Yield Responses of Six White Clover Clones to Virus Infection Under Field Conditions

C. LEE CAMPBELL, Assistant Professor, and JAMES W. MOYER, Associate Professor, Department of Plant Pathology, North Carolina State University, Raleigh 27695

ABSTRACT

Campbell, C. L., and Moyer, J. W. 1984. Yield responses of six white clover clones to virus infection under field conditions. Plant Disease 68:1033-1035.

One hundred twenty vegetatively propagated plants from each of six Tillman white clover seedlings were grown in the field under natural virus-infection pressure during 1981 and 1982. Assay on *Chenopodium quinoa* indicated that 33% of plants were infected during 1981 and 85% of plants were infected by September 1982. Enzyme-linked immunosorbent assay revealed incidence of alfalfa mosaic virus (AMV), clover yellow vein virus (CYVV), and peanut stunt virus (PSV), with predominance of CYVV and PSV. Differences ($P < 0.10$) between yields of virus-free and virus-infected plants were found for two, three, and five of the clones on 17 May, 11 June, and 8 July 1982, respectively. Maximum yield reductions were 47.2, 33.9, and 57.1% at the three harvest dates, respectively.

White clover (*Trifolium repens* L.), especially the large ladino type, is an important perennial forage species used in legume-grass pastures in most humid, temperate regions and is the principal grazing forage legume in North Carolina. It is a high-quality nutritive feed for cattle, provides nitrogen for the sward, and can enhance soil stabilization and reduce erosion. Unlike many crops that are harvested annually, white clover is harvested or grazed several times during each of several growing seasons. Consequently, yield is a function of plant growth between harvests or grazings and of plant persistence over an extended period.

Diseases, insect damage, and unfavorable environmental conditions and management practices adversely affect growth of ladino clover. These factors have been implicated as major determinants of yield loss and poor persistence (2,8,10), and virus diseases are a major component of this decline complex.

Lucas and Harper (11) isolated viruses from samples of all symptomatic plants and from 50% of nonsymptomatic clover plants obtained during a statewide survey of pastures and experimental plots in North Carolina. Alfalfa mosaic virus (AMV), peanut stunt virus (PSV), and clover yellow vein virus (CYVV) were present in 50, 45, and 20% of the samples, respectively. Tobacco ringspot virus (TRSV) and white clover mosaic virus (WCMV) were also infrequently detected. Barnett and Gibson (1) confirmed the widespread occurrence of virus-infected white clover in a survey of 19 pastures in eight southern states; eight pastures representing different stand ages and geographical areas were sampled in North Carolina. As many as 86% of the plants sampled from pastures were virus-infected; AMV, CYVV, PSV, and WCMV were detected in 7, 15, 14, and 5 pastures, respectively.

Virus diseases have been shown to reduce yield from white clover plants. In greenhouse and field studies, Kreitlow et al (10) showed that mechanical inoculation with AMV and bean yellow mosaic virus (BYMV) reduced yields from five ladino clover clones by 23–55%. Gibson et al (5) quantified yield loss attributed to virus infection of inoculated ladino white clover in filtered-air enclosures designed to exclude aphids. PSV resulted in a greater reduction in forage yield (22–40%) than CYVV (9–26%); AMV induced losses intermediate between those caused by PSV and CYVV. Losses to PSV and CYVV infection varied according to the time of year harvests were made.

Most measurements of yield reduction in white clover caused by virus diseases have been made in greenhouses, controlled-environment chambers, or filtered-air chambers on mechanically inoculated plants or infected clones (5,6,9,10). Our objectives were to quantify virus-induced losses in white clover under natural virus infection pressure in the field and to measure the responses of individual clones derived from the cultivar Tillman to virus infection.

MATERIALS AND METHODS

Twenty seedlings were arbitrarily selected from a population of Tillman white clover and grown in a greenhouse. Twenty-five stolon cuttings (1–1.5 cm) were taken from each stock plant and rooted for 2 wk in pasteurized sand. Rooted cuttings were inoculated with an effective *Rhizobium* strain (Nitragin, Milwaukee, WI), transplanted into peat pots 5.7 cm in diameter containing a pasteurized soil mix of sandy loam and sand (3:1, v/v), and grown in the greenhouse for 3 wk.

Twenty plants, free of viruses detectable in *Chenopodium quinoa* Willd., from each of six randomly selected clones (T3, T7, T12, T15, T17, and T19) were transplanted to a field site in Wake County, NC, with Cecil clay soil (pH 6.0) on 23 April 1981. Plants were arranged in blocks of six plants (one of each clone) with 1.07 m (3.5 ft) between plants and 1.52 m (5 ft) between blocks. Blocks were arranged in a 4 × 5 pattern. Plants were watered as needed.

Viruses were assayed by arbitrarily removing two or three fully expanded leaves and extracting in a buffer appropriate for the assay with a mechanical leaf squeezer. Tissues for bioassay were extracted in 0.05 M potassium phosphate buffer, pH 7.2, and assayed on *C. quinoa*. Enzyme-linked immunosorbent assays (ELISA) were conducted using the protocol of Clark and Adams (3). Individual clover plants were assayed for virus infection on *C. quinoa* on 26 May and 26 August 1981 and 26 April and 17 September 1982. On 26 August 1981 and 26 April and 22 September 1982, clover plants were assayed for CYVV and PSV by ELISA

Paper 9288 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh 27650. A contribution in support of Regional Project S-127, Forage Legume Viruses.

Supported in part by a grant from the National Crop Loss Design Committee, USDA/CSRS.

Use of trade names does not imply endorsement by the North Carolina Agricultural Research Service of the products named or criticism of similar ones not mentioned.

Accepted for publication 23 July 1984 (submitted for electronic processing).

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.

and additionally for AMV in the 22 September 1982 assay. Purified antibody and enzyme-conjugated antibody for ELISA were supplied by O. W. Barnett, Clemson University, or M. McLaughlin, USDA Forage Unit, Mississippi State University.

Foliage of each plant was harvested on 17 May, 11 June, and 8 July 1982 by clipping with hedge shears to a height of 6–8 cm. Shears were dipped in a 10% aqueous Clorox solution for 30–60 sec between plants to minimize mechanical spread of viruses. Harvested material was dried for 24 hr at 100 C before weighing.

Plants were classified as "virus-free" or virus-infected for the entire 1982 season on the basis of results of the 26 April 1982 *C. quinoa* assay. Means for each clone within a harvest were compared using an appropriate t test for equal or unequal sample size after testing for equality of variance between treatments by an F test (12).

RESULTS

Virus incidence. Assay of clover plants on *C. quinoa* indicated that 0, 20, 34, 63, and 103 plants were virus-infected on 16 April, 26 May, and 26 August 1981 and 26 April and 17 September 1982, respectively (Table 1). One plant of clone T7 and one of clone T12 were killed by *Sclerotium rolfsii* Sacc. At the beginning of the second (1982) growing season (26 April 1982 *C. quinoa* assay), 7, 8, 14, 10, 6, and 10 plants of clones T3, T7, T12, T15, T17, and T19, respectively, were virus-free, but by the 17 September 1982 *C. quinoa* assay, only 0, 2, 5, 5, 1, and 2 plants of these clones were "virus-free."

ELISA for CYVV and PSV showed that 34 and 40 plants were infected by one or both of these viruses on 26 August 1981 and 26 April 1982, respectively (Table 2). By 22 September 1982, ELISA for AMV, CYVV, and PSV indicated that 81 of the clover plants were infected by one or more of these viruses. PSV incidence was greater than CYVV incidence on 26 August 1981, with few double infections. PSV and CYVV incidence were nearly similar on 26 April 1982, with few double infections. On 22 September 1982, incidence of CYVV was higher than that of PSV or AMV and more doubly infected plants were found.

Clover yield. Harvests were not made during 1981 because of unusually dry weather conditions and the relatively small size of plants due to the late-spring planting. Most plants remained distinct throughout the study; however, when necessary, plants were physically cut to maintain individual integrity.

On 17 May 1982, "virus-free" plants had a numerically greater mean yield than virus-infected plants for clones T3, T12, T15, T17, and T19 but not for clone T7 (Table 3). Mean yields per plant were greater for "virus-free" plants than for virus-infected plants for clones T15 (P = 0.10) and T19 (P = 0.04). Percent difference in yield—[("virus-free"–virus-infected)/virus-infected] × 100—ranged from an increase of 4.9 for plants of clone T7 to a suppression of 47.2 for plants of clone T19.

At the second harvest (11 June 1982), yields of "virus-free" plants were numerically larger than yields of virus-infected plants for clones T3, T7, T15, T17, and T19. Mean yields between treatments were significantly different for clones T3 (P = 0.07), T15 (P = 0.06), and T19 (P = 0.05). Percent difference in yield ranged from an increase of 4.8 for plants of clone T12 to a reduction of 33.9 for plants of clone T19.

Mean yield of "virus-free" plants was numerically greater than yield of virus-infected plants for all clones by the third harvest (8 July 1982). Means were statistically different for clones T3 (P = 0.07), T12 (P = 0.05), T15 (P = 0.03), T17 (P = 0.09), and T19 (P = 0.04). Means for clone T7 were different at P = 0.16. Percent difference in yield between virus-free and virus-infected plants was 53.7, 29.5, 42.1, 41.8, 57.1, and 40.3 for clones T3, T7, T12, T15, T17, and T19, respectively.

DISCUSSION

Gibson et al (6) reported that white clover plants were not killed by virus infection in controlled-environment studies. Our results concurred with these findings; however, current-season yield was severely limited by virus infection in our study. Percent reduction in yield increased over time for plants of all clones tested, with a final range (8 July 1982) of 29.5–57.1%.

Our study is unique because it relied on natural virus spread and infection of clover plants under field conditions to provide "treatments." The experimental plot was surrounded by pasture-type and alfalfa fields, which is typical in a pasture/forage production ecosystem in the piedmont region of North Carolina. Although not assayed to determine the presence of specific viruses, clover plants in nearby mixed stands of clover and tall fescue (*Festuca arundinacea* Schreb.)

Table 1. Assay of 20 plants of each of six white clover clones for virus infection by mechanical inoculation on *Chenopodium quinoa*

Clover clone	Assay date: 16 Apr. 1981[a] No.+[b]	16 Apr. 1981[a] No.−	26 May 1981 No.+	26 May 1981 No.−	26 Aug. 1981 No.+	26 Aug. 1981 No.−	26 Apr. 1982 No.+	26 Apr. 1982 No.−	17 Sept. 1982 No.+	17 Sept. 1982 No.−
T3	0	20	2	18	7	13	13	7	20	0
T7	0	20	2	18	6	13	11	8	17	2
T12	0	20	5	15	3	16	5	14	14	5
T15	0	20	5	15	5	15	10	10	15	5
T17	0	20	4	16	7	13	14	6	19	1
T19	0	20	2	18	6	14	10	10	18	2
Total	0	120	20	100	34	84[c]	63	55	103	15

[a] Before transplanting into field plots.
[b] No.+ = *C. quinoa* plants showing local lesions and/or systemic symptoms 7–10 days after inoculation; No.− = *C. quinoa* plants not showing symptoms.
[c] Missing plants killed by *Sclerotium rolfsii* infection.

Table 2. Assay of 20 plants of each of six white clover clones for virus[a] infection by enzyme-linked immunosorbent assay

Clover clone	Assay date: 26 August 1981 Negative	CYVV only	PSV only	CYVV + PSV	26 April 1982 Negative	CYVV only	PSV only	CYVV + PSV	22 September 1982 Negative	AMV	CYVV	PSV	AMV + CYVV	AMV + PSV	CYVV + PSV	AMV + CYVV + PSV
T3	16	1	3	0	10	4	6	0	2	0	7	3	0	0	8	0
T7	15	1	3	1	10	4	6	0	4	0	7	2	0	0	6	0
T12	18	0	2	0	17	0	2	0	12	0	2	4	0	1	0	0
T15	16	0	4	0	15	1	4	0	9	0	4	6	0	0	1	0
T17	16	1	3	1	12	4	2	2	5	0	6	3	0	0	5	1
T19	16	1	3	0	14	3	2	1	5	0	9	3	0	0	3	0
Total	96	4	28	2	78	16	21	3	37	0	35	21	0	1	23	1

[a] AMV = alfalfa mosaic virus, CYVV = clover yellow vein virus, and PSV = peanut stunt virus.

served as potential sources of inoculum and potential reservoirs for aphid vectors.

Results of our study indicate the potential damage from virus infection of white clover in pasture systems. Twenty-nine percent of the plants were virus-infected within 4 mo of being placed in the field and 87% were virus-infected within 17 mo (Table 1). These results are in general agreement with survey findings of previous investigations (1,4,11).

AMV, CYVV, and PSV were detected by ELISA in plants of all six clover clones (Table 2). Initial incidence of PSV was greater than that of CYVV; however, final incidence of CYVV was higher than that of PSV, and incidence of AMV was very low. ELISA for AMV, CYVV, and PSV on 22 September 1982 identified 37 plants as not infected by one of these viruses, whereas only 15 plants were identified as free of viruses detectable on *C. quinoa* on 17 September 1982. This difference in number of "virus-free" plants between the assays suggests the presence of a virus or viruses in plants that were not detected by ELISA. Red clover vein mosaic virus (RCVMV), AMV, CYVV, and PSV were detected by ELISA in 1983 in clover bait plants placed in an area adjacent to the location of this yield loss study as part of the S-127 (Forage Legume Viruses) Regional Project survey of viruses and vectors (M. McLaughlin, *personal communication*). Thus, RCVMV may account for part of the difference in the incidence of virus-infected plants detected by *C. quinoa* assay and ELISA.

Yield (as measured by foliage dry weight) was determined on a per-plant basis for plants growing in a pure clover stand. Standard deviations of the mean yield for each clone-treatment combination indicate a certain amount of variability among plants even within each combination. Although yields were higher than might be expected for individual clover plants in a mixed tall fescue-clover pasture because of competition within the pasture system, differences existed in the magnitude of yield and yield loss among the clones arbitrarily selected from the Tillman population. Plants of clone T17 consistently had the highest yields; plants of clone T7 consistently had the lowest yields. Also, clover clones differed in tolerance to virus disease, eg, percent yield loss was lower for virus-infected plants of clone T7 than for any other clone. Variability among clones, with regard to such traits as yield and virus tolerance, could be expected because Tillman is a six-clone synthetic (7) and *T. repens* is an autotetroploid. It is not possible, however, to distinguish between genetic differences and differences resulting from infection by one or more viruses, because presence of each virus in each plant was not monitored at each harvest.

Although most plants were virus-infected by 17 September 1982, differences in yield between plants designated as "virus-free" and virus-infected on the basis of the 26 April 1982 *C. quinoa* assay were detected throughout the growing season. Virus infection during the growing season may have reduced the magnitude of the detected yield differences. Another possibility is that current-season virus infection of clover plants may not be as significant a determinant of yield as the plant status with regard to virus infection at the beginning of the growing season. The impact of current-season virus infection and the time of this infection on yield needs further investigation before sound conclusions can be made.

Our study provides quantitative evidence of the severity of virus diseases on yield of white clover under natural virus-infection pressure in the field. These results emphasize the continued need for the design of new management programs for white clover virus diseases and especially for the development of new white clover cultivars. Future studies should identify the effects of specific viruses on clover yield under field conditions in a grass-clover system and should examine the potential usefulness of virus tolerance to reduce the impact of virus infection on clover yield and persistence.

Table 3. Foliar dry weight measurements for "virus-free" and virus-infected[a] plants of six white clover clones at three harvest dates

Clover clone	Virus-free			Virus-infected			Difference (%)	Probability of a numerically larger value of t[c]
	n[b]	$\bar{x}$	SD	n	$\bar{x}$	SD		
				17 May 1982				
T3	6	166	73.6	13	137	121.2	17.5	>0.50
T7	8	82	31.3	11	86	40.0	−4.9	>0.50
T12	13	119	49.0	5	106	54.5	10.9	>0.50
T15	9	95	24.1	10	73	27.5	23.1	0.10
T17	5	282	86.7	14	153	90.1	15.5	>0.50
T19	10	168	91.9	10	92	42.2	47.2	0.04
				11 June 1982				
T3	6	174	56.8	13	129	35.9	25.8	0.07
T7	8	114	44.1	11	90	31.8	21.1	0.21
T12	13	125	54.2	5	131	111.4	−4.8	>0.50
T15	9	134	31.5	10	97	41.4	27.6	0.06
T17	5	249	202.0	14	188	114.1	24.5	>0.50
T19	10	159	52.0	10	105	58.5	33.9	0.05
				8 July 1982				
T3	6	216	119.0	13	100	37.1	53.7	0.07
T7	8	105	54.2	11	74	21.0	29.5	0.16
T12	13	121	46.9	5	70	25.0	42.1	0.05
T15	9	141	55.3	10	82	33.0	41.8	0.03
T17	5	203	114.7	14	87	26.2	57.1	0.09
T19	10	129	56.4	10	77	48.9	40.3	0.04

[a] Plants were classified as virus-free or virus-infected for the growing season on the basis of results of the 26 April 1982 assay on *Chenopodium quinoa*.

[b] n = Number of plants in that classification, $\bar{x}$ = arithmetic mean of the harvest weights (in g/plant), and SD = standard deviation of the mean.

[c] Means were compared by using an appropriate t test for unequal or equal sample size after testing the null hypothesis $H\sigma:\sigma_1^2 = \sigma_2^2$ by an F test (12).

ACKNOWLEDGMENTS

We thank O. W. Barnett, Clemson University, and Mike McLaughlin, USDA Forage Research Unit, Mississippi State University, for supplying the ELISA materials. We also sincerely appreciate the assistance of C. R. Harper, J. T. Johnson, C. K. Ragland, and W. M. Thal in these studies.

LITERATURE CITED

1. Barnett, O. W., and Gibson, P. B. 1975. Identification and prevalence of white clover viruses and the resistance of *Trifolium* species to these viruses. Crop Sci. 15:32-37.
2. Chamblee, D. S., Lucas, L. T., and Campbell, W. V. 1976. Persistence of Ladino clover as affected by physical management, disease and insects. South. Branch Am. Soc. Agron. Abstr. Tech. Pap. 2:1.
3. Clark, M. V., and Adams, A. N. 1977. Characteristics of the microplate method of the enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475-483.
4. 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.
5. Gibson, P. B., Barnett, O. W., Burrows, P. M., and King, F. D. 1982. Filtered-air enclosures exclude vectors and enable measurement of effects of viruses on white clover in the field. Plant Dis. 66:142-144.
6. 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.
7. Gibson, P. B., Beinhart, G., and Halpin, J. E. 1969. Registration of Tillman white clover. Crop Sci. 9:522.
8. 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.
9. Kreitlow, K. W., and Hunt, O. J. 1958. Effect of alfalfa mosaic and bean yellow mosaic viruses on flowering and seed production of ladino clover. Phytopathology 48:320-321.
10. Krietlow, K. W., Runt, O. J., and Wilkins, H. L. 1957. The effect of virus infection on yield and chemical composition of Ladino clover. Phytopathology 47:390-394.
11. Lucas, L. T., and Harper, C. R. 1972. Mechanically transmissible viruses from ladino clover in North Carolina. Plant Dis. Rep. 56:774-776.
12. Steel, R. G. D., and Torrie, J. H. 1980. Principles and Procedures of Statistics. 2nd ed. McGraw Hill, New York. 633 pp.