Viruses Infecting Six Species of Perennial Clover (*Trifolium* spp.) in Field Evaluations of Plant Introductions and Cultivars

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ABSTRACT

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Enzyme-linked immunosorbent assay (ELISA) was used to detect bean yellow mosaic virus (BYMV), clover yellow vein virus (CYVV), white clover mosaic virus (WCMV), and red clover vein mosaic virus (RCVMV) in 1-yr-old plants established in the field for germ plasm evaluations and in feral clover plants growing adjacent to cold frames and evaluation fields. Severe virus infections were observed in accessions of *Trifolium hybridum, T. pratense,* and *T. repens* that coincided with a higher proportion of multiple infections found in these species. *T. ambiguum* and *T. medium* were less affected and fewer multiple infections were detected. *T. alpestre* was intermediate in its response to virus infections in the field. All four viruses were detected in *T. pratense* and three (CYVV, WCMV, and RCVMV) in feral *T. repens* plants growing adjacent to cold frames and evaluation fields.

Plant introductions of perennial *Trifolium* spp. are evaluated for field performance as they are seed-increased at the Northeast Regional Plant Introduction Station. Among the characteristics evaluated are their responses to viral infections as they occur naturally in the field and these vary from year to year, affecting the accessions' vigor, survival, and seed production in varying degrees. The observations reported here were made to identify at least some of the viruses present during field evaluations of plant introductions and to obtain a better understanding of their role in field performance of accessions.

MATERIALS AND METHODS

Plants or stolons were collected at random in June and July 1981 regardless of symptom expression from feral individuals adjacent to cold frames used to harden clover transplants, from the experimental evaluation field (Smith Farm), and from fields 8 km away used to evaluate other plant germ plasm (Darrow Farm). They were planted singly in Cornell mix (2) in an insect-proof greenhouse and assayed at least twice within 6 mo using the direct enzyme-linked immunosorbent assay (ELISA) serological method (3). Leaf samples were ground at 1:5 (w/v) in phosphate-buffered saline, pH 7.5, with 0.001 M ethylene diamine tetracetic acid and 1% polyvinylpyrrolidone. A total of 335 plants, of which 91% were *T. repens* and 9% *T. pratense*, were assayed for bean yellow mosaic virus (BYMV), clover yellow vein virus (CYVV), white clover mosaic virus (WCMV), and red clover vein mosaic virus (RCVMV). Control virus isolates and their antisera were provided by O. W. Barnett, Clemson University, Clemson, SC. BYMV-Ky 204-1 was used as the control BYMV. The other strains were as reported by Barnett and Gibson (1).

Leaf samples were collected in June and July 1982 from 1-yr-old plants of *T. alpestre* L., *T. ambiguum* Bieb., *T. hybridum* L., *T. medium* L., *T. pratense* L., and *T. repens* L. and assayed as described. Single-plant samples were taken from each of six replicates containing 16 plants placed 30 cm apart in a square. The species were replicated in a triple 6 × 7 rectangular lattice design. The field planting was established in June 1981 with seedlings that had been grown for 1 mo in seedling beds in an insect-proof greenhouse, then transplanted to individual pots and transferred to cold frames for 2 wk for a hardening period. As is usual with the seed-increase and evaluation plantings, *Rhizobium* inoculum was not applied to the seeds before planting and a preplant granular fertilizer (10-20-20, NPK) was broadcast at 200 lb/A.

RESULTS AND DISCUSSION

The feral *T. repens* plants collected adjacent to the cold frames assayed negative for BYMV; 28% were positive for CYVV, 87% were positive for WCMV, and RCVMV was detected in 40% of the samples. Most of the plants had multiple infections, and in a few cases, virus was not detected. At the Smith Farm, where the experimental field was established, no BYMV was detected, CYVV was detected in 48% of the samples, and a high proportion of the plants tested, 86 and 88% assayed positive for WCMV and RCVMV, respectively. More plants from this area assayed positive for three viruses and many assayed positive for two. BYMV was found only in feral *T. pratense* collected at the Darrow Farm adjacent to fields for seed increase of other germ plasm. Most plants from this area were infected with WCMV and RCVMV. CYVV was detected in 27% of the plants tested and multiple infections were also common in this area.

Table 1 summarizes results of assays from plants in the six clover species in the field. RCVMV was the virus most consistently found in all clover introductions and cultivars. CYVV was detected in a few cases in *T. pratense, T.*

Table 1. Incidence of viruses in single-plant samples of six perennial *Trifolium* plant introductions, (PI) and cultivars under field evaluations

Species PI no. or cultivar	Virus[a] BYMV	CYVV	WCMV	RCVMV
Trifolium alpestre				
206484	0	0	33	66
314116	16	0	33	33
325479	16	33	0	83
325480	50	33	16	66
325497	16	83	33	50
325498	16	0	0	50
T. ambiguum				
229624	0	16	16	50
225827	0	0	16	33
238154	0	0	16	33
277535	0	0	0	16
405122	0	0	0	16
Townsend (C-2 Kura)	0	0	0	33
T. hybridum				
184548 (2 rep)	40	100	40	60
206481-A (2 rep)	50	100	0	100
255891 (5 rep)	60	100	40	60
257273 (4 rep)	75	100	0	100
266044 (5 rep)	40	100	20	100
T. medium				
325481	0	16	16	33
325498	0	0	0	33
G-15175	0	0	0	16
G-22502	0	0	0	16

(*continued on next page*)

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Table 1. (*continued from preceding page*)

Species PI no. or cultivar	Virus[a] BYMV	CYVV	WCMV	RCVMV
T. pratense				
Pennscott	16	16	16	50
Kenland	33	0	33	16
Kenstar	33	0	50	0
Florex	50	0	33	50
Arlington	83	0	50	83
172484	50	0	50	83
172484-S	33	0	33	100
202716	83	0	33	83
202716-S	83	0	33	50
231781	50	0	33	50
231781-S	83	0	50	16
221523	50	0	16	83
257274	33	0	33	16
266047	16	0	33	66
304781 Hermes	66	0	33	83
211523 (4 rep)	75	0	0	75
T. repens				
Ladino Gigante	0	83	50	100
Tillman	0	83	50	66
Common Idaho	0	100	50	83
Fries-Grongier	0	83	50	66

[a] Percentage of plants infected.

ambiguum, and *T. medium* but was found in most replicates of *T. repens* and in all samples of *T. hybridum*. WCMV was detected in only a few samples of *T. ambiguum* and *T. medium* but was often found in *T. pratense* and *T. repens*. BYMV was not detected in *T. ambiguum, T. medium*, and *T. repens* but was found in a relatively high proportion of replicates in *T. hybridum* and *T. pratense*. In the samples tested, selections of *T. pratense* introductions did not differ markedly from their original seed sources in the number of viral infections detected.

T. hybridum introductions were clearly the most severely affected by virus infections. Accessions of this species developed mild to severe mosaic, veinclearing, and stunting in the cold frames and had very poor survival 1 yr after planting in the field. All four viruses were detected in these introductions and multiple infections by two and three viruses were common. *T. pratense* was represented by 94 samples of 5 cultivars and 11 plant introductions and selections. Severity of symptoms in some cases was as marked as in *T. hybridum* but much less uniform. Twelve percent of the *T. pratense* samples assayed negative for all four viruses tested and no samples were positive for all; 36% were positive for only one virus and 46% assayed positive for two. Only 5% assayed positive for three viruses. *T. repens* cultivars were uniformly affected and 55% of them assayed positive for three viruses. Only one sample was negative for all four viruses tested. Most samples (79%) assayed positive for two or three viruses. The least affected species were *T. ambiguum* and *T. medium*. In the *T. ambiguum* introductions, only 8% reacted positive for two viruses and the rest for only one virus (25%) or none (66%). Seventy-four percent of the samples in *T. medium* were negative for all viruses tested and 26% assayed positive for one. The response of *T. alpestre* to viral infection was more severe than those observed in *T. ambiguum* and *T. medium* and more multiple infections (36%) were detected. Leaf necrosis and mosaic were commonly associated with viral infections in this species.

No assays were made of the seedlings before transfer to cold frames, but during their growth in the greenhouse, symptoms of virual infection were not observed. Symptoms became apparent in *T. hybridum* introductions the second week in the cold frames. These plants are assumed to have become infected by aphids migrating from infected feral plants nearby. BYMV was not detected in most feral plants collected but detection of this virus in many samples of accessions of *T. alpestre, T. hybridum*, and *T. pratense* indicates that alternate hosts other than clover near evaluation fields may be more numerous than assays of feral clovers tested indicate. The high proportion of feral *T. repens*, a species not susceptible to BYMV (8), in the samples resulted in very low detection of this virus. The viruses detected in the evaluation fields have also been reported in the northeastern region of the United States and adjacent areas of Canada (4–7).

It is apparent that there was considerable variation in viral infections among the perennial *Trifolium* spp. Multiple infections occurred within 1 yr of planting in the field, and under the experimental conditions, they appeared to coincide with more severe responses in the affected plants. Their detection and recognition of their possible role in germ plasm field performance should help in selecting virus-resistant accessions more adequate for the northeastern region.

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