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The Identity of Barley Yellow Dwarf Virus Isolates in Cereals and Grasses from Mainland Australia

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Abstract

Four serologically distinct types of barley yellow dwarf virus (BYDV) were identified infecting cereals and grasses collected around mainland Australia. On the basis of serological relatedness to North American type-isolates, the Australian isolates were classified as MAV-, PAV-, RMV- and RPV-like. However, aphid transmission studies showed that, while the Australian PAV-, RPV-, and RMV-like isolates had conventional vector relationships, the Australian MAV-like isolates had *Rhopalosiphum padi* as a major vector species. Differences in the *Sitobion* species occurring in North America and Australia made comparisons of the transmissibility of MAV- and PAV-like isolates by these vectors impractical. Isolates serologically like PAV were by far the most common viruses in each State surveyed, with the next most common varying from State to State. The identifications of RMV- and MAV-like isolates are the first confirmed records of these types of BYDV in Australia.

Introduction

Barley yellow dwarf (BYD) was first recognized as a virus disease of cereals and grasses by Oswald and Houston (1953) in California, U.S.A. In Australia, first reports of its occurrence were based on symptoms seen in wheat and oats growing in New South Wales (Smith 1957) and on aphid transmission tests from wheat to oat indicator plants (Butler *et al.* 1960).

More recent studies have shown that the causal viruses, collectively referred to as barley yellow dwarf virus (BYDV), comprise a group of variously related luteoviruses (Shepherd *et al.* 1976). In North America, this group has been subdivided into five virus types, primarily on the basis of their principal aphid vectors (Rochow 1969). The five types are exemplified by five U.S. isolates designated by the initial letters of their principal aphid vector or vectors, namely: RPV, transmitted principally by *Rhopalosiphum padi*; RMV by *R. maidis*; MAV by *Macrosiphum* (= *Sitobion*) *avenae*; SGV by *Schizaphis graminum*; and PAV by both *R. padi* and *M. avenae*. Each of these isolates probably represents a group that includes a range of variants that differ from each other with respect to some feature. The subdivision by aphid vector specificity is also supported by other properties such as serological specificity, ultrastructural and cytopathological effects, electrophoretic mobility of dsRNAs, and specificity of cDNA clones (Aapola and Rochow 1971; Gill and Chong 1979; Lister and Rochow, 1979; Gildow *et al.* 1983; Hsu *et al.* 1984; Barbara *et al.* 1987). Apart from the vectors listed, at least another 19 aphid species are known to transmit one or more BYDV isolates (A'Brook 1981).

In only two previous studies have antisera specific to the U.S. isolates, together with aphid transmission tests, been used to identify BYDV types in Australia in relation to those found in North America. Waterhouse and Helms (1985) tested a small number of cereals and grasses from two sites in New South Wales and found only PAV-like isolates, while Guy *et al.* (1986, 1987) extensively sampled pasture and other grasses in Tasmania and found predominantly PAV-like isolates, with some mixed infections containing both PAV- and RPV-like isolates.

We describe here the identity of BYDV isolates from cereals and grasses collected throughout

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mainland Australia during 1985 and 1986, as determined serologically by enzyme-linked immunosorbent assay (ELISA) using antisera to U.S. isolates, and biologically by transmission with a range of aphid species.

Materials and Methods

Cereal and Grass Collections

Single cereal plants with or without symptoms resembling those of BYDV, and native and introduced grasses, were collected from a range of sites around mainland Australia and returned in polythene bags to the laboratory, where they were identified, fumigated to kill aphids, and grown on in the greenhouse. Samples from Western Australia (collected by T. N. Khan), were air dried at room temperature prior to being sent to Victoria, a method shown by Lister *et al.* (1985) as suitable for subsequent testing for BYDV by ELISA.

The cereals included wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and oats (*Avena sativa* L.), and the grasses included wild oats (*Avena fatua* L.), brown top (*Agrostis tenuis* sibth.), soft brome (*Bromus molliformis* Lloyd), cocksfoot (*Dactylis glomerata* L.), a wallaby grass (*Danthonia* sp.), fescue (*Festuca rubra* L.), barley grass (*Hordeum leporinum* Link), Italian ryegrass (*L. multiflorum* Lam), perennial rygrass (*Lolium perenne* L.), paspalum (*Paspalum dilatatum* Poir.), canary grass (*Phalaris* sp.), and winter grass (*Poa pratensis* L.).

Transmission Tests

Four cereal aphid species were used in attempts to transmit BYD viruses from field samples of cereals and grasses collected in eastern Australia to oats (*A. sativa* cv. Algeribee), and from glasshouse-grown inoculated oats to oats. They were the oat aphid (*Rhophalosiphum padi* L.), the corn aphid (*R. maidis* Fitch), the rose-grain aphid (*Metopolophium dirhodum* Walk.), and the English grain aphid (*Sitobion miscanthi* Takahashi).

BYDV-free cultures of these aphids were reared on barley (*H. vulgare* cv. Lara) seedlings grown on nutrient-soaked cotton wool in closed polystyrene cups (Ridland *et al.* 1988). First instar apterae from these cultures were placed on test leaf material (i.e. field samples, known positives, or healthy controls) and allowed an acquisition feed for 48 h at 18°C. They were then transferred, 2 or 3 aphids per indicator plant, to oat indicator seedlings in polystyrene cups and held for a further 96 h at 18°C for the transmission feed. The aphids were then removed with a fine artist's brush and the seedlings were potted into soil and maintained in an insect-screened greenhouse for observation of symptoms after four and eight weeks. Leaf samples collected at four and eight weeks after aphid infestation, were tested by ELISA to confirm the serological identity of the isolate or isolates transmitted. Initially, at least five replicate transmissions were attempted from each field sample with each aphid species. In subsequent tests on representative isolates transmitted from field samples to oats, 20 replicate transmissions were attempted for each isolate with each aphid species.

Serological Tests

Polyclonal or monoclonal antisera to four well-characterized isolates from North America, namely, MAV, RPV and RMV (Rochow 1970) and a PAV-like isolate, IL-PAV (D'Arcy and Hewings 1986) were used for all serological tests. Positive control antigen sources consisted of extracts of air-dried leaves containing subcultures of a PAV-like isolate (P-PAV, Hammond *et al.* 1983), MAV and RPV (Rochow 1970) maintained at Purdue University, and of fresh leaves containing an RMV-like isolate from Queensland, transmitted only by *R. maidis* and serologically related to Rochow's RMV (Greber and Sward, unpublished).

The polyclonal MAV immunoglobulin used reacted heterologously with PAV-like isolates in ELISA (Fargette *et al.* 1982), though less so than homologously. The immunoglubin from monoclonal antiserum MAV-1 (Hsu *et al.* 1984) was reported to react specifically with MAV and not at all with PAV, so this was used where polyclonal MAV reactions suggested the presence of MAV-like isolates. ELISA was done in Immulon M-129 B microtiter plates (Dynatech Inc.), with 200 μl samples in each well, by standard double antibody sandwich (DAS) or indirect (ID) procedures (Clark *et al.* 1986). Immunoglobulins (Ig's) were prepared by ammonium sulfate precipitation from the antisera. Polyclonal (rabbit) antisera were cross-absorbed with preparations from healthy oats to remove activity to normal host proteins, either prior to Ig preparation, or during ELISA, by diluting conjugate in healthy plant extracts (Lister and Rochow 1979). Stock Ig preparations were stored at 1 mg/ml (A_{280} = 1·4), and used at a dilution of 1/1000. Coating Ig's were diluted in sodium carbonate coating buffer, pH 9·6; conjugates were diluted in phosphate-

buffered saline containing 0·05% Tween 20 and 0·02% ovalbumin (conjugate buffer), or in sap extracts as above; second (monoclonal) antibodies were diluted in conjugate buffer.

Conjugates were made by labelling the stock Ig's with alkaline phosphatase by a one-step treatment with 0·06% glutaraldehyde for 4 h at room temperature (Clark *et al.* 1986). The substrate was *p*-nitrophenyl phosphate at 1 mg/ml in diethanolamine/HCl buffer at pH 9·8. Reactions were stopped by adding 50 µl of 3 M NaOH. Some sample extracts were prepared from fresh leaf by squeezing them in a roller press (Erich Pollahne, West Germany), and diluting the expressed sap with 0·1 M phosphate buffer, pH 7·0, but most were made by grinding fresh or dry leaf in liquid nitrogen, then re-grinding in 0·1 M phosphate buffer, pH 7·0, at 1:10, w/v (Lister *et al.* 1985). Timing of the steps in ELISA varied, but for convenience was usually as follows:

(*a*) DAS-coating, 2–3 h (37°C); sample application, 4–6 h (37°C); conjugate, overnight (4°C); substrate 30–60 min (20°C).

(*b*) ID-coating, 2–3 h (37°C); sample application, 4–6 h (37°C); second (monoclonal) antibody, overnight (4°C); rabbit anti-mouse conjugate (Sigma), 2 h 37°C); substrate 30–60 min (20°C).

Test results (Absorbance at 405 nm) were read in a Dynatech MicroELISA minireader. Duplicate wells were used for each sample. Mean absorbances equal to, or exceeding twice the 'background' values for extracts from non-infected control plants or from check plants infected with hetrologous isolates, were regarded as positive.

Table 1. ELISA estimates of BYDV infections in cereal and grass samples collected in Victoria during 1985

Sample source and type	Number of plants tested	Number of plants infected	Number of plants reacting with Igs indicated			
			PAV	MAV	RPV	RMV
	Balliang area — W. Central, Sept. 1985; 4 sites					
Oats	18	18	12	4	3	0
Barley	15	8	5	3	0	1
Grasses	9	2	1	0	1	0
Subtotals	42	28	18	7	4	1
	Balliang area — W. Central, Oct. 1985; 3 sites					
Oats	40	25	23	3	1	0
Barley	5	0	0	0	0	0
Grasses	11	2	2	0	1	0
Subtotals	56	27	25	3	2	0
	Ballarat area — W. Central, Sept. 1985; 5 sites					
Wheat	19	2	1	0	1	0
Oats	24	21	14	2	10	0
Barley	1	0	0	0	0	0
Grasses	26	7	5	0	2	1
Subtotals	70	30	20	2	13	1
	Leongatha, S. Central, Oct. 1985; 1 site					
Grasses	10	1	1	0	0	0
	Burnley, S. Central, Dec. 1985; 1 site					
Wheat	7	7	7	0	0	0
	Horsham area — Wimmera: Aug. 1985; 1 site					
Wheat	15	15	14	0	2	0
Totals	200	108	85	12	21	2

Results

Table 1 summarizes ELISA test results of cereal and grass samples collected from 15 sites within Victoria. Of 200 samples tested, 108 (54%) tested positive for BYDV. PAV-like types predominated (present in 79% of the infected plants), followed by RPV-like (19%), MAV-like (11%), and RMV-like (2%). Ten plants (9%) reacted positively to antisera of more than one BYDV type. Seven oat plants, one ryegrass, and one wheat plant indexed positive for both

PAV-and RPV-like types, and one oat plant indexed positive for PAV-, RPV- and MAV-like types.

Table 2 summarizes some typical DAS-ELISA test results obtained from a subsample of

Table 2. Double antibody sandwich ELISA results for four barley yellow dwarf serotypes in cereals and grasses collected at Balliang, Victoria

Sample	ELISA value (A405) with indicated polyclonal Igs[A]			
	IL-PAV	MAV	RPV	RMV
1c/1 oats	1·04	0·20	0·01	0·00
1g/1 ryegrass	0·33	0·09	0·96	0·08
1h/1 oats	0·26	0·12	0·05	0·09
1i/1 barley	1·71	0·42	0·02	0·04
1i/2 barley	1·82	0·44	0·02	0·02
1j/1 oats	0·55	0·12	0·03	0·00
1k/1 barley	1·36	0·38	0·07	0·00
1l/1 oats	0·10	0·11[b]	0·19	0·01
2a/1 oats	0·16	0·72[b]	0·02	0·00
3a/1 barley	1·24	0·26	0·02	0·02
3a/2 barley	0·13	0·44[b]	0·02	0·03
3a/3 barley	0·03	0·19[b]	0·03	0·03
3b/1 oats	0·19	0·06	0·08	0·03
3d/2 barley	0·03	0·03	0·03	0·20
4a/1 oats	0·12	0·55[b]	0·02	0·00
4a/2 oats	0·13	0·35[b]	0·04	0·02
4a/3 oats	1·81	0·43	0·00	0·00
4a/4 oats	1·61	0·29	0·00	0·00
4a/5 oats	2·00	0·41	0·01	0·00
4a/6 oats	1·20	0·25	0·01	0·01
4a/7 oats	1·78	0·29	0·01	0·00
4a/8 oats	>2	0·49	0·28	0·01
4a/9 oats	0·82	0·05	0·00	0·01
4a/10 oats	0·13	0·57[B]	0·02	0·01
4a/11 oats	1·38	0·19	0·00	0·00
4b/1 barley	0·38	0·16	0·01	0·02
4b/2 barley	0·10	0·44[B]	0·01	0·01
PAV check	1·12	0·11[B]	0·00	0·00
MAV check	0·14	1·40[B]	0·02	0·07
RPV check	0·02	0·02	0·65	0·01
RMV check	0·01	0·02	0·01	0·31
Healthy control	0·02	0·02	0·01	0·01

[A]Homologous polyclonal coating and conjugate Ig's were used throughout.
[B]Samples for which reactions with MAV.Ig exceeded those with IL-PAV.Ig.

the September 1985 collection from Balliang in southern Victoria. These illustrate how DAS-ELISA values from field samples were interpreted as indicating the serological relationships of the viruses present. For example, results for the oat coded 2a/1 and the barley coded 4b/2 indicated the presence of MAV- and PAV-like types, while the results for the barley coded 3d/2 indicated the presence of an RMV-like type. An indirect ELISA using the MAV-1 monoclonal antibody was required to confirm the presence of the MAV serotype (Lister and Sward 1987).

Table 3 summarizes some results of ELISA tests for oat indicator plants following test transmissions of virus isolates from field samples by vectors of BYDV. Sample 2a/1 was identified as MAV-like on the basis of serology, but, unexpectedly, was transmitted only by *R. padi*. Sample 3d/2 was identified serologically as RMV-like and was transmitted by *R. maidis*. Sample 4b/2 was identified as containing a mixture of MAV- and PAV-like types, from which the MAV-like component was transmitted by *R. padi* and *S. miscanthi* but not by *M. dirhodum*, and the

PAV-like component was transmitted by *M. dirhodum* and *S. miscanthi* but not by *R. padi* in this series of tests.

Table 3. ELISA of oat plants following aphid transmission tests of barley yellow dwarf virus isolates from cereals and grasses collected at Balliang, Victoria

Sample	Aphid species used for transmission	ELISA values (A405) with Igs indicated) IL-PAV	MAV-1	NY-RMV
2a/1 oats	*R. padi*	0·05	0·05	—[A]
2a/1 oats	*R. padi*	0·05	0·38	—
2a/1 oats	*R. padi*	0·06	0·37	—
2a/1 oats	*R. padi*	0·08	0·23	—
2a/1 oats	*R. padi*	0·05	0·31	—
2a/1 oats	*S. miscanthi*	0·05	0·04	—
2a/1 oats	*S. miscanthi*	0·02	0·03	—
2a/1 oats	*S. miscanthi*	0·02	0·03	—
2a/1 oats	*S. miscanthi*	0·02	0·02	—
2a/1 oats	*S. miscanthi*	0·02	0·03	—
3d/2 barley	*R. maidis*	0·00	0·00	0·44
3d/2 barley	*R. maidis*	0·02	0·00	0·40
3d/2 barley	*R. maidis*	0·01	0·00	0·04
3d/2 barley	*R. maidis*	0·00	0·00	0·03
3d/2 barley	*R. maidis*	0·01	0·00	0·31
4b/2 barley	*R. padi*	0·06	0·33	—
4b/2 barley	*R. padi*	0·04	0·16	—
4b/2 barley	*R. padi*	0·05	0·34	—
4b/2 barley	*R. padi*	0·01	0·00	—
4b/2 barley	*R. padi*	0·02	0·00	—
4b/2 barley	*M. dirhodum*	1·17	0·00	—
4b/2 barley	*M. dirhodum*	0·06	0·00	—
4b/2 barley	*M. dirhodum*	0·01	0·01	—
4b/2 barley	*M. dirhodum*	1·56	0·00	—
4b/2 barley	*M. dirhodum*	0·01	0·00	—
4b/2 barley	*S. miscanthi*	0·71	0·00	—
4b/2 barley	*S. miscanthi*	0·51	0·13	—
4b/2 barley	*S. miscanthi*	0·01	0·39	—
4b/2 barley	*S. miscanthi*	0·01	0·00	—
4b/2 barley	*S. miscanthi*	0·01	0·00	—
PAV check		1·00	0·00	0·01
MAV check		0·09	0·52	—
RMV check		—	—	0·60
Healthy control		0·00	0·01	0·00

[A]Not specifically tested with Ig indicated.

The transmission of four specific BYDV isolates representing the four main serological types identified in Victoria was tested with the four cereal aphid species (Table 4). *R. padi* was an efficient vector of all except the RMV-like isolate, whilst *R. maidis* efficiently transmitted only the RMV-like isolate. *S. miscanthi* and *M. dirhodum* transmitted only the MAV- and PAV-like isolates, *S. miscanthi* inefficiently and *M. dirhodum* with moderate efficiency.

Table 5 summarizes the ELISA results of cereal and grass samples collected from 31 sites throughout mainland Australia and mailed to us for testing. The grass samples had no specific BYDV-like symptoms and were collected at random, but most cereal samples were from plants showing symptoms suggesting BYDV infection. Of 121 samples, 64 (53%) were positive for BYDV. PAV-like serotypes predominated, occurring in 94% of infected plants; MAV-like types occurred in 8%, RMV-like types occurred in 3%, and RPV-like types in 2%. There were mixtures

of BYDV types in four plants, all grasses. Three of these were infected with MAV- and PAV-like isolates, and one was infected with PAV- and RPV-like isolates.

Table 4. Frequency and percentage transmission of Victorian barley yellow dwarf virus isolates by four cereal aphid species

Serological type	Code and/or geographical source of isolate[A]	Aphid species used for transmission			
		R. padi	*R. maidis*	*S. miscanthi*	*M. dirhodum*
MAV	2a/1, Balliang Sept. 1985	94/117[B] (80)[C]	0/45 (0)	3/57 (5)	27/49 (55)
PAV	Separated from VI[D] Glenormiston, 1982	30/33 (91)	0/14 (0)	2/41 (5)	17/25 (68)
RMV	3d/2, Balliang Sept. 1985	0/29 (0)	32/50 (64)	0/25 (0)	0/11 (0)
RPV	Leongatha, May 1985	94/106 (89)	1/38 (3)	0/20 (0)	0/19 (0)

[A]All isolates were originally obtained from infected oat plants.
[B]The numerator is the number of successful transmission (confirmed by ELISA), the denominator is the number of attempted transmissions.
[C]Percentage transmission.
[D]VI was a mixed infection of RPV- and PAV-like isolates (Sward and Lister 1987).

At least one example of each BYDV serotype was further tested for aphid transmission by the four aphid species. In each case the vector relationships parallelled those found for the four representative Victorian isolates shown in Table 4.

Discussion

This study confirms and extends earlier evidence that BYDV is widespread in cereals and grasses in Victoria, and also provides new information on its occurrence elsewhere in Australia. Fifty-four per cent of the plants sampled in Victoria were infected, but as most were selected because they showed BYDV-like symptoms, this figure is not indicative of the occurrence of BYDV on a statewide basis. Similarly, 53% of the plants sampled throughout the rest of mainland Australia were infected with BYDV, but again, most were selected on the basis of symptoms.

Considering the grass samples alone, 21% of those collected in Victoria indexed positive for BYDV, compared with 86% of those collected in New South Wales. Grasses do not usually show distinct symptoms of BYDV and the plants were sampled at random, and although the sample size is small, these figures can be taken as an estimate of the level of infection in the grasses in the localities concerned, although not necessarily representative of the infection levels statewide. In Tasmania, Guy *et al.* (1986) found that 13% of pasture grasses sampled at random from five sites were infected. However, they suggested that the general level of infection in Tasmanian pasture grasses was probably much higher, as their sampling was biased towards pastures less than two years old. In a later study, Guy *et al.* (1987) sampled annual and perennial grasses from throughout Tasmania and found approximately 9% were infected with BYDV, although a higher incidence of BYDV (up to 31%) occurred in some subfamilies of grasses, notably the Bambusoideae. In New Zealand, Latch (1977) found that more than 50% of perennial ryegrass plants were infected in pastures that were 6 to 30 years old. In Europe, the United Kingdom, USA and Canada, infection levels in grasses varied from less than 3% to 77% depending on various factors, including climate and the predominant grass species (Catherall 1963; Fargette *et al.* 1982; Grafton *et al.* 1982; Paliwal 1982).

As indicated above, the amounts of infection in the cereals examined from Victoria (60%) and other parts of mainland Australia (56%) were overestimated, because of bias in plant

Table 5. ELISA estimates of barley yellow dwarf virus infections in various survey samples from mainland Australia

Sample source and type	Number of plants tested	Number of plants infected	Number of plants reacting with Igs indicated: PAV	MAV	RPV	RMV
W. Australia, Oct. 1985; 7 sites						
Wheat	9	9	9	0	0	0
Oats	4	3	3	0	0	0
Barley	5	4	4	0	0	0
Subtotals	18	16	16	0	0	0
W. Australia, Oct. 1985; 2 sites						
Wheat	4	4	4	0	0	0
Oats	15	15	15	0	0	0
Barley	4	1	1	0	0	0
Subtotals	23	20	20	0	0	0
N.S.W., Nov 1985; 7 sites						
Wheat	12	0	0	0	0	0
Oats	8	1	1	0	0	0
Grasses	16	13	13	2	1	0
Subtotals	36	14	14	2	1	0
S.E. Queensland, Oct. 1984 and Nov. 1985; 7 sites						
Oats	2	2	2	0	0	0
Barley	1	1	1	0	0	0
Grasses	6	6	4	0	0	2
Subtotals	9	9	7	0	0	2
N. Queensland, Dec. 1985; 6 sites						
Oats	5	1	0	1	0	0
Grasses	22	2	1	2	0	0
Subtotals	27	3	1	3	0	0
N.S.W. Aug. 1986; 1 site						
Wheat	1	0	0	0	0	0
Barley	3	0	0	0	0	0
Subtotals	4	0	0	0	0	0
W. Australia, Dec. 1986; 1 site						
Barley	1	1	1	0	0	0
Oats	3	1	1	0	0	0
Subtotals	4	2	2	0	0	0
Total	121	64	60	5	1	2

selection. By comparison, Sward and Lister (1987) found that only 2% of wheat plants sampled at random from 26 sites throughout Victorian wheat districts were infected, although some individual crops had up to 20% infection. However, they considered that the amount of infection would have been considerably greater had the plants been sampled later in the season after the spring cereal aphid flights, as were the samples in the collections considered here.

In the present surveys, PAV-like types were by far the most common among cereal and grass samples from mainland Australia, and indeed, in Western Australia they were the only type identified. Similarly, in recent studies of grasses in Tasmania, Guy *et al.* (1986, 1987) respectively, reported that 98% and 60% of the BYDV isolates obtained were PAV-like. The next most common isolate types in the Victorian samples were RPV (19% of the infections

found), followed by MAV (11%). RMV-like types were least common, occurring in only 2% of infections. Our identifications of MAV- and RMV-like isolates constitute the first confirmed reports of isolates of these types in Australia.

Smith and Plumb (1981) reported that more than 73% of cereal aphids trapped at two sites in Victoria in 1977–78 were *R. padi*. The next most common were *S. miscanthi* and the least common, *R. maidis*. The distribution and relative proportions of BYDV types are largely dictated by the abundance and activity of the vector aphid species. It is not surprising that BYDV types with *R. padi* as the major vector were the predominant types, although it is not clear why PAV-like isolates were more common that the other types with *R. padi* as the major vector. In areas of the U.S.A. where PAV-like types are common, both *Sitobion avenae* and *R. padi* are present as vectors. In Victoria, the local *Sitobion* species does not efficiently transmit the PAV-like isolates; however, other cereal aphid species, such as *Rhopalosiphum rufiabdominalis*, may play an important role in the local epidemiology of particular BYDV types (Ridland and Sward, unpublished).

Plants containing mixtures of BYDV types constituted 9% of the infected samples collected in Victoria. The common combination of RPV- and PAV-like isolates frequently occurred in oats showing severe symptoms. This mixture of BYDV types has been identified previously in Victoria (Sward and Lister 1987) and has been separated into its component BYDV types by serial aphid transfers, thus confirming that it is a mixture and not an undescribed serotype (Waterhouse *et al.* 1986). Guy *et al.* (1987) also found RPV- and PAV-like types occurring together in a range of grass subfamilies, and concluded that they must frequently be cotransmitted by the aphid vector, as mixed infections occurred far more often than would have been expected if they had infected plants independently. Mixtures of MAV- and PAV-like isolates were also common, and it is probable that at least some of these occurred following cotransmission by infective *R. padi* or by *M. dirhodum*.

A range of BYDV isolates collected over 20 years from New York State and studied at Cornell were found to have serological affinities consistent with the vector specificities defined for the five characterized BYDV types from North America (Rochow 1979). The aphid transmission studies reported here have provided further important information on the nature of the Australian BYDV isolates and highlight a number of important differences from the North American types. The RPV- and RMV-like isolates appeared to have vector relationships similar to those of the type isolates from North America (Rochow 1970); the RMV-like isolates were transmitted specifically by *R. maidis* and the RPV-like isolates by *R. padi*. However, in one experiment a single transmission of an RPV-like isolate by *R. maidis* was obtained, whereas Rochow (1969) obtained no transmission of his RPV by *R. maidis* in 37 serial transfers carried out over six years. This could reflect a subtle difference between Rochow's RPV and the Australian RPV-like isolates. Conversely, we obtained no transmission of an Australian RMV-like isolate with any of the other three aphid species used, whereas Rochow (1969) reported 11% transmission of his RMV with *R. padi*.

It was not possible to test the transmission of the Australian isolates identified serologically as PAV-like or MAV-like by *Sitobion avenae*, which does not occur in Australia. We were, however, able to test the related species *S. miscanthi* as a vector, and this transmitted Australian MAV- and PAV-like isolates only at the low frequency of 5%. In this regard, the recent identification of the Tasmanian *Sitobion* species as *S. fragariae* (Guy *et al.* 1986) now raises questions regarding the occurrence of both *S. miscanthi* or *S. fragariae* throughout Australia, and their relative efficiency as vectors. The Australian MAV-isolates differed significantly from Rochow's MAV in that they were transmitted by *R. padi* (Lister and Sward 1987), and in this respect resembled Rochow's PAV. Australian MAV-and PAV-like isolates were also transmitted by *M. dirhodum* (a species recently arrived in Australia (Carver 1984)), and it will be of interest to monitor their distribution in relation to the spread of this aphid.

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Survival and Fecundity of Wild Radish (*Raphanus raphanistrum* L.) Plants in Relation to Cropping, Time of Emergence and Chemical Control

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Abstract

During consecutive seasons, wild radish (*Raphanus raphanistrum* L.) seedling emergence decreased exponentially with increasing time after the emergence of lupin crops. Initial survival of seedlings was markedly reduced by pre-emergence applications of simazine at 0·75 kg a.i. ha^{-1}. In the absence of herbicide, however, the presence of a lupin crop did not have a negative effect upon early survival. Probabilities of reproduction of wild radish plants decreased with later emergence within treatments; no plants which emerged later than 21 days after crop emergence produced seeds.

Seed production by wild radish was considerably higher when lupins were sown late. Regardless of sowing date, the application of triazine herbicides reduced the amount of seeds produced to the point where grain contamination was insignificant. However, the few plants which escaped herbicide treatment produced large numbers of seeds. Virtually no seeds were produced when additional post-emergence applications of simazine (0·375 kg a.i. ha^{-1}) were made. It is argued that the major role of post-emergence application in this crop-weed system is to prevent reproduction by plants which escape the pre-emergence application, rather than to control late-emerging plants.

Introduction

In addition to causing yield reductions in narrow-leafed lupins (*Lupinus angustifolius* L.) and interference with crop harvesting, wild radish (*Raphanus raphanistrum* L.) seed and pod material are often contaminants of harvested lupin grain in Western Australia. During 1986, Western Australian grain handling authorities imposed dockages ranging from $5 per tonne for lupin grain with 2–4% foreign matter to $20 per tonne with 8–10% contamination. Seed lots with more than 8% wild radish contamination were not accepted (R. Broom, personal communication). Such stringent requirements for seed purity reduce the economic threshold for control of wild radish below that pertaining to grain yield. This problem is similar to instances where weeds such as *Avena fatua* L. and *Galium aparine* L. occur in seed crops (Cussans 1980).

Since patterns of wild radish emergence are often asynchronous (Piggin *et al.* 1978; Reeves *et al.* 1981; Amor 1985; Cheam 1986), strategies for control depend on the relative importance of yield response and seed contamination. Piggin *et al.* (1978) found that early spraying of wild radish in wheat (4–6 weeks after emergence) gave the best yield responses, but resulted in some contamination due to late-emerging wild radish plants. Late spraying (post-tillering) resulted in lower yield responses but usually no contamination.

The demographic approach is widely used in ecological studies (Harper 1977) and has proven a valuable aid for assessing and improving strategies for weed management (Mortimer 1983). Because few demographic data are available for populations of wild radish, the aims of the work reported here were to determine the effects of time of emergence and herbicide application upon the survival and seed production of wild radish plants in lupin crops.

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