t/ha for the tall, late-maturing Siromo, to 1.78 for the mid-season Micky and the early-flowering Kaye. Yield increased 2.4% per annum in the period 1989–2001. The main limitations to yield in the four test years were respectively spring drought, mid-spring frost, heavy Sclerotinia attack and weeds. Some yield increase was due to earlier flowering, but this was a one-off gain. It appears that resistance to environmental stresses and increased efficiency of resource utilisation provide much larger and more prolonged responses to selection.

Nematospora seed rot

A yeast was identified by its variable cell size in rotted seeds grown overseas and at Ginninderra Experiment Station. Distinctive long, thread-like, thick-walled spores also were observed: these strongly resemble those described for *Nematospora sinecauda* by Holley *et al.* (1984). It was eliminated by hot water treatment of seeds and by control of the presumed vector, Rutherglen bug.

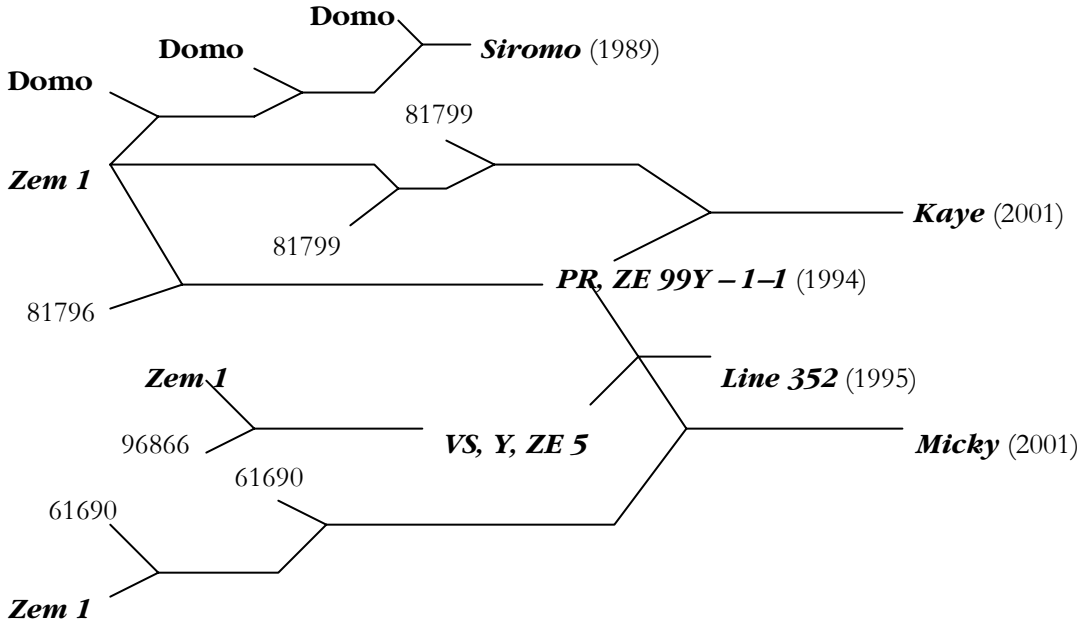

Figure 34: Cultivars with their year of release under each name.
The Commonwealth Plant Introduction accessions in standard type are high erucic and susceptible to Pseudomonas; all are from India, except for 96866 from China.
Italic type indicates low erucic acid lines and bold, resistant lines.

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