Viruses Detected in Forage Legumes in Idaho

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ABSTRACT

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A limited survey was conducted to identify viruses infecting white clover (*Trifolium repens*) and other forage legumes in Idaho. Of 93 samples collected in 11 counties, 48 tested positive by ELISA for one or more viruses, including alfalfa mosaic, clover yellow mosaic, clover yellow vein, red clover vein mosaic, and white clover mosaic.

Productive seed fields of white clover (*Trifolium repens* L.) flourished in central west Valley County and foremost northern Boundary County, Idaho, during the 1940s to the 1960s. Yields gradually declined thereafter, and by 1965 little seed was produced. A concurrent decline in seed and forage production of red clover (*T. pratense* L.) in Idaho was reported by Watson and Guthrie (45) to be due primarily to infection by clover yellow mosaic virus (CYMV) and white clover mosaic virus (WCMV), followed by *Fusarium* species.

The low white clover seed yields prompted a breeding program to select clones that were more prolific seed producers. Evaluation of cultivars for seed productivity showed variation between and among cultivars. A breeding program using recurrent selection resulted in clonal progenies that produced 24–170% more seed than the Idaho common white clovers. Ten of these clones were combined into the synthetic cultivar Star (13). During the latter stages of the breeding program, clones in the breeder's seed increase plots became infected with unknown viruses and seed yields declined materially. The viruses were identified as alfalfa mosaic virus (AMV) and clover yellow vein virus (CYVV), and all mother clones of Star were subsequently freed of both viruses by meristem-tip culture as evaluated by enzyme-linked immunosorbent assay (ELISA) (*unpublished*). White clover production and persistence may be seriously reduced by CYVV infection (5,11,15).

The effects of virus diseases on white clover production and breeding efforts in Idaho, the occurrence of CYVV, and the fact that the virus diseases of white clover in Idaho had not been examined in over 20 yr prompted us to conduct a limited survey of viruses infecting white clover during 1982–1983. This paper presents results of that survey, reviews

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the viruses of white clover and other forage legumes in Idaho, and discusses these findings in relation to present knowledge of forage legume virus nomenclature and distribution in North America.

MATERIALS AND METHODS

Sample collection. White clover samples, consisting of fresh leaves, petioles, and stolons, were collected in 11 counties of western and northern Idaho during May–July 1982 and 1983 (Table 1). Samples were arbitrarily collected from pastures, hayfields, research plots, and lawns and along roadsides. Each sample of approximately 35 g of fresh tissue was collected in a 178 × 203 mm polyethylene bag by turning the labeled bag inside out over the hand and gathering leaves, petioles, and stems into the bag. A few samples were also collected from red clover, alsike clover (*T. hybridum* L.), yellow sweet clover (*Melilotus officinalis* (L.) Lam.), alfalfa (*Medicago sativa* L.), and black medic (*M. lupulina* L.). Immediately after collection, the samples were packed in ice, brought to the laboratory in Moscow, Idaho, and stored at −20 C. Samples were subsequently freeze-dried 18–24 hr, sealed in plastic bags, and shipped by express mail to Mississippi

Table 1. Viruses identified from forage legumes collected in Idaho during 1982 and 1983

Species[a]	Collection site	County	Viruses identified[b]				
			AMV	CYMV	CYVV	RCVMV	WCMV
Trifolium repens	Old pasture, E. Eagle	Ada	−	+	−	−	+
	Golf course, Eagle	Ada	−	+	−	−	+
	Desmet	Benewah	−	+	−	−	−
	Sandpoint Beach	Bonner	−	+	−	−	+
	Bonners Ferry	Boundary	−	+	+	−	+
	Old pasture, Middleton	Canyon	+	−	−	−	+
	Old turf, Parma Station	Canyon	−	+	+	−	+
	Old pasture, Meridian	Canyon	−	−	−	−	+
	Caldwell	Canyon	−	+	+	−	+
	Old pasture, Meridian	Canyon	−	−	−	−	+
	Golf course, Grangeville	Idaho	−	+	+	−	+
	Dalton Gardens	Kootenai	−	−	+	−	+
	Dalton Gardens	Kootenai	−	−	+	+	+
	Blackwell Hill	Kootenai	−	−	+	−	−
	Heritage Place	Kootenai	−	−	−	−	+
	Old pasture, E. Moscow	Latah	−	+	+	−	+
	UI campus, Moscow	Latah	−	+	+	−	+
	UI Plant Sci. Farm, Moscow	Latah	+	+	−	−	−
	UI Plant Sci. Farm, Moscow	Latah	−	−	−	+	−
	UI Plant Sci. Farm, Moscow	Latah	+	−	+	−	−
	UI Plant Sci. Farm, Moscow	Latah	+	−	+	−	−
	UI Plant Sci. Farm, Moscow	Latah	+	−	+	−	−
	Fair Grounds	Latah	−	−	−	−	+
	Ferdinand	Lewis	−	−	+	−	+
	Winchester	Lewis	−	+	+	−	+
	Culdesac	Nez Perce	−	−	−	−	+
	Old pasture, McCall	Valley	−	−	−	−	+
	West McCall	Valley	−	+	−	−	+
	Pasture, NE McCall	Valley	−	+	+	−	+
	North Donnelly	Valley	−	−	+	−	+
(30/61)			(5)	(14)	(16)	(2)	(23)
T. hybridum	Roadside	Boundary	−	−	+	−	−
	Old turf, Parma Station	Canyon	−	−	+	−	+
	Caldwell	Canyon	−	+	−	−	+
	UI Plant Sci. Farm, Moscow	Latah	−	−	+	−	−
	UI Plant Sci. Farm, Moscow	Latah	−	−	+	−	+
	UI Plant Sci. Farm, Moscow	Latah	−	−	−	−	+
(6/10)			(0)	(1)	(4)	(0)	(4)
Mixed *T. repens* and *hybridum*	N. Bonners Ferry	Bonner	−	−	+	−	−
	Old pasture	Canyon	−	+	+	−	+
(2/2)			(0)	(1)	(2)	(0)	(1)
T. pratense	Old pasture, E. Eagle	Ada	−	+	−	−	−
	Clarksfork	Bonner	−	−	+	−	−
	Clarksfork	Bonner	−	−	+	−	−
	UI Plant Sci. Farm, Moscow	Latah	−	+	−	−	−
	Park, Winchester	Lewis	−	−	−	−	+
	Pasture, Donnelly	Valley	−	−	−	−	+
(6/11)			(0)	(2)	(2)	(0)	(2)
Medicago sativa	N. Eagle	Ada	+	−	−	−	−
	Wilford Hwy.	Fremont	+	−	−	−	−
(2/3)			(2)	(0)	(0)	(0)	(0)
M. lupulina	Nez Perce	Lewis	−	−	+	−	−
(1/3)			(0)	(0)	(1)	(0)	(0)
Melilotus officinalis	N. Bonners Ferry	Bonner	−	−	+	−	−
(1/3)			(0)	(0)	(1)	(0)	(0)
All species (48/93)			(7)	(18)	(26)	(2)	(30)

[a]Only positive samples listed (number of positive samples/number of samples collected and tested).
[b]Based on positive ELISA results; number of positive samples for each virus is shown in parentheses for each species and for combined species. AMV = alfalfa mosaic virus, CYMV = clover yellow mosaic virus, CYVV = clover yellow vein virus, RCVMV = red clover vein mosaic virus, WCMV = white clover mosaic virus.

State, Mississippi, where they were stored at −20 C pending virus testing.

Virus testing. Samples were removed from storage and triturated in 0.02 M sodium phosphate buffer, pH 7.3, containing 0.05 % (v/v) Tween 20 (PBT) and 0.02 M 2-mercaptoethanol with mortar and pestle. Homogenates were tested for the presence of viruses by double-antibody sandwich ELISA as previously described (32). Antisera to AMV, bean yellow mosaic virus (BYMV KY204-1), CYMV, CYVV-Pratt, WCMV, peanut stunt virus (PeSV), and red clover vein mosaic virus (RCVMV) were from laboratory stocks as previously described (32). Antisera to cucumber mosaic virus (CMV) and tobacco streak virus (TSV) were provided by J. W. Demski, University of Georgia, Experiment, and R. W. Fulton, University of Wisconsin, Madison, respectively. All antisera were prepared for and used in ELISA as described previously (31). All ELISA was done with 96-well polystyrene microtiter plates coated with antibody (60 μl per well, 2.5 μg/ml in 0.5 M sodium carbonate buffer, pH 9.6, 1 hr at 5 C). Wells were rinsed three times for 3 min each with PBT between steps. Freshly prepared test samples (approximately 200 μl per well) were incubated overnight at 5 C. Alkaline phophatase conjugates diluted 1:400–800 in PBT (50 μl per well) were incubated 6 hr at 5 C. Substrate solution (100 μl per well) was incubated at room temperature 1 hr. Absorbance measurements were made on a BioTek Model 307 EIA Reader at 405 nm. Positive and negative control samples, consisting of fresh leaf tissue of alsike clover or tobacco (*Nicotiana tabacum* L. 'Burley 21' for PeSV and TSV) were included with all tests. Test wells were rated positive if their absorbance exceeded the mean absorbance plus two standard deviations of negative control values.

RESULTS

The 93 forage legume samples collected and tested for viruses by ELISA comprised 61 white clover, 11 red clover, 10 alsike clover, 2 mixed white and alsike clover, 3 alfalfa, 3 black medic, and 3 sweet clover samples. Results of virus tests were positive for 48 samples. Information on the collection sites and test results for virus-positive samples is presented in Table 1. The relatively large sample size for all the collections precluded expression of the results on a per plant basis, and although mixed infections are likely, they cannot be discerned from these results. Because the limited survey was not intended to be comprehensive for either the species or counties from which samples were collected, information on the virus-negative samples is not presented. Positive tests occurred most frequently for WCMV, CYVV, CYMV, and AMV, in that order. Positive tests for RCVMV occurred with two white clover samples. BYMV, CMV, PeSV, and TSV were not detected in any samples. Multiple virus-positive tests occurred with 21 of 30 virus-positive white clover samples: eight of CYVV + CYMV + WCMV, four of CYMV + WCMV, three of CYVV + WCMV, three of AMV + CYVV, and one each of AMV + WCMV, AMV + CYMV, and CYVV + RCVMV + WCMV. Three multiple positive tests from six positive alsike clover samples were two of CYVV + WCMV and one of CYMV + WCMV. One of two mixed samples containing white and alsike clovers was also positive for three viruses (CYMV + CYVV + WCMV), but the distribution of these viruses between the two species within these samples could not be determined.

DISCUSSION

These results document the occurrence of CYVV in white clover in Idaho. In 1969, in the first report of CYVV in North America (40), Pratt noted the occurrence of CYVV in eastern and western Canada and suggested the possibility that a previously reported BYMV isolate from bean (*Phaseolus vulgaris* L.) in Idaho (44) and one from white clover in California (20) may have been CYVV isolates. Although similar in many properties to BYMV, CYVV was recently proposed as a distinct member of the BYMV subgroup of potyviruses (8). Historically, white clover-infecting isolates of the BYMV/CYVV type were referred to as severe strains of BYMV (44), necrotic strains of BYMV (17,19,20), subgroup-1 strains of BYMV (23), or CYVV (6,9,40,41). Although the nomenclature used in these reports is certainly important, especially for clarity and continuity in the literature, it is relatively less important than the fact that these virus isolates were recognized as distinct from typical BYMV. In Idaho and other northwestern states, severe isolates of BYMV have been recognized and distinguished from typical BYMV strains for over 50 yr (14,18,19,21,26, 27,38,48).

The perennial nature of white clover and other forage legumes in Idaho makes them likely reservoirs for viruses that may infect other crop species. Forage legumes have been demonstrated to play a significant role in the epidemiology of virus diseases of cultivated annual legumes such as bean (17,18). Although legume virus workers in the Pacific Northwest have been aware that CYVV occurred generally in white clover in that area, the present work documents the occurrence of CYVV in white clover in Idaho. In the northeastern United States, CYVV, formerly referred to as a severe strain of BYMV, causes devastating diseases of bean and pea (*Pisum sativum* L.), and recent genetic studies of pea indicate that resistance to BYMV and CYVV may be conferred by two distinct, but closely linked, genes (41). Resistance in bean to the typical and severe strains of BYMV as they occur in western Oregon has also been shown to be independently inherited (42). Differing levels of infection with BYMV and CYVV within alsike clover germ plasm lines (which suggests differences in genetics of resistance) have also been reported in the northeastern United States (4), and concurrent research detected both viruses in plant introduction trials of several *Trifolium* species (3). In the latter report, the natural host ranges differed in that BYMV predominated in red clover and only CYVV occurred in white clover. A similar distribution has been reported in the southeastern United States (7,29,33). Distinguishing these viruses remains critical to our understanding of the genetics of resistance (4,10,41,43) and epidemiology (17,18) of diseases caused by members of this important potyvirus subgroup.

Our failure to detect PeSV in white clover is of interest, since PeSV is common in white clover in the southeastern states (29,32), where it was identified in 24% of white clover plants in an 11-state bait plant test (33). Although the number of samples in the present study was relatively smaller, experience with PeSV in white clover in the Southeast suggests that if PeSV was common in white clover in Idaho, it would have been detected in this study. To date, PeSV, with the exception of an isolate from bean in Washington (35), has been reported only as far north and west as Iowa (30). On the other hand, CYMV, which was commonly detected in this survey, is widely reported in the Northwest (2,39) but is uncommon east of the Mississippi River (2,7,22,32,33).

A review of earlier studies showed the presence of AMV, CYMV, and WCMV in Idaho. In the mid-1930s, Pierce (37,38) identified AMV in red clover, sweet clover, and alsike clover; BYMV (yellow bean mosaic and pea mosaic strains) in red clover, alsike clover, yellow sweet clover, and white lupine (*Lupinus albus* L.); and WCMV in white clover, red clover, alsike clover, sweet clover, alfalfa, and yellow trefoil (black medic) (*Medicago lupulina* L.). In 1953, AMV and CMV (12,46) were reported in alfalfa from Idaho, and in 1963, Zaumeyer (47) reported AMV to be "common and widespread" in alfalfa in southern Idaho. In the early 1960s, Pratt (39) identified CYMV and WCMV in clover samples (species not identified) from Idaho, Agrawal et al (2) identified WCMV in white clover collected in Idaho, and Guthrie and Slinkard (16) implicated CYMV in reduced seed yields of red clover, noting that red clover stands often contained 50–100% virus-infected plants

after 2 yr of growth. Other reports of viruses in legume crops in Idaho include BYMV and TSV in common pea (14,36,38) and bean (44,49). AMV, BYMV, CYMV, and WCMV have also been identified in forage legumes in other northwestern states and western Canada (1,2,18,20,24,28,34,39,47). More recently, Kaiser et al (25) reported TSV in white sweet clover (*Melilotus alba* Desr.) in eastern Washington.

Although the present study emphasized white clover, viruses were also detected in six other forage legume species (Table 1). The limited sample size, however, precludes extrapolation of these results as representative of these species in Idaho. Absence of BYMV in the survey may be partially due to the preponderance of nonhost samples of white clover, although alsike and red and yellow sweet clovers (24 samples total) are hosts of BYMV (4,33). Results of the present study are believed to be the first to document the possible occurrence of RCVMV in white clover in Idaho. Because the identification of RCVMV is based on ELISA results from only two samples, however, it is reported as a possible identification. In addition, the possible incidence of RCVMV in Idaho may not be accurately represented by the relatively low incidence of RCVMV-positive white clover samples reported here and the fact that it was not detected from three red clover samples tested. A more extensive survey of red clover is needed to fully assess the incidence of BYMV and RCVMV in Idaho. The low number of *Melilotus* samples and the fact that these were yellow sweet clover rather than white sweet clover may have precluded detection of TSV, since Kaiser et al (25) found TSV in white sweet clover but not in yellow sweet clover in eastern Washington. Although only three alfalfa samples were included in the present study, two contained AMV, confirming the occurrence of this virus in alfalfa in Idaho.

The number and geographic distribution of viruses detected among the forage legumes collected, the apparently contrasting occurrences of CYMV and PeSV in white clover in Idaho and the southeastern United States, and the importance of virus diseases in forage legume seed production point to the the need for more extensive research on viruses of forage legumes in Idaho.

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