Major Gene Resistance to Blackleg in *Brassica napus* Overcome Within Three Years of Commercial Production in Southeastern Australia

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ABSTRACT

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The infection by *Leptosphaeria maculans* of *Brassica napus* cultivars with major gene resistance derived from *Brassica rapa* subsp. *sylvestris* was studied in southeastern Australia. Following the commercial release of these cultivars in Australia in 2000, plants with stem cankers were first reported in 2002 at two geographically isolated regions in South Australia and New South Wales. In 2003, this study showed that the major gene resistance had been overcome in an area of approximately 50,000 ha in South Australia and in two fields in New South Wales (0.5 and 30 ha). There was no relationship between disease severity and incidence in 2003 and the proximity to the sites where resistance breakdown occurred in 2002. At some locations, the frequency of isolates able to overcome the *B. rapa* subsp. *sylvestris*-derived resistance had increased between 2002 and 2003. Isolates cultured from canola cultivars with either *B. rapa* subsp. *sylvestris*-derived resistance or polygenic resistance showed host specificity when inoculated onto cultivars with *B. rapa* subsp. *sylvestris*-derived or polygenic resistance, respectively. The most likely cause of the resistance breakdown was the rapid increase in frequency of *L. maculans* isolates virulent on this particular resistance source. The selection pressure leading to this increased frequency was probably mediated by the planting of cultivars harboring the major resistance gene in the same locations for a 3-year period, and the ability of the pathogen to produce large numbers of asexual and sexual spores.

Additional keywords: oilseed rape, *Phoma lingam*

Blackleg, caused by the pathogen *Leptosphaeria maculans* (anamorph = *Phoma lingam* (Tode:Fr.) Desmaz.), is one of the most important diseases of *Brassica napus* L. var. *oleifera* (Metzger) Sink. (canola, oilseed rape, and colza) worldwide (13,34). The primary source of inoculum is airborne ascospores released from infected stubble following rainfall. In Australia, ascospore release begins after the first autumn rains and coincides with the emergence of new crops. Hyphae from germinating ascospores enter the cotyledons or leaves via stomata or wounds, initially colonizing spaces between the mesophyll cells (11). From the leaf, the fungus grows within the vascular tissue to the stem base where it becomes necrotrophic, causing a basal canker which results in reduced water and nutrient uptake, or plant death if necrosis is severe (12).

Host resistance to *L. maculans* has been identified at the seedling (leaf lesion) and

adult (stem canker) growth stages. Resistance in the seedling and adult growth stages can be under the same (15,27,36) or different genetic control (3,25), and can be either monogenic (single major gene) or polygenic (quantitative trait loci). Gene-for-gene interactions have been described whereby resistance genes in the host (expressed in cotyledons) are matched by complementary avirulence genes in the fungus (1,2). Until recently, Australian cultivars relied on polygenic adult plant resistance, which provides protection only against stem canker while leaves remain susceptible to infection (20). Hereafter in this article, these cultivars are described as "polygenic" cultivars. The stem canker phase of the disease is the most important because this symptom is associated with yield loss (22). The term "resistance breakdown" is used in this article to refer to the ability of isolates in the field to form stem cankers on cultivars with *B. rapa* subsp. *sylvestris*-derived resistance.

To improve resistance of *B. napus* cultivars to *L. maculans*, related *Brassica* spp. have been screened to provide sources of resistance (8,10,20,28,30). Due to the presence of similar genomes in different *Brassica* spp., resistance loci identified in one species can be introgressed into *B.*

napus (33). The three amphidiploid species, *B. napus* (AACC), *B. juncea* (AABB), and *B. carinata* (BBCC) are derived from crosses between the three diploid species, *B. oleracea* (AA), *B. nigra* (BB), and *B. rapa* (CC) as described by the Triangle of U (33). Crouch and colleagues (8) produced a synthetic *B. napus* line by crossing *B. oleracea* subsp. *alboglabra* with an accession of *B. rapa* subsp. *sylvestris* that had a high level of resistance to *L. maculans*. The F₁ progeny resulting from a cross between the synthetic line and a *B. napus* line was highly resistant to *L. maculans* when tested under field conditions in Australia and England (8). This resistance derived from *B. rapa* subsp. *sylvestris* (hereafter referred to as *sylvestris*-derived resistance) was then incorporated successfully into *B. napus* cultivars agronomically suited to Australia, resulting in lines with an extremely high degree of resistance to *L. maculans*. Resistance was conferred by a single dominant gene (*LepR3*; 15,35) and provided the plant with almost complete protection from infection of the leaf by inhibiting spore germination and mycelial growth, hence reducing the formation of stem canker (18,31). A number of cultivars containing this source of resistance were released commercially in 2000 for different Australian environments.

In 2002, canker symptoms not previously observed on cultivars with *sylvestris*-derived resistance were identified at two canola trial sites in southeastern Australia: Cudal in New South Wales (148°45'E, 33°17'S) and the Lower Eyre Peninsula in South Australia (135°43'E, 34°15'S) (9). In addition, Li et al. (19) identified isolates of *L. maculans* in Western Australia capable of attacking these cultivars. The locations in South Australia, New South Wales, and Western Australia each were separated by more than 1,000 km (Fig. 1). The initial site in 2002 on the Lower Eyre Peninsula was an area of 0.5 ha. In 2003, nearly every field sown with cultivars with *sylvestris*-derived resistance within the 50,000-ha canola production area was severely affected and, in 2004, no cultivars with *sylvestris*-derived resistance were grown in the Lower Eyre Peninsula region.

Cultivars with *sylvestris*-derived resistance released commercially in 2000 were highly resistant to *L. maculans*, with an Australian Blackleg Rating (ABR) of be-

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tween 8.0 and 9.0, where a rating of 1.0 is highly susceptible and 9.0 is highly resistant (Canola Association of Australia. Anonymous. 2000). The ABR is a national system that ranks all commercially released *B. napus* cultivars according to their survival due to stem canker resistance in experiments conducted across Australia at sites with high levels of *L. maculans* inoculum (20). In 2004, these cultivars were still rated as highly resistant but a warning that they may be highly susceptible in some areas was published with the ratings. These warnings reflected the fact that none of the trial sites used to determine the 2004 ABR values were in regions where the breakdown of *sylvestris*-derived resistance occurred in 2003.

Breakdown of major gene resistance in *B. napus* to *L. maculans* has been reported previously in France. In a wide-scale survey of commercial fields in France between 1990 and 2000, 1,011 isolates of *L. maculans* were assessed for the presence of three avirulence genes (*AvrLm1*, *AvrLm2*, and *AvrLm4*; 29). The proportion of isolates harboring *AvrLm1* declined rapidly in the years after the 1998–99 season, when cultivars with the corresponding resistance gene *Rlm1* accounted for 43.7% of the acreage sown to *B. napus* in France. In a second study, *B. napus* lines with major gene resistance introgressed from either *B. juncea* or *B. nigra* were tested in the field to determine the durability of the resistance source (5). The lines were sown into stubble of the resistant lines and infected susceptible lines collected from the previous year. Resistance in lines containing major gene resistance introgressed from *B. juncea* was overcome in 3 years, whereas lines with resistance derived from *B. nigra* remained effective after 4 years.

In this article, we report the findings of a survey aimed at determining (i) the distribution of *L. maculans* isolates able to attack *B. napus* lines with *sylvestris*-derived resistance and (ii) the prevalence of diseased plants in field experiments and commercial crops of cultivars with *sylvestris*-derived or polygenic resistance in southeastern Australia. We also report changes in host specificity of the pathogen population in response to sowing of cultivars with *sylvestris*-derived resistance. The results are discussed in relation to the durable deployment of different sources of resistance to *L. maculans*.

MATERIALS AND METHODS

Survey of commercial fields of *B. napus* cultivars with *sylvestris*-derived resistance. A survey of cultivars with *sylvestris*-derived resistance (Surpass 400, Surpass 603CL, Surpass 501TT, and Hyola 60) was conducted in commercial crops and field experiments in 2003 and 2004 (Fig. 1). In 2003, the area within a 100-km radius of the sites at Cudal in New South Wales and Lower Eyre Peninsula in South

Australia, where stem cankers first were observed in cultivars with *sylvestris*-derived resistance in 2002, was surveyed. In addition, commercial fields in canola-producing regions of Victoria, southern New South Wales, and around Bordertown in South Australia were examined. In 2004, the survey was conducted in South Australia, Victoria, and southern New South Wales.

In 2003, crops at several distances from sites where stem cankers first were observed in plants of cultivars with *sylvestris*-derived resistance in 2002 were examined. Commercial crops of cultivars with *sylvestris*-derived resistance were identified within a 100-km radius of the site on Lower Eyre Peninsula in South Australia and Cudal in New South Wales (Fig. 1). In all, 31 fields were assessed on Lower Eyre Peninsula and 33 fields were assessed near Cudal. Both of these areas are intensive cropping zones, with canola grown in fields every 2 to 4 years in rotation with cereals and, sometimes, a grain legume. Based on the proximity of crops to the sites where stem cankers first were observed in 2002, fields were grouped into categories: 0 to 5 km, 5 to 10 km, 10 to 20 km, 20 to 30 km, 30 to 50 km, or 50 to 100 km. This experiment was not repeated in 2004 because cultivars with *sylvestris*-derived resistance were no longer widely grown in either South Australia or New South Wales after results from the 2003 survey were communicated to growers.

In 2003 and 2004, a survey was carried out in canola-producing areas of South Australia, Victoria, and New South Wales to establish the prevalence of isolates capable of causing disease in cultivars with *sylvestris*-derived resistance (Fig. 1). In all, 99 sites were assessed in 2003 (including those sites at Lower Eyre Peninsula and Cudal described above) and 52 sites in 2004. In 2003, the majority of sites assessed were commercial fields of cultivars with *sylvestris*-derived resistance. Sites were distributed across Victoria and New South Wales but were only located at the Lower Eyre Peninsula and Bordertown in South Australia. In 2004, the disease severity and incidence in cultivars with either polygenic resistance or *sylvestris*-derived resistance were assessed at experimental sites. The cv. Beacon (ABR 6.0) with polygenic resistance and cv. Surpass 501TT (ABR 8.5) with major gene *sylvestris*-derived resistance were examined at most sites. Where these cultivars were not present, cultivars with polygenic and *sylvestris*-derived resistance with an ABR similar to cvs. Beacon and Surpass 501TT were assessed instead. The size of sites ranged from 0.5 ha for some experimental sites to over 100 ha for commercial crops.

The severity and incidence of *L. maculans* infection was assessed on 60 or 100 mature plants per site just prior to windrowing. Plants assessed in commercial

fields were chosen by walking a “W” transect, taking four paces and selecting the third plant from the right foot (15 or 25 plants per leg of the “W”). Plants assessed in trial plots were selected by taking 20 consecutive plants in the same row beginning at least 1 m from the end of the plot. Three replicate plots were assessed at each trial site. Plants were cut transversely at the crown and then visually assessed for blackleg symptoms. Each plant was scored as having 0, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% internal infection (disease severity; 22). The average disease severity and incidence of plants with disease severity $\geq 5\%$ was then calculated for each field and trial site.

Collection of *L. maculans* isolates. Isolates of *L. maculans* derived from single ascospores were collected from infected canola stubble from 19 fields at 13 different locations in southeastern Australia during a 3-year period (2002 to 2004; Fig. 1; Table 1). *L. maculans* is a heterothallic fungus with two mating types; therefore, the ascospore-derived isolates cultured from stubble in one year (e.g., 2002) originate from sexual recombination between isolates that infected the crop in the previous season (e.g., 2001).

Following the commercial release of cultivars with *sylvestris*-derived resistance in 2000, isolates were cultured from the stubble of several cultivars collected in 2002 and 2003 from *L. maculans* disease nurseries and commercial fields in Victoria, New South Wales, and South Australia to monitor the presence and frequency of isolates able to overcome the *sylvestris*-derived resistance in different areas (Table 1). These sites were representative of canola-producing areas in Australia. Isolates collected in 2003 and 2004 were tested for virulence concurrently with the field surveys. After the breakdown of the *sylvestris*-derived resistance during the 2003 growing season, isolates were collected from the stubble of cultivars with polygenic or *sylvestris*-derived resistance from commercial fields in Victoria and South Australia in 2004. The ability of these isolates to attack both types of resistance was then tested. All stubble collected in 2004 from South Australia was from areas where the breakdown of cultivars with *sylvestris*-derived resistance had occurred in 2003.

Stubble pieces bearing *L. maculans* pseudothecia were wetted and suspended over water agar plates (20 g of agar per liter of water) for 2 h for ascospore discharge. Several single ascospores of *L. maculans* per stubble piece were collected. Individual ascospores were transferred to Campbell's V8 juice agar plates (100 ml of V8 juice and 20 g of agar per liter of water, pH 6) and incubated at 20°C with 12 h of light. Isolates were stored in sterile water and on dried cellulose discs at 20°C with silica gel.

Virulence of *L. maculans* isolates. *Cotyledon test.* Isolates collected from 2002 and 2003 stubble were tested for pathogenicity on the cotyledons of *B. napus* cv. Surpass 400 (Table 1). This test provides a reliable indication of the ability of an isolate to form stem cankers on cultivars with *sylvestris*-derived resistance (16). The total number of isolates tested from 2002 stubble was 122 (35 from South Australia, 26 from Victoria, and 61 from New South Wales). In all, 104 isolates from 2003 stubble were tested (49 from South Australia, 40 from Victoria, and 15 from New South Wales). A well-characterized Australian isolate, IBCN 18 (formerly named M1), collected in 1986, was included as a control. Isolates were each inoculated onto 12 plants grown from

commercial seed of cv. Surpass 400 at 10 days after sowing as described previously (27). Plants were grown in a glasshouse maintained at a constant temperature of 22°C. Pycnidiospores were harvested from 12-day-old cultures grown on 10% V8 agar as described above. The plates were flooded with sterile water, then scraped with a scalpel to liberate pycnidia. After 1 h, the pycnidiospore suspension was filtered through Miracloth and the concentration of spores adjusted. Pycnidiospore suspensions of 1×10^7 spores/ml were applied as a 10- μ l droplet onto four pinprick wounds on the cotyledons (total of 48 inoculations per isolate). The 12 plants per punnet were inoculated with one isolate and placed into a high humidity container with free water at 22°C. After 48 h,

the droplets of inoculum had dried up and plants were removed from the humidity chamber. Punnets were watered carefully to avoid cross-contamination of isolates. To allow full expansion of the cotyledons, developing true leaves were removed.

Disease was rated between 12 and 14 days post inoculation with a 0-to-9 scale as described previously by Koch et al. (14), where 0 = no darkening around wounds; 1 = limited blackening around wounds, lesion diameter 0.5 to 1.5 mm; 3 = dark necrotic lesions, 1.5 to 3 mm; 5 = dark lesions, 3 to 6 mm, brownish on lower surface; 6 = as in 5, but less necrotic; 7 = gray-green lesions of limited size or large necrotic lesions; 8 = spreading gray-lesions with no or few pycnidia; and 9 = large gray-green lesions with profuse

Fig. 1. Sites in southeastern Australia from which *Leptosphaeria maculans* isolates were collected, as well as where cankers were first observed on stems of blackleg-affected cultivars with *Brassica rapa* subsp. *sylvestris*-derived resistance (Lower Eyre Peninsula, South Australia, and Cudal, New South Wales).

sporulation. Lesions were not scored for plants that had senesced by 12 to 14 days post inoculation when disease was scored. Isolates were classified as having either low (cotyledon score 0 to 2), moderate (2 to 4), high (4 to 6), or very high (6 to 9) cotyledon scores (14). The percentage of isolates in each category was then determined for each state (South Australia, New South Wales, and Victoria) for each year.

Stem test. To determine whether isolates exhibited host specificity in their ability to develop stem cankers in cultivars with major gene *syvestris*-derived resistance or cultivars with polygenic resistance, isolates collected from South Australia and Victoria from stubble in 2004 were tested for the development of stem canker in cv. Beacon (ABR 6.5) with polygenic resistance, cv. Surpass 400 (ABR 9.0), and cv. Q2 (ABR 1.0), which was used as a susceptible control. In all, 33 isolates were tested, 18 collected from stubble of polygenic cultivars in Victoria and 15 from stubble of cultivars with *syvestris*-derived resistance in South Australia (Table 1). Twenty-eight days after sowing, plants at the one-leaf stage were sprayed with a pycnidiospore suspension (1×10^7 spores/ml with 0.05% Tween

20) until run-off. Pycnidiospore suspension was collected as described above. Six individual plants of each cultivar were inoculated with each isolate. Plants were then placed in a humidity chamber for 48 h before transfer to a shade house, where they were fertilized and watered as required. Severity of stem canker development was assessed 18 weeks postinoculation by cutting the plant at the crown and scoring disease severity using a 0 to 100% scale as described above.

Statistical analyses. Stem canker severity data and disease incidence data collected from the survey of commercial crops were square-root transformed and arcsin transformed, respectively, prior to analysis (22). Analyses were performed using REML, the algorithm in Genstat version 6.1 (26) for unbalanced data sets. All values presented in tables and figures are of untransformed data.

RESULTS

Breakdown of *syvestris*-derived resistance in the field. There were no significant differences between the severity and incidence of blackleg recorded in cultivars with *syvestris*-derived resistance at dis-

tances up to 100 km from the original sites where cankers first were observed in 2002 in either the Cudal or Lower Eyre Peninsula regions (*data not shown*). However, there was a significant difference in the level of disease between the two regions. High levels of disease were recorded on Lower Eyre Peninsula at sites up to 100 km from the original site, with an average disease severity of 60 and incidence of 94% across the 31 fields surveyed. At a distance of 0 to 5 km from the Lower Eyre Peninsula site, the disease severity was 63 and the incidence was 97% and, at 50 to 100 km, the disease severity was 47 and the incidence was 87%. In contrast, around Cudal in New South Wales, the average disease severity across the 33 fields surveyed was 2 and the incidence was 10%. Disease severity was less than 5 and the incidence below 20% at all distances.

The severity and incidence of blackleg recorded in the survey of cultivars with *syvestris*-derived resistance differed between states in 2003 and 2004 (Table 2). In South Australia, disease severity was constant between 2003 and 2004 but disease incidence declined. In both years, all sites examined in South Australia had infected

Table 1. Source of *Leptosphaeria maculans* isolates from southeastern Australia^x

Location	Year stubble collected	<i>Brassica napus</i> cultivar	ABR of cultivar in year grown ^y	No. of isolates tested
Struan, South Australia	2002	Polygenic and <i>syvestris</i> -derived ^z	...	35
Wonwondah, Victoria,	2002	ATR-Beacon	6.0	7
Lake Bolac, Victoria,	2002	TI1 Pinnacle	5.5	19
Farm 1, Thuddungra, New South Wales	2002	TI1 Pinnacle	5.5	27
Farm 2, Thuddungra, New South Wales	2002	ATR-Grace	6.5	34
Struan, South Australia	2003	Polygenic and <i>syvestris</i> -derived	...	34
Lower Eyre Peninsula, South Australia	2003	Polygenic and <i>syvestris</i> -derived	...	15
Wonwondah, Victoria	2003	ATR-Beacon	6.0	12
Lake Bolac, Victoria	2003	TI1 Pinnacle	6.0	28
Lockhart, New South Wales	2003	ATR-Beacon	6.0	8
Grenfell, New South Wales	2003	TI1 Pinnacle	6.0	7
Wonwondah, Victoria	2004	ATR-Grace	6.5	3
Laharum, Victoria	2004	TI1 Pinnacle	5.5	3
Geelong, Victoria	2004	TI1 Pinnacle	5.5	4
Moyhu, Victoria	2004	ATR-Grace	6.5	5
Bordertown, South Australia	2004	Ag-Emblem	7.0	3
Lower Eyre Peninsula, South Australia	2004	Surpass 400	8.5	5
Farm 1, Bordertown, South Australia	2004	Surpass 603CL	8.5	5
Farm 2, Bordertown, South Australia	2004	Surpass 603CL	8.5	5

^x Pathogenicity of isolates collected in 2002 and 2003 was tested on cotyledons, while isolates collected in 2004 were tested for ability to cause stem cankers.

^y ABR = Australian Blackleg Rating; all commercially released cultivars are ranked according to their survival in trials conducted across Australia, with 1.0 being highly susceptible and 9.0 being highly resistant.

^z Cultivar from which isolates were collected is unknown.

Table 2. Presence of blackleg in *Brassica napus* cultivars with *B. rapa* subsp. *syvestris*-derived resistance at sites in the canola-producing areas of South Australia (SA), Victoria (Vic), and New South Wales (NSW) in 2003 and 2004

State	2003				2004			
	No. of sites	Infected ^y	Severity ^z	Incidence (%) ^z	No. of sites	Infected ^y	Severity ^z	Incidence (%) ^z
SA	33	33	60.0 ± 2.7 a	94.1 ± 1.2 c	15	15	48.7 ± 9.0 a	66.0 ± 9.5 d
NSW	47	44	1.7 ± 0.7 a	8.8 ± 2.3 c	16	14	5.8 ± 1.6 b	34.4 ± 6.1 d
Vic	19	19	4.1 ± 0.9 a	19.6 ± 3.5 c	21	18	3.6 ± 0.6 a	14.6 ± 2.0 c
Mean of all states	99	96	22.3 ± 3.0	40.3 ± 4.2	52	47	18.6 ± 4.2	36.9 ± 4.7

^y Infected sites (Infected) are those that had ≥1 plant with blackleg disease symptoms in the stem.

^z Severity is the area of the stem cross-section with disease symptoms (0 to 100%) and incidence is the percentage of plants with disease severity ≥5% per site. Values are the average ± standard error for infected sites. Disease severity values (a or b) and disease incidence values (c or d) followed by the same letter across a row (i.e., between years) are not significantly different at $P = 0.05$.

plants and also high disease severity. All sites in South Australia in 2003 had over 50% of plants infected, whereas 11 of 15 of the sites had more than 50% of plants

infected in 2004. The level of disease recorded in Victoria remained constant, with an average severity of 3.7 and incidence of 17.2% over the 2 years. In Victoria, one

site recorded over 50% of plants infected but the mean disease severity was only 5.2. In New South Wales, the severity of disease remained low; however, the incidence of blackleg increased from 8.8% in 2003 to 34.4% in 2004. In 2003, one site had 92% of plants infected, with a mean severity of 31.5, while another site had 47% of plants infected, with a mean severity of 10.5. Both of these fields had been sown with cultivars with *sylvestris*-derived resistance in adjacent fields for three consecutive seasons. In 2004, four sites had over 50% of plants infected; however, the disease severity was less than 20 at each site (*data not shown*).

When blackleg severity was compared in cultivars with polygenic or *sylvestris*-derived resistance at the same site in 2004, the disease severity differed between sites and states (Fig. 2A). At all Victorian sites, disease severity was low, with an average severity of less than 25 in the polygenic cultivar and less than 10 in the cultivar with *sylvestris*-derived resistance. A similar trend was observed at New South Wales sites, with disease severity higher in the polygenic compared with the cultivar with *sylvestris*-derived resistance. Two sites in South Australia had an average disease severity of less than 2.0 in both the polygenic cultivar and the cultivar with *sylvestris*-derived resistance; however, at the majority of the remaining sites, the disease severity was greater than 20 in the cultivar with polygenic resistance and greater than 55 in the cultivar with *sylvestris*-derived resistance.

The incidence of plants with blackleg in cultivars with polygenic and *sylvestris*-derived resistance followed a trend similar to that of disease severity, with the highest levels observed at sites in South Australia and the lowest at Victorian sites (Fig. 2B). In Victoria, disease incidence in the polygenic cultivar ranged from 2 to 60% and 0 to 23% in the cultivar with *sylvestris*-derived resistance. As in Victoria, most sites in New South Wales had a higher incidence of blackleg in the polygenic cultivar than in the cultivar with *sylvestris*-derived resistance, although the range was greater. The incidence of blackleg recorded at sites in South Australia was similar in cultivars with polygenic or *sylvestris*-derived resistance, and both cultivars had an incidence greater than 50% at the majority of sites.

Virulence of *L. maculans* isolates. The frequency of *L. maculans* isolates able to cause high and very high levels of disease on the cotyledons of Surpass 400 differed between states, with a shift in frequency observed from 2002 to 2003 for isolates from South Australia and Victoria (Fig. 3). The percentage of isolates collected from South Australia causing high and very high disease scores increased from 14.3 to 38.8% and 0 to 16.3%, respectively. There was no change in the frequency of isolates

Fig. 2. A, Blackleg severity and B, incidence in *Brassica napus* cultivars with polygenic or *B. rapa* subsp. *sylvestris*-derived resistance at sites in Victoria (○), South Australia (□), and New South Wales (▲) in 2004. Each point represents data collected from one site. Severity is the area of the stem cross-section with disease symptoms (0 to 100%) and incidence is the percentage of plants with disease severity $\geq 5\%$ per site.

causing moderate disease scores but a large decline from 48.6 to 8.2% in isolates causing low disease scores. In Victoria, there was a similar increase in the proportion of isolates causing very high disease scores. During the 2 years, isolates from New South Wales showed a similar spectrum of disease scores on cotyledons of cv. Surpass 400. No New South Wales isolates gave very high disease scores. Isolate IBCN 18 gave medium scores in both 2002 (3.51 ± 0.19) and 2003 (3.19 ± 0.23), and also formed stem lesions on cv. Surpass 400 (data not shown).

Isolates collected from the stubble of cultivars with polygenic or *sylvestris*-derived resistance in 2004 showed host specificity when inoculated onto cultivars with polygenic or *sylvestris*-derived resistance and scored for stem canker severity (Fig. 4). Most isolates from stubble of polygenic cultivars caused more severe stem symptoms on the cvs. Q2 and Beacon than on Surpass 400, whereas isolates from stubble of cultivars with *sylvestris*-derived resistance caused more severe canker symptoms on Surpass 400 than on Q2 and Beacon (Fig. 4). The isolates cultured from stubble of cultivars with polygenic or *sylvestris*-derived resistance both caused very severe disease on cv. Q2 (severities of 98.5 ± 0.7 and 89.1 ± 3.3 , respectively), with most plants dead prior to maturity. On the moderately resistant cv. Beacon, isolates from stubble of polygenic cultivars had an average disease severity of 70.0 ± 5.9 , whereas isolates from stubble of cultivars with *sylvestris*-derived resistance had an average disease severity of 36.2 ± 6.9 . In contrast, the average disease severity of isolates from cultivars with polygenic or *sylvestris*-derived resistance inoculated on cv. Surpass 400 was 19.7 ± 5.4 and 89.3 ± 5.2 , respectively.

DISCUSSION

Our survey data show a significant decrease in efficacy of the major gene resistance to *L. maculans* derived from *B. rapa* subsp. *sylvestris* in some areas of south-eastern Australia. This breakdown of resistance has been rapid, as in the years prior to our study, cultivars with *sylvestris*-derived resistance were highly resistant to the development of stem canker (31). The breakdown of major gene resistance described in this article appears to be more dramatic than the breakdown of resistance conferred by the *Rlm1* gene in winter *B. napus* in France, which was widespread rather than limited to particular regions (29). This is not surprising, given that the temperate climate in which *B. napus* is grown in Australia allows for a high degree of recombination in the fungus during summer and for production of large amounts of ascospore inoculum. The temperate conditions and long growing period for canola also allow for more severe disease development (32). Another explana-

tion for the large yield losses and very rapid resistance breakdown in Australia is that the general level of polygenic resistance of the Australian spring *B. napus* cultivars into which the *sylvestris*-derived resistance was introduced may have been less than that of the European winter *B. napus* cultivars, leading to severe losses when the resistance source was overwhelmed. Isolates of *L. maculans* able to overcome the *sylvestris*-derived resistance were collected from cultivars with *sylvestris*-derived resistance in Western Australia in 2001 (19), and also in 2002 and 2004 (17). However, results from field surveys of disease severity in Western Australia have not been published and severe yield losses were not reported until 2004 (W. Smith, Agronomic Acumen, Western Australia, personal communication).

Prior to 2003, cultivars with *sylvestris*-derived resistance were rated highly resistant, with an ABR of between 8.0 and 9.0. Very few leaf lesions or stem cankers were observed on cultivars with *sylvestris*-derived resistance (31); however, the pathogen was isolated from within infected stems at two field experiments in New South Wales in 2002 (J. Kirkegaard, CSIRO Plant Industry, personal communication). Indeed, Marcroft et al. (21) found that stubble of cultivars with *sylvestris*-derived resistance collected in December 2001 hosted pseudothecia and released ascospores, albeit at a much lower frequency than polygenic cultivars, suggesting that some infection of plants had occurred. The reduction in pseudothecia and subsequent ascospore release from the stubble of cultivars with *sylvestris*-derived resistance suggest strong selection for

isolates able to overcome the *sylvestris*-derived resistance. The spores produced from these isolates infect the following crop and would compose a large proportion of the pathogen population in areas where the cultivars with *sylvestris*-derived resistance were widely grown. This may be the reason for the sudden and severe breakdown in areas such as Lower Eyre Peninsula, where cultivars with *sylvestris*-derived resistance constituted a large proportion of the area sown to canola.

Isolates of *L. maculans* able to overcome the *sylvestris*-derived resistance were most likely widespread in the Australian population prior to the commercial release of cultivars with *sylvestris*-derived resistance in 2000. This is suggested by (i) the presence of isolates able to cause moderate to severe lesions on the cotyledons of cv. Surpass 400, which has *sylvestris*-derived resistance, in stubble of *B. napus* crops grown in locations across three states in 2001; (ii) the detection of disease in cultivars with *sylvestris*-derived resistance in geographically distant areas; and (iii) the lack of a relationship between proximity to the sites where the breakdown was first reported in 2002 and the level of disease recorded in crops with *sylvestris*-derived resistance up to 100 km away in 2003. Indeed, isolates collected from *B. napus* in the early 1970s were able to form leaf lesions on cv. Surpass 400 (17), and the control isolate (IBCN 18) used in the cotyledon testing, which was collected in 1986, forms leaf lesions and stem cankers on cv. Surpass 400 (H. L. Hayden and B. J. Howlett, data not shown).

The initial increase in area sown to cultivars with *sylvestris*-derived resistance

Fig. 3. Pathogenicity of *Leptosphaeria maculans* isolates collected in 2002 and 2003 from Victoria, South Australia, and New South Wales able to cause disease on *Brassica napus* cv. Surpass 400. Symptoms are classed as low, moderate, high, or very high depending on the mean lesion score based upon the lesion descriptions of Koch et al. (14). The number of isolates used to calculate the frequency is shown in each bar.

and subsequent selection of isolates able to overcome the resistance in these crops resulted in a shift in the pathogen population. In the locations where the breakdown was most severe in 2003, cultivars with *sylvestris*-derived resistance were grown intensively during the previous seasons, thereby creating strong pressure to select those isolates capable of overcoming the

resistance. Evidence of a shift in the pathogen population was observed in the increase in frequency from 2002 to 2003 of *L. maculans* field isolates able to cause disease on the cotyledons of a cultivar with *sylvestris*-derived resistance. Many of the most damaging and dangerous pathogens undergo a combination of sexual and asexual reproduction, termed a "mixed" repro-

duction system (24). *L. maculans* exhibits a mixed mode of reproduction, which may have contributed to the increase in frequency of isolates able to overcome the *sylvestris*-derived resistance. Sexual reproduction may have increased the proportion of selected isolates in the population through recombination, with the resultant ascospores infecting newly sown crops. Although the role of pycnidiospores in relation to the development of blackleg epidemics is thought to be minimal (34), the asexual multiplication phase may increase to a high level the frequency of isolates in the population able to overcome the resistance through clonal reproduction. Selection combined with the mixed mode of reproduction proves a more probable explanation of the increased frequency of isolates able to cause disease on cultivars with *sylvestris*-derived resistance than the introduction of these isolates via transmission on seed. The spread of *L. maculans* via infected seed is extremely rare because the majority of canola seed sold commercially in Australia is now treated with a fungicidal seed dressing. Barrins (4) did not find any seed infected with *L. maculans* after testing approximately 28,000 seed untreated with fungicide from 14 different grain depots from across Victoria and Western Australia in 2001.

The frequency of isolates able to infect cultivars with *sylvestris*-derived resistance and subsequent levels of disease recorded in the survey of commercial crops differed between states. The frequency of highly virulent isolates increased in both Victoria and South Australia from 2002 to 2003, but did not change in New South Wales. In contrast, the level of disease observed in field surveys was much greater in South Australia than in either Victoria or New South Wales. One possible explanation for the high frequency of virulent isolates collected from Victoria is that the stubble was collected from trial sites where canola lines with *sylvestris*-derived resistance had been grown in close proximity for a number of years. Hence, the selection on the population at these sites may not reflect the larger *L. maculans* population in Victoria, where low disease levels were recorded in field surveys in 2003 and 2004. Another explanation for the high disease levels in South Australia may be that farmers adopted the cultivars with *sylvestris*-derived resistance immediately following their release, much more quickly than they were adopted by farmers in other states. The host specificity in the stem canker tests of isolates collected in 2004 and the high disease levels recorded in the cultivar with *sylvestris*-derived resistance compared with the polygenic cultivar at many South Australian sites in 2004 is consistent with extremely strong selection pressure placed on the *L. maculans* population due to the wide-scale use of cultivars with *sylvestris*-derived resistance.

Fig. 4. Severity of blackleg in the stem at plant maturity caused by 33 individual isolates collected from the stubble of a cultivar with polygenic (●) or *Brassica rapa* subsp. *sylvestris*-derived resistance (○) and inoculated onto different cultivars. Each isolate was inoculated onto cv. Surpass 400; **A**, cv. Q2, the highly susceptible control; and **B**, the moderately resistant polygenic cv. Beacon.

L. maculans has a number of characteristics that are indicative of a pathogen with a high risk of overcoming resistance genes. McDonald and Linde (23,24) proposed that a high evolutionary potential of a pathogen is associated with a mixed reproduction system, a high potential for genotype flow (i.e., long-distance dispersal), and a large population size. These, in combination with the widespread use of a major gene for resistance, would create a situation in which the pathogen was highly likely to overcome the resistance. *L. maculans* has a large population size with airborne ascospores and reproduces both sexually on stubble and asexually within the host crop. This high evolutionary potential is supported by the findings that Australian populations of *L. maculans* have high levels of gene and genotypic diversity (H. L. Hayden and B. J. Howlett, unpublished). Another example of a fungal pathogen with high evolutionary potential overcoming host resistance is *Mycosphaerella graminicola* (anamorph *Septoria tritici*), a foliar pathogen of wheat. Within 5 years of the release in Oregon of a cultivar with high resistance (cv. Gene), isolates were identified that could specifically overcome this resistance (7). Despite the reduction in commercial production of cv. Gene, these isolates have persisted in the population. *M. graminicola* has a high degree of gene and genotypic diversity and a lifecycle very similar to that of *L. maculans*, with sexual recombination occurring on infected debris, airborne dispersal of sexual spores, and asexual multiplication within the crop (6).

Host plant resistance, in combination with cultural practices, has been the strategy for controlling *L. maculans* in Australia. However, the dramatic breakdown of major gene resistance presented here highlights the need for strategies to deploy resistance sources so that they are effective for as long as possible. McDonald and Linde (23) suggest that, in situations where the probability of resistance breakdown is high, breeders should focus on the use of quantitative (polygenic) resistance, renewed regularly. Although this has been a relatively successful strategy for the Australian canola industry to date, cultivars with polygenic resistance can still incur significant yield loss in high disease situations and erosion of resistance over time.

It is assumed that the useful life of a major gene for resistance ends once it has been overcome by a pathogen. However, Rouxel et al. (29) found the avirulence gene, *AvrLm4*, in over 30% of French isolates after it previously was thought to be very rare or absent in France. If this increase coincided with the withdrawal of cultivars harboring the corresponding resistance gene *Rlm4*, this finding suggests that a fitness deficit may be associated with the *avrLm4* allele. The increase in *AvrLm4*-bearing isolates raises the prospect

that resistance genes that previously had been overcome could be redeployed in the future. The current situation on the Lower Eyre Peninsula in which cultivars with *sylvestris*-derived resistance are no longer being grown and the high frequency of isolates with the ability to overcome the *sylvestris*-derived resistance provides an excellent long-term opportunity to understand the evolutionary forces involved in shaping the *L. maculans* populations in Australia.

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