Relationships of Virus Infections to Field Performance of Six Clover Species

R. ALCONERO, B. FIORI, and W. SHERRING, Northeast Regional Plant Introduction Station, Geneva, NY 14456

ABSTRACT

Alconero, R., Fiori, B., and Sherring, W. 1986. Relationships of virus infections to field performance of six clover species. Plant Disease 70:119-121.

Eighty-one *Trifolium ambiguum* accessions, representing the germ plasm collection for that species, and selected accessions of *T. alpestre, T. hybridum, T. medium, T. pratense,* and *T. repens* were evaluated in the field from 1981 through 1983 for overall vigor, persistence, yield, and susceptibility to virus infections. Infections by bean yellow mosaic virus, clover yellow vein virus, white clover mosaic virus, and red clover vein mosaic virus were common in many accessions. Accessions of *T. ambiguum* and *T. medium* were the least affected by virus infections and persisted better than other species. Symptoms of virus infection appeared early and spread rapidly in accessions of *T. hybridum, T. pratense,* and *T. repens* and were associated with loss of vigor and death of most of the plants by 1983. *T. alpestre* accessions were only moderately affected by viruses but showed neither the rapid growth of *T. hybridum, T. pratense,* or *T. repens* nor the persistence of *T. ambiguum* or *T. medium.* Several accessions of *T. ambiguum* compared favorably with developed cultivars of *T. pratense* after 2 yr of field exposure.

Field evaluation records of perennial clovers (*Trifolium* spp.) at the Northeast Regional Plant Introduction Station show considerable differences among accessions for winterhardiness, spring and fall vigor, and susceptibility to disease. In some cases, where information is available, the records show different responses by the same accession when grown in different years for seed increase. Yearly variations in climatic and in other types of stress probably are the major cause of these differences. It is apparent that at least in some species, field performance must be evaluated for more than 1 yr to obtain an accurate measure of their value or the relative merit of their accessions. Some species of perennial clover at Geneva behave in the field as vigorous biennials with a rapid growth habit and an equally rapid decline after the first year. Others take longer to become established but may persist better.

The field performance of representative accessions in six species of clover during three growing seasons from 1981 through 1983 is reported. Because some accessions are especially susceptible to virus infections and other biotic stresses, we applied pesticides to study their effects on plant losses and performance in the field. Incidence of the viruses detected has been reported elsewhere (1).

Accepted for publication 28 June 1985.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

MATERIALS AND METHODS

Clover species evaluated. *Trifolium pratense* L. (red clover) has the largest number of accessions in the perennial clover germ plasm collections. In our comparison of species, red clover was represented by 16 accessions: five were cultivars (Pennscott, Kenland, Kenstar, Florex, and Arlington), six were accessions and their local selections from previous evaluations by D. Dolan (*personal communication*) for vigor and apparent freedom from virus infections, and five were unselected accessions.

T. repens L. (white clover) was represented by five cultivars (Ladino Gigante, Common Idaho, Fries-Groninger, Tillman, and Nordic). *T. alpestre* L. and *T. hybridum* L. (alsike clover) were each represented by five accessions. *T. medium* L. (zig-zag clover) had four accesions and *T. ambiguum* L. (kura clover) had five accessions and one selection (Townsend C-2). *T. ambiguum* was also represented by 81 accessions in a separate planting.

Field plantings. Two field plantings were established in June 1981 with seedlings grown for 1 mo in sand beds in an insectproof greenhouse. Seedlings were transplanted to individual pots for 6 wk before being transferred to cold frames for 2 wk of hardening. Each accession was represented by three replicates, with each replicate consisting of 16 3-mo-old plants spaced 30 cm apart in a square plot and each plot separated by 60 cm. The 81 accessions of *T. ambiguum* were planted in a randomized complete block design. About 5 m away, representatives of the six species were planted together in a 6 × 7 rectangular lattice design in two replicated blocks 960 cm apart. One block received a series of pesticide applications and the other was untreated. Both plantings received one granular treatment of 10-20-20 fertilizer broadcast at 224 kg/ha.

Pesticide treatments. Three weeks before planting, the soil was treated with methyl bromide at 76.4 kg/ha and kept covered with plastic for 48 hr. Three days before planting, disulfoton, fensulfothion, and benomyl were incorporated into the soil at 1.1, 8.4, and 48.9 kg/ha, respectively. Foliar sprays of triadimefon, applied monthly, and methoxychlor, malathion, and benomyl applied biweekly, were used throughout each growing season at 0.14, 3.4, and 0.51 kg/ha, respectively. Disulfoton was broadcast biweekly at 1.1 kg/ha and fensulfothion was applied at 8.4 kg/ha during May of each year after the planting year.

Measures of field performance. Plant vigor was evaluated in terms of winterhardiness, spring recovery, fall recovery, and ground cover. Ratings were based on a scale of 0–9, where 0 = poor and 9 = vigorous canopy totally covering the plot. A composite vigor index was obtained by averaging the combined ratings for each accession. Plant survival was determined in each replicate during July of 1982 and 1983. Yields were obtained manually by harvesting shoots 3 cm above the soil line from each square plot in October 1982. Shoots were dried at 50 C for 48 hr before weighing. Poor survival by 1983 precluded additional yield observations in the mixed-species planting.

Virus disease incidence. Estimates of virus infection were based on symptom expression in plants within each plot in October 1981, about 4 mo after planting, and in July 1982. In addition, direct double-sandwich enzyme-linked immunosorbent assays (ELISA) were made of plants in each plot regardless of symptom expression during June and July 1982 for bean yellow mosaic virus (BYMV), clover yellow vein virus (CYVV), white clover mosaic virus (WCMV), and red clover vein mosaic virus (RCVMC) as reported elsewhere (1).

RESULTS AND DISCUSSION

Association of virus infections and field performance. Symptoms of virus infection were detected very early and may have resulted partly from infections that occurred during the hardening period in the cold frames (1). About 4 mo after field establishment, all *T. hybridum* plants showed symptoms of virus infection, many of them with severe reactions. A large proportion of *T. repens* plants, except cultivar Tillman, had mild symptoms. The incidence of symptoms in *T. pratense* accessions varied from 2 to 100%, and no symptoms were observed at this time in *T. alpestre, T. ambiguum,* and *T. medium* (Table 1). One year after planting (July 1982), *T. alpestre, T. ambiguum,* and *T. medium* accessions remained relatively free of symptoms, but a significant increase was observed in *T. pratense*, and all plants of *T. repens* and *T. hybridum* were affected. ELISA at this time detected RCVMV and WCMV in all six species. CYVV was found in all plants of *T. hybridum*, in most *T. repens* plants, in half of the *T. alpestre* accessions, and in only a few samples of *T. ambiguum, T. medium,* and *T. pratense*. BYMV was most prevalent in *T. hybridum* and *T. pratense* but absent in *T. ambiguum, T. medium,* and *T. repens* (1). Assays for BYMV, CYVV, WCMV, and RCVMV in July 1982 in the planting with 81 *T. ambiguum* accessions only resulted in similar types and degrees of viral infection to those already reported for the six accessions of this species (1). Some virus infections may not have been detected by the ELISA survey because of the limited number of antisera used or in latent infections by measuring incidence of virus infections on the basis of symptom expression.

Table 1. Field responses of six perennial *Trifolium* species grown without pesticides during 1981–1983, Geneva, NY

Accessions and cultivars	Virus symptoms (av. % incidence) 1981	Virus symptoms (av. % incidence) 1982	Av. % plants remaining 1982	Av. % plants remaining 1983	Av. composite vigor index 1982	Av. dry wt per plot (g) 1982
T. pratense						
Pennscott	53	97 ab[y]	75	27 cdefg	5.2[z]	828 g
Kenland	23	32 efg	87	45 ghijk	7.1	755 fg
Kenstar	10	15 ghi	87	60 kl	7.4	1,967 i
Florex	8	44 def	87	42 efghijk	6.8	1,592 h
Arlington	2	27 efgh	94	56 ijkl	7.2	1,531 h
172484	52	94 ab	75	8 abc	4.5	741 fg
172484S	76	96 ab	62	10 abcd	5.6	665 fg
202716	84	94 ab	75	6 ab	5.2	729 fg
202716S	8	50 de	75	8 abc	5.5	805 g
22523	60	79 abc	56	2 a	2.8	369 abcdef
231781	25	100 a	68	10 abcd	5.8	585 cdefg
231781S	18	68 bcd	100	25 bcdef	5.3	1,513 h
257274	100	100 a	68	8 abc	3.6	613 defg
266047	100	100 a	18	0 a	1.2	158 ab
304781	100	100 a	37	0 a	3.3	421 bcdef
406641	14	58 cd	31	8 abc	0.6	247 abcd
T. repens						
Ladino Gigante	92	100 a	100	14 abcd	6.9	641 efg
Tillman	39	100 a	100	22 bcde	7.6	614 defg
Common Idaho	100	100 a	100	2 a	6.3	843 g
Fries-Groninger	98	100 a	100	0 a	5.8	403 cdef
Nordic	57	100 a	50	35 efgh	6.6	213 abc
T. alpestre						
206484	0	22 fghi	81	54 hijkl	2.9	175 ab
295352	0	16 ghi	80	54 hijkl	2.2	120 ab
314116	0	30 efg	94	54 hijkl	4.4	271 abcde
325479	0	30 efg	75	29 defg	4.0	169 ab
325480	0	62 cd	81	37 efghi	3.0	134 ab
325497	0	15 ghi	81	39 efghij	2.9	115 ab
T. ambiguum						
229624	0	0 i	56	43 fghijk	2.8	66 ab
225827	0	0 i	75	70 lm	6.4	221 abc
238154	0	0 i	50	58 jkl	5.2	216 abc
277535	0	0 i	31	45 ghijk	4.2	180 ab
405122	0	0 i	87	83 mn	6.9	383 abcdef
Townsend C-2	0	2.6 hi	81	79 mn	6.6	411 bcdef
T. hybridum						
184548	100	100 a	18	0 a	0.9	108 ab
206481-A	100	100 a	10	2 a	0.0	44 ab
255891	100	100 a	25	0 a	0.9	46 ab
257273	100	100 a	18	0 a	0.6	51 ab
266044	100	100 a	6	0 a	1.9	36 ab
T. medium						
325481	0	8 ghi	94	93 n	7.3	861 g
325498	0	6 ghi	81	81 mn	7.3	911 g
G-15175	0	6 ghi	87	81 mn	4.4	278 abcde
G-22502	0	3 ghi	100	87 mn	6.6	563 cdefg

[y]Mean values followed by the same letter(s) within a column do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.
[z]Average index of ratings for ground cover, winterhardiness, and spring and fall recovery based on a scale of 0–9, where 0 = poor and 9 = vigorous canopy.

Table 1 illustrates the average virus incidence in accessions as determined by symptoms in 1981 and 1982, average percentage of plants remaining in 1982 and 1983, and average composite vigor index and dry weight per plot in grams in 1982. The species most affected by viruses, *T. pratense, T. hybridum,* and *T. repens,* showed a rapid decline in survival between 1982 and 1983. The less affected species, *T. alpestre, T. ambiguum,* and *T. medium*, had a larger proportion of plants remaining by 1983, 2 yr after field planting. Among *T. pratense* accessions, virus incidence, survival, vigor, and yield were highly variable, but cultivars generally outperformed plant introductions. A notable exception was PI 231781S, which performed comparatively well in 1982 but had lost 75% of its plants by 1983 and was clearly in decline as were the other accessions in *T. pratense* (Table 1). Virus incidence in 1981 and 1982 was not statistically correlated with the 1982 yield but was negatively correlated with 1982 and 1983 accession survival.

The limited number of accessions representing each species in the mixed-species planting probably does not adequately reflect the best features or the range of responses possible under field conditions. This may be best appreciated by comparing yields of the six *T. ambiguum* accessions tested in the mixed-species planting (Table 1) with those shown by the 81 accessions of the species evaluated in the separate planting (Fig. 1). The yields of the six *T. ambiguum* accessions ranged from 66 to 411 g dry weight per plot in 1982, considerably less than those of most *T. pratense* accessions tested, although their composite vigor index was comparable to the best of *T. pratense*. Among the 81 *T. ambiguum* accessions, 10 yielded 900 g or more in 1982 (PIs 405119, 405120, 405121, 405123, 405124, 440686, 440689, 440709, and G-26478 and Townsend selection C-30). Although it was apparent after 1982 that *T. pratense, T. repens,* and *T. hybridum* were seriously affected by viruses and were in decline, *T. ambiguum* was increasing in vigor. Figure 2 shows an increase in the number of *T. ambiguum* accessions with a rating of 7–8 and a decrease in the number of accessions with ratings of 1–6 for ground cover from 1982 to 1983. The average composite vigor index for *T. ambiguum* accessions in 1982 (Table 1) was affected by the apparent death of the crown in some original plants in each plot, but subsequent regrowth

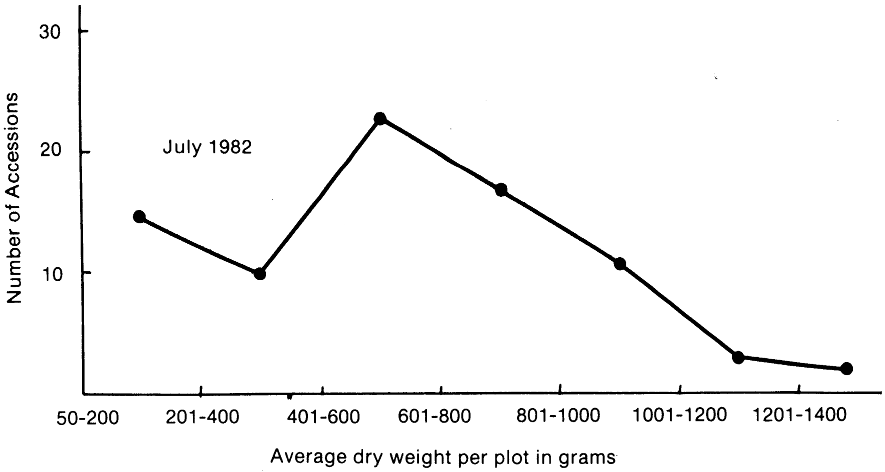

Fig. 1. Yields for 81 accessions of *Trifolium ambiguum*. Values represent averages of three replicates per accession.

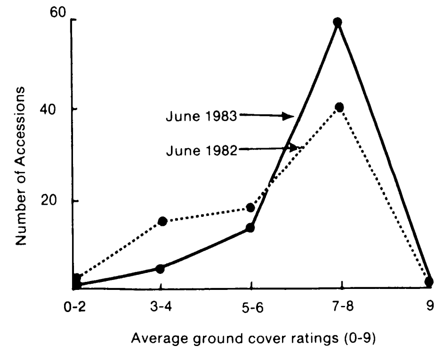

Fig. 2. Ground cover ratings for 81 accessions of *Trifolium ambiguum*. Values represent averages of three replicates per accession.

and emergence of rhizomic shoots from these plants showed that they had survived the winter and spring stresses. Inconsistencies in plant survival values in 1982 and 1983 for *T. ambiguum* may be explained by this type of recovery, which was not observed in the other species (Table 1).

The four *T. medium* accessions had higher percentages of virus incidence than *T. ambiguum* in 1982, but average composite vigor index and yield were generally better. In 1983, survival rate, expressed as the average percentage of plants remaining, was significantly higher than for any other species tested (Table 1). An evaluation of a larger number of accessions of *T. medium* is needed to determine whether its apparent high vigor, persistence, and high resistance to virus infection also occurs in a wider representation of the species.

The rhizomatous species, *T. alpestre, T. ambiguum,* and *T. medium*, persisted better under our field conditions and were less affected by viruses than the stoloniferous *T. repens* and the two species, *T. hybridum* and *T. pratense*, which have neither rhizomes nor stolons. We do not know whether the rhizomatous habit is a significant advantage over others in clovers for winter survival and is necessarily associated with virus resistance.

Effects of pesticides on field performance. No statistically significant effects were detected between pesticide treatments and survival, vigor, or yield in the accessions tested. Our results differ from reports of other beneficial effects of pesticides in white and red clovers under greater pest pressures (6,7). During the experimental period reported here, insects and diseases other than those caused by viruses were of relatively minor importance.

The importance of virus infections in the persistence and vigor of *T. pratense* and *T. repens* is well documented (3–5,8,9). Our observations corroborate those studies as well as others describing the resistance of *T. ambiguum* to virus diseases (2). In addition, our results suggest that this species, although slower to become well established in the field than *T. pratense* and *T. repens*, is superior in persistence and some accessions compare favorably in yield once established. Our results emphasize that evaluation of field performance and comparisons of species of clover should include relatively large numbers of representative accessions that are observed for more than one year.

ACKNOWLEDGMENTS

We thank J. Barnard for his assistance in the design of field experiments and the statistical analysis of the data and C. Tolley and J. Oughterson for their technical help.

LITERATURE CITED

1. Alconero, R. 1983. Viruses infecting six species of perennial clover (*Trifolium* spp.) in field evaluations of plant introductions and cultivars. Plant Dis. 67:1270-1271.
2. Barnett, O. W., and Gibson, P. B. 1975. Identification and prevalence of white clover viruses and resistance of *Trifolium* species to these viruses. Crop Sci. 15:32-37.
3. Cope, W. A., Walker, S. K., and Lucas, L. T. 1978. Evaluation of selected white clover clones for resistance to viruses in the field. Plant Dis. Rep. 62:267-270.
4. Gibson, P. B., Barnett, O. W., Skipper, H. D., and McLaughlin, M. R. 1981. Effects of three viruses on growth of white clover. Plant Dis. 65:50-51.
5. Goth, R. W., and Wilcoxson, R. D. 1962. Effect of bean yellow mosaic virus on survival and flower formation in red clover. Crop Sci. 2:426-429.
6. James, J. R., Lucas, L. T., Chamblee, D. S., and Campbell, W. V. 1980. Influence of fungicide and insecticide applications on persistence of ladino clover. Agron. J. 72:781-784.
7. Leath, K. T., Zeiders, K. E., and Byers, R. A. 1973. Increased yield and persistence of red clover after a soil drench application of benomyl. Agron. J. 65:1008-1009.
8. Newton, R. C., and Graham, J. H. 1960. Incidence of root-feeding weevils, root rot, internal breakdown, and virus and their effect on longevity of red clover. J. Econ. Entomol. 53:865-867.
9. Smith, R. R., and Maxwell, D. P. 1971. Productivity and quality responses of red clover (*Trifolium pratense* L.) infected with bean yellow mosaic virus. Crop Sci. 11:272-274.