Viruses Infecting Forage Legumes in Tennessee

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ABSTRACT

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Bean yellow mosaic virus (BYMV), clover yellow vein virus (CYVV), peanut stunt virus (PSV), and red clover vein mosaic virus (RCVMV) were identified by enzyme-linked immunosorbent assay of clover bait plants in Tennessee. Species used as bait plants were alfalfa (*Medicago sativa*), arrowleaf clover (*Trifolium vesiculosum*), alsike clover (*T. hybridum*), crimson clover (*T. incarnatum*), red clover (*T. pratense*), subterranean clover (*T. subterraneum*), and white clover (*T. repens*). Viruses were detected in all clover species but not in alfalfa; however, not all viruses infected all species. White clover was not infected by BYMV nor was red clover by CYVV. The highest incidence of infections was by PSV, followed in order by RCVMV, CYVV, and BYMV.

Tennessee has about 5 million acres of permanent grass pasture, of which about 3 million acres contain lespedeza (*Lespedeza* spp.) and about 1 million acres contain white clover (*Trifolium repens* L.). In 1978, 1.25 million acres of hay crops were harvested and sales of cattle and dairy products were more than $319 and $216 million, respectively (2). Despite the importance of legumes in forage and hay crop production, information on the virus diseases of forage legumes in Tennessee was not available before this study. The identities of viruses commonly infecting forage legumes are well documented in the six states forming Tennessee's northern, eastern, and southern borders.

Alfalfa mosaic virus (AMV) is reported from white clover in North Carolina (3,9), Georgia, and Alabama (3); bean yellow mosaic virus (BYMV) from red clover (*T. pratense* L.) in Kentucky (6) and arrowleaf clover (*T. vesiculosum* L.) in Mississippi (7); clover yellow mosaic virus (CYMV) from white clover in Kentucky (1,6); clover yellow vein virus (CYVV) from white clover in Virginia (3), North Carolina (3,9), Georgia, Alabama, and Mississippi (3), and from arrowleaf clover in Mississippi (7); peanut stunt virus (PSV) from red clover in Kentucky (6), white clover in Virginia (3,17), North Carolina (3,5,9), Georgia, Alabama, and Mississippi (3), and from arrowleaf clover in Mississippi (7); white clover mosaic virus (WCMV) from red clover in Kentucky (6) and white clover in North Carolina (3,9), Georgia, Alabama, and Mississippi (3); and tobacco ringspot virus from red clover in Kentucky (6) and from white clover in North Carolina (9). Cucumber mosaic virus (CMV) was reported from crown vetch in Virginia (14) but in a later study of plants collected from the same plot, Tolin and Miller suggested that the virus described as CMV was probably PSV (18).

In 1968, McCord and Gudauskas (10) described an isolate of BYMV from vetch, *Vicia sativa* L., in Alabama. Their description noted, among other characters, that the vetch virus induced white or tan local lesions with red borders in *Chenopodium amaranticolor* Coste & Reyn.; did not infect *Phaseolus vulgaris* L. 'Great Northern'; did infect white clover; induced more severe symptoms on most hosts than did BYMV; and induced larger and more numerous intranuclear inclusions in broadbean, *V. faba* L., than did BYMV. From their description, I believe that the vetch virus was probably an isolate of closely related CYVV, which was not recognized in North America until 1969 (16).

Of the viruses occurring in bordering states, AMV, BYMV, CYVV, and PSV are transmitted in nature in a nonpersistent manner by several species of aphids; they were, therefore, considered potentially more important and of greater interest in the present study. In this paper, I report the identification and relative incidence of these viruses and of another aphid-transmitted virus, red clover vein mosaic virus (RCVMV), as they occurred in forage legume bait plants from five locations in Tennessee.

MATERIALS AND METHODS

Seed of the bait plants were those distributed for use in Southern Regional Research Project S-127, Forage Legume Viruses. Plants were grown from seed in steam-sterilized soil in 5-cm-diameter clay pots in a greenhouse at Knoxville. Six to 10 seedlings were started per pot, then thinned to one per pot. Plants 6–8 wk old were transplanted 8–18 October 1979 to small field plots at five widely separated locations from Jackson in west Tennessee to Greeneville in east Tennessee, a distance of about 500 km (Fig. 1). Twenty-five plants of each of four perennial species of forage legumes (alfalfa, *Medicago sativa* L. 'Apalachee'; alsike clover, *T. hybridum* 'Common'; red clover cv. Kenland; and white clover cv. Tillman) were arranged randomly with 0.61-m spacing in plots measuring 7.62 × 7.62 m at the West Tennessee Experiment Station, Jackson; the Middle Tennessee Experiment Station, Spring Hill; the Plateau Experiment Station, Crossville; and the Tobacco Experiment Station, Greeneville. Plants of each of the perennial species and three annual species (arrowleaf clover cv. Yuchi; crimson clover, *T. incarnatum* L. 'Dixie'; and subterranean clover, *T. subterraneum* 'Mt. Barker') were arranged with 0.61-m spacing in a randomized complete block design with 11 replications at the University of Tennessee Plant Science Farm, Knoxville.

Winter survival of the bait plants was assessed in March 1980, and dead or missing plants were replaced between 20 April and 6 May with 6- to 8-wk-old greenhouse-grown seedlings. Replacement plants were grown as described except that specific *Rhizobia* inoculants (supplied by J. C. Burton, Nitragin Co., Milwaukee, WI) were incorporated into the soil before seeding. Plants were sampled for virus testing between 20 May and 25 June 1980. Leaf samples were packed on ice for transport to the laboratory in Knoxville.

Viruses were identified by enzyme-linked immunosorbent assay (ELISA) under optimum conditions as described by McLaughlin et al (12). One or 2 days

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Table 1. Distribution among sampling sites of forage legume viruses detected in perennial bait plants in Tennessee

Site	No. of plants	Number of infected plants[a]									
		BYMV	CYVV	PSV	RCVMV	BYMV CYVV	CYVV PSV	CYVV RCVMV	PSV RCVMV	CYVV PSV RCVMV	Total
Greeneville	100	0	5	10	2	1	0	1	0	0	19
Crossville	98	3	3	18	5	0	2	0	2	0	33
Spring Hill	99	2	10	9	1	0	4	0	1	1	28
Jackson	100	0	0	0	13	0	0	0	0	0	13
Total	397	5	18	37	21	1	6	1	3	1	93

[a] Plants were also tested for alfalfa mosaic virus, but none was detected. Viruses detected were bean yellow mosaic virus (BYMV), clover yellow vein virus (CYVV), peanut stunt virus (PSV), and red clover vein mosaic virus (RCVMV). Detection was by enzyme-linked immunosorbent assay. Bait plants were alfalfa, *Medicago sativa*; alsike clover, *Trifolium hybridum*; red clover, *T. pratense*; and white clover, *T. repens*; no infections were detected in alfalfa.

Table 2. Distribution of forage legume viruses among annual and perennial bait plant species at Knoxville, TN

Species	No. of plants	Number of infected plants[a]						
		CYVV	PSV	RCVMV	CYVV PSV	CYVV RCVMV	PSV RCVMV	Total
Alfalfa	11	0	0	0	0	0	0	0
Alsike clover	11	0	0	0	0	0	1	1
Arrowleaf clover	10	0	3	0	2	1	1	7
Crimson clover	7	0	1	2	0	0	0	3
Red clover	11	0	0	1	0	0	0	1
Subterranean clover	11	1	2	4	0	0	1	8
White clover	11	0	4	1	1	0	1	7
Total	72	1	10	8	3	1	4	27

[a] Plants were also tested for alfalfa mosaic virus, bean yellow mosaic virus, clover yellow mosaic virus, and white clover mosaic virus, but none was detected. Viruses detected by enzyme-linked immunosorbent assay were clover yellow vein virus (CYVV), peanut stunt virus (PSV) and red clover vein mosaic virus (RCVMV).

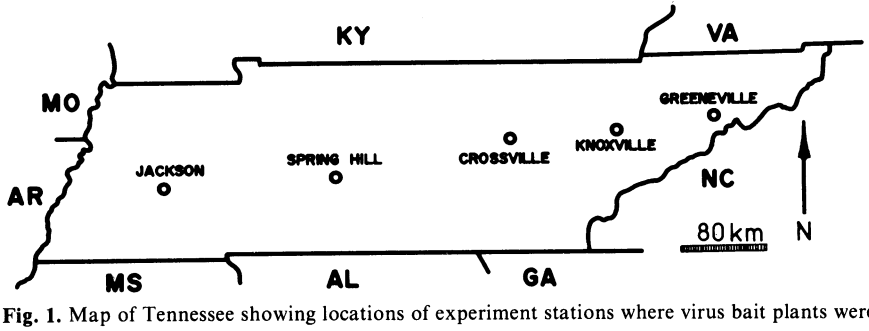

Fig. 1. Map of Tennessee showing locations of experiment stations where virus bait plants were grown.

after collection, six to eight young leaves from different areas of each plant were ground with 3 ml of PBS-Tw-ME-DIECA buffer (0.02 M phosphate, 0.15 M NaCl, 0.003 M KCl, pH 7.3, containing 0.05% Tween 20, 0.02 M 2-mercaptoethanol, and 0.02 M sodium diethydithiocarbamate) using mortars and pestles. Antisera and alkaline phosphatase-conjugated immunoglobulins to AMV, BYMV, CYMV, CYVV, PSV, RCVMV, and WCMV were supplied by R. H. Baum and O. W. Barnett, Clemson, SC. All plants were tested for AMV, BYMV, CYVV, PSV, and RCVMV, and plants from the Knoxville plot were also tested for CYMV and WCMV. ELISA results were determined visually 1–1.5 hr after substrate addition by comparison with control tests of healthy and known infected leaf samples.

RESULTS

Plants set in the field in the fall were not nodulated. Winter mortality averaged 44% and ranged from 5% at Jackson to 64% at Crossville, with 44% at Greeneville, 48% at Knoxville, and 58% at Spring Hill. Mortality among species was alfalfa, 34%; alsike clover, 45%; red clover, 52%; white clover, 47%; crimson clover, 46%; subterranean clover, 27%; and arrowleaf clover, 46%. Replacement plants set in the field in the spring were well nodulated.

One hundred single-virus infections and 20 multiple-virus infections were detected among the 469 bait plants tested at the five sampling sites. The numbers of plants with single infections were 5 BYMV, 19 CYVV, 47 PSV, and 29 RCVMV. The numbers of plants with multiple infections involving each virus were 1 BYMV, 13 CYVV, 17 PSV, and 10 RCVMV. Six of the 120 infected plants had BYMV (5%), 32 CYVV (27%), 64 PSV (53%) and 39 RCVMV (33%). Tests for AMV at all locations and for CYMV and WCMV at Knoxville were negative. The distribution of viruses among locations is shown in Tables 1 and 2 and their distribution among bait plant species in Tables 2 and 3. The numbers of virus-infected plants detected among fall- and spring-planted bait plants by location were, respectively, Greeneville, 10 and 9; Knoxville, 20 and 7; Crossville, 12 and 21; Spring Hill, 16 and 12; and Jackson, 13 and 0. Totals were 71 and 49.

Of 110 white clover plants tested, 50 of 57 infected plants had single or multiple infections of PSV, representing 78% of total PSV infections. The incidence of RCVMV was higher in alsike and white clovers than in red clover, with single or multiple infections in 13, 10, and 7 plants respectively (Tables 2 and 3). The incidence of CYVV in alsike clover with single or multiple infections in 19 and 9 plants, respectively, accounting for 28 of the 32 total CYVV infections, was double that in white clover. Of 111 alsike clover plants tested, 19 of 35 infected plants had CYVV.

DISCUSSION

These results provide further evidence of the importance of *T. repens* in PSV ecology (17) and, conversely, the importance of PSV in *T. repens* ecology (3). Such a high incidence of PSV infections after 9 mo or less in the field is cause for concern because PSV infections significantly damage white clover plants and probably contribute to lack of persistence of white clover in pastures (4).

The apparent associations between PSV and white clover and between CYVV and alsike clover may be the result of differential susceptibility of the host plants, preferential selection of host plants by aphids transmitting the viruses, or both. Evidence of both such mechanisms was found with BYMV and CYVV. As also found in this study, BYMV does not infect white clover (15), even when inoculated mechanically or by aphids (M. R. McLaughlin, *unpublished*); but it commonly infects red clover (6,8).

Table 3. Distribution of forage legume viruses among perennial bait plant species at Greeneville, Crossville, Spring Hill, and Jackson, TN

Species	No. of plants	Number of infected plants[a]									
		BYMV	CYVV	PSV	RCVMV	BYMV CYVV	CYVV PSV	CYVV RCVMV	PSV RCVMV	CYVV PSV RCVMV	Total
Alfalfa	99	0	0	0	0	0	0	0	0	0	0
Alsike clover	100	3	16	1	11	1	1	1	0	0	34
Red clover	99	2	0	1	6	0	0	0	0	0	9
White clover	99	0	2	35	4	0	5	0	3	1	50
Total	397	5	18	37	21	1	6	1	3	1	93

[a] Plants were also tested for alfalfa mosaic virus, but none was detected. Viruses detected were bean yellow mosaic virus (BYMV), clover yellow vein virus (CYVV), peanut stunt virus (PSV), and red clover vein mosaic virus (RCVMV). Detection was by enzyme-linked immunosorbent assay.

White clover apparently is resistant to infection by BYMV. On the other hand, red clover, which is susceptible to infection by CYVV, is rarely found infected with CYVV in nature (8,15). Vector preferences may account for this situation.

AMV was not detected in forage legumes in this study. However, it was identified in burley tobacco in 1980 in a field plot located about 400 m from the bait plant test at Greeneville (13).

Lack of virus infections in alfalfa was unexpected: in similar studies conducted in surrounding states in 1978 (11), alfalfa accounted for 4% of the virus infections detected among bait plants of the same forage legume species used here. In that study, however, the incidence of virus infection in alfalfa was lower than in other forage legumes.

Except for RCVMV, the viruses identified in Tennessee also occur in most southeastern states (3,5–7,9,11,17). Most of these reports did not include data on RCVMV; however, Jones and Diachun (6) tested 550 red clover plants with symptoms of virus disease and detected no RCVMV. Comparisons of the incidences of AMV, BYMV, CYVV, and PSV in Tennessee with those reported from other states may be made only with certain qualifications. In North Carolina, Lucas and Harper (9) reported AMV, PSV, and CYVV in 50, 45, and 20%, respectively, of over 300 white clover plants, two-thirds of which were selected for virus disease symptoms. They further noted that 25% of these plants had multiple infections of AMV and PSV.

Barnett and Gibson (3) tested 240 white clover plants from North Carolina pastures and found 15% infected with AMV, 10% with CYVV, and 7% with PSV. In the same study, however, the incidences of infections among 636 white clover plants from eight southeastern states were PSV, 21%; CYVV, 14%; and AMV, 11%. They also noted multiple infections in 37% of plants tested. In the Kentucky study of red clover (6), BYMV infected 76% of the plants tested and PSV 14%; CYVV was not included in the survey, no AMV was detected, and 10% of plants tested had multiple infections. In a study of 894 plants, representing approximately equal numbers of the same bait plant species used in the present study and covering nine southeastern states, BYMV was detected in 15% of the plants, CYVV in 11%, PSV in 10%, and AMV in $<1\%$ (11). Therefore, the species tested, the predominant forage legume species in the area where samples are collected, and collection of samples from plants with or without symptoms all influence the relative incidences of forage legume viruses detected and must be considered when comparing results of different studies.

This study documents the occurrence of several viruses of forage legumes in Tennessee and indicates potentially serious disease situations. The association of PSV and white clover is of particular significance. The importance of white clover as a component of improved pastures in Tennessee and its probable role as a reservoir host of PSV are important reasons for continued research on the epidemiology of forage legume virus diseases.

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