The Effects of Clover Yellow Vein Virus and Peanut Stunt Virus on Yield of Two Clones of Ladino White Clover

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ABSTRACT

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Two clones, T7 and T17, of ladino white clover were examined in a transplanted clover-tall fescue system under field conditions for response to infection by peanut stunt virus (PSV), clover yellow vein virus (CYVV), and PSV + CYVV in 1982 and 1983. Plants infected by either PSV, CYVV, or the two viruses together had lower dry weight yields than plants of initially virus-free clover. PSV was the more severe virus and resulted in greater yield loss for both clones; however, clone T17 was less susceptible to yield reduction by PSV than was T7. Conversely, clone T7 was less susceptible to yield loss from infection by CYVV than was T17. The combination of both viruses led to the same yield reduction as PSV alone; however, PSV + CYVV plots exhibited the highest mortality rate during the second growing season. In the 1983 field plots, fescue had greater yield at the second and third harvest when grown with CYVV or CYVV + PSV-infected clover than when grown with initially virus-free clover. Yield and components of growth (stolons per plant, length per stolon, nodes per centimeter of stolon, rooting nodes per stolon) of virus-free, CYVV, PSV, and PSV + CYVV-infected plants of clone T17 were also evaluated in controlled-environment studies. Virus and temperature effects were significant for all components. A virus by temperature interaction ($P < 0.05$) occurred for final root and shoot dry weight, average number of nodes per centimeter of stolon, and average length per stolon. Reductions in components of growth due to virus infection were greatest in PSV and PSV + CYVV treatments, and overall growth of clover was greatest at 20 C. The different coat protein antigen levels, as determined by ELISA, indicated differences in titer and distribution of CYVV between plants of clones T7 and T17. Clone T17, which exhibited the greater yield reduction when infected with CYVV, had higher virus titer, in general, than clone T7. Older leaves of plants of clone T17 had higher virus titer than comparable leaves of plants of clone T7.

Virus diseases are significant factors in reducing yield and persistence of white clover (*Trifolium repens* L.) (10,11,14,15). Viruses have been detected in symptomatic and asymptomatic white clover samples throughout the southeastern United States where the most commonly identified viruses were peanut stunt virus (PSV), alfalfa mosaic virus, and clover yellow vein virus (CYVV) (1,16).

Virus infection in pure stands of clover reduced yield by 48.4% in greenhouse tests and 54.3% in field tests (14). In further studies (15), yield loss due to infection by alfalfa mosaic virus and bean yellow mosaic virus, in both greenhouse and field tests, ranged from 23 to 55%. In filtered-air enclosures, infection with each of several

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viruses reduced clover yield, with infection by PSV causing the greatest reduction (10). Yield reductions from 20 to 50% were found (5) among six clones of Tillman white clover in spaced-plant field stands under natural virus infection pressure which was due primarily to CYVV and PSV.

It has been established that infection by viruses such as PSV, alfalfa mosaic virus, and CYVV reduces the growth and survival of white clover plants in the field and greenhouse (4,5,8,11). It has not been documented, however, how virus infection influences the normal clover-fescue cropping system. Further, we have observed apparent tolerance (to symptoms) to CYVV infection (5) and additional information is needed before this potentially useful form of resistance can be utilized.

To better understand the influences of CYVV and PSV on clover yield and the components of clover growth that contribute to yield and to seek plausible biological explanations for a differential clone reaction to virus infection, a series of field, greenhouse, and laboratory investigations were initiated. The specific purposes of this research were: to quantify the effects of PSV and CYVV on yield of two clones, T7 and T17, of ladino clover in a transplanted clover-tall fescue grass system; to determine the effects of these viruses on components of growth of clone T17; and to determine the relative titer of CYVV in plant parts of clones T7 and T17 to account for differences between the clones in yield reduction due to this virus.

MATERIALS AND METHODS

Plant material. Two clones (T7 and T17) of the white clover cultivar Tillman, a ladino type (4), were maintained in the greenhouse in separate Saran screen cages as "virus-free," CYVV-infected, PSV-infected, and PSV + CYVV-infected. Plants for each treatment were derived from a single plant of each clone. Clover was propagated by rooting 1.0- to 2.5-cm-long cuttings in pasteurized sand. Rooted cuttings were inoculated with *Rhizobium* spp. The virus status of each plant was determined by enzyme-linked immunosorbent assay (ELISA) for CYVV or PSV and/or assay on *Chenopodium quinoa* Willd. before each experiment.

Yield assessment. Rooted cuttings of clover were transplanted into Metro-Mix potting medium (Grace Horticultural Products, W. R. Grace & Co., Cambridge, MA), and tall fescue (*Festuca arundinacea* Schreb.), cultivar KY 31, was also grown from seed in Metro-Mix until transfer to field plots. Field plots (Cecil clay soil, pH 6.0) were fumigated with methyl bromide (9.5 kg/100 m^2) 1–2 wk prior to planting. Two separate field plot experiments were planted at adjacent locations in May and August of 1982. Each "circle" or plot was separated by 0.91 m and contained in a circular area (0.61 m in diameter) by metal garden edging. Two clover cuttings and four fescue seedlings were transplanted into each plot. Each experiment consisted of the eight randomly placed treatments, replicated 10 times. Ten additional replications of the "virus-free" treatment were included in the August 1982 planting, and plots in this second experiment were surrounded by a border crop of rye which served to intercept potential virus vectors and thus to increase the likelihood of having "virus-free" plots at the end of the season.

Furadan (22.4 kg/ha) and malathion (3.9 ml/L) were applied as needed for insect control in field plots. Fertilizer (0-10-30, N-P-K) (≈112 kg/ha) was applied at planting to both fields. Irrigation was provided as needed.

Plants in plots were harvested by clipping to a height of 7.6 cm and the clipped clover and fescue components were separated. Clipping shears were immersed for 30-60 sec in 10% Clorox between plots to minimize mechanical spread of virus. Three harvests were made during June-August each year at approximately monthly intervals. Harvested material was dried for 24 hr at 100 C and weighed.

Clover plants were assayed several times during the growing season by using ELISA (7,13) or local lesion assay on *C. quinoa*. Antisera and conjugate were provided by O. W. Barnett of Clemson University. Absorbance at 405 nm was measured with a TiterTek Multiskan ELISA-plate reader (Flow Laboratories, Inc., McLean, VA).)

Data were analyzed by using analysis of variance (17,18) and Duncan's new multiple range test for mean separation ($P = 0.05$). For the purpose of statistical analysis, all plots established in May 1982 were utilized. All initially virus-infected plots and 10 healthy plots were selected from the 20 "virus-free" plots established in August 1982 for statistical analysis. Selection of these 10 "virus-free" plots was based on ELISA conducted for CYVV and PSV on May 1983 and presence of harvestable clover at the first harvest.

Components of growth. Additional analysis of the effect of virus infection on growth components was conducted at the Southeastern Plant Environment Laboratory. Rooted cuttings of healthy and virus- infected (CYVV, PSV, or CYVV + PSV) plants of clone T17 were transplanted into a pasteurized loam soil:sand (3:1, v/v) mix contained in 10-cm-diameter plastic pots. Eight pots per treatment were randomly placed in each of three controlled-environment chambers (0.91 × 1.22 × 1.22 m). Temperature was maintained at 10, 20, or 30 C with a variation about the set point of 0.38 C and relative humidity was 60–70%. A 12-hr light/dark photoperiod was utilized with an illuminance at pot level of 430–480 klux provided by cool-white fluorescent and incandescent lamps. Experiment duration was 83, 83, and 85 days, respectively, in each of the three runs, and stolons of all plants were kept manually within the pot area. Chamber temperature was randomly assigned for each run.

Foliage from all plants was clipped to a height of 2.5–3.0 cm on day 41 or 42 after set up and used in a local lesion assay on *C. quinoa*. Foliage was subsequently clipped to 2.5–3.0 cm on day 62 or 63 and on day 83 or 85. At the termination of each experiment, shoot dry weight, root dry weight, number of stolons per plant, length per stolon, number of nodes per stolon and number of rooted nodes per stolon were recorded for each plant. Number of nodes per centimeter of stolon was calculated.

Data were analyzed by analysis of variance (18). Runs, temperatures, and virus treatments were considered as sources of variation in the fixed-effects model. In the analysis of variance, significance of the temperature effect was determined by using the runs by temperature interactions as the error term; significance of the virus effect and virus by temperature interaction was determined by using an error term comprised of the virus by runs and virus by runs by temperature interactions. When a significant F value was obtained for a growth value with respect to virus treatment, single-degree-of-freedom linear contrasts were used to make comparisons: virus-free versus others, CYVV versus PSV, CYVV and PSV versus PSV + CYVV. Data from a plant were considered in the analysis only if the local lesion assay on *C. quinoa* was negative for the virus-free treatment and was positive for the PSV, CYVV, or PSV + CYVV-infected treatment; otherwise data for a plant were considered as missing values. Missing values were included in PSV treatments for eight plants in run 1 and for 12 plants in run 2 and in the PSV + CYVV treatment for nine plants in run 1.

Distribution of CYVV. Plants of each clone infected with CYVV were established from rooted cuttings and grown in greenhouse flats; four plants were placed in each flat. One stolon from each of 10 plants per clone was removed for analysis by ELISA over the time interval from 27 August 1983 through 30 October 1983. On the average, one stolon of each clone was processed each week; however, since each stolon came from a separate plant, stolons are considered to be replicates and time was not considered as a source of variability. For each stolon, all trifoliolate leaves, all petioles, tissue from three positions on the stolon, and root tissue from three positions along the stolon (if present) were assayed. Thus, a total of 10 stolons was analyzed for each clover clone.

A cork borer was used to remove a 5-mm-diameter portion from the center of each leaflet of a trifoliolate leaf and the three portions were then pooled to become the sample tested at that leaf position. Leaf positions were numbered from the distal leaflet such that the youngest, fully expanded trifoliolate leaf was leaf position 1 and numbers continued in ascending order toward the plant center. Position 1 was thus the youngest leaf and position 10 the oldest leaf in the study. A 4-mm-diameter cork borer was used to remove internode tissue from stolon sections, and a 13-mm-diameter cork

borer was used to obtain sections from the middle position of the petiole. Root samples 1 cm in length were removed arbitrarily. All samples were diluted (1:100) with buffer solution and homogenized in a ground glass tissue homogenizer. This dilution was determined during preliminary dilution experiments to be in the linear range of absorbance versus virus concentration. Absorbance readings at 405 nm (A_{405nm}) were compared to healthy samples by analysis of variance (17,18).

RESULTS

Yield assessment. Virus assays of clover plants prior to planting were generally consistent with intended treatments with several exceptions for intended PSV-infected plants. In several instances, initial assays of plants derived as cuttings from known PSV-infected plants were negative for PSV after both ELISA and bioassay on *C. quinoa*. We believe that these plants were most likely derived from stolon sections in which the virus was absent; however, plants (not greater than two per experiment) were considered as missing. Assays at various times after planting also indicated spread of both PSV and CYVV throughout field plots in 1982 and 1983. In plots established in May 1982, all plants in the initially "virus-free" plots were virus infected by September 1982. In plots established in August 1982, six plants in the intended "virus-free" plots were virus infected by 21 September. Poor persistence of clover (especially in the treatments established with infected plants) in the field plots established in August 1982 was evident from the number of missing plants (14–18 per treatment) by the time of assay on 6 October 1983.

Initial infection of plants by virus reduced yield ($P = 0.05$) for both clover clones (Tables 1 and 2). Time of harvest was a significant factor ($P = 0.05$) affecting forage yield in 1983, but not in 1982; a virus by time of harvest interaction was not detected in either year. For example, in July 1983, yields were much greater than yields in June and in August (Table 2). In 1982, yields were fairly similar for the three harvests (Table 1).

The two clones responded differently to infection by the viruses. In general, yield of plants of clone T7 was reduced less by CYVV compared to "virus-free" plants than was yield of plants of clone T17. The opposite was generally true concerning PSV infection.

PSV was the more severe of the two viruses. PSV infection reduced clover yield for both clones significantly more than CYVV-infection in 1982 (Table 1), but not in 1983 (Table 2). Yield reduction induced by CYVV and PSV in combination was not different ($P = 0.05$) from that induced by PSV infection alone during either year.

Virus infection of clover had no effect on the yield of fescue in 1982; however, a significant effect ($P = 0.05$) was found in 1983 (Table 3). For example, in July and August, fescue in all virus-infected plots yielded significantly more than fescue in initially virus-free plots.

Components of growth. Final root and shoot dry weights, averaged over the four treatments, were greatest at 20 C and least at 10 C (Table 4). For both weight measurements, the effects of virus and temperature were highly significant ($P = 0.01$). All three linear contrasts tested led to rejection of the null hypothesis. Thus, dry weights were greater in virus-free plants than in virus-infected plants; CYVV-infected plants had greater dry weights than PSV-infected plants; and the double virus infection reduced dry weights more than infection by either virus alone. A significant ($P = 0.01$) temperature by virus interaction occurred for shoot and root dry weight. For both measurements, proportional increase in weight between 10 and 20 C and decrease in weight between 20 and 30 C was greatest in the PSV + CYVV treatments and least in the CYVV treatments. Apparently, plants with the double virus infection were more sensitive to temperature and CYVV-infected plants were less sensitive to temperature than virus-free or PSV-infected plants.

Temperatures and virus treatment affected ($P < 0.01$) the number of stolons per plant, length per stolon, number of nodes per centimeter of stolon, and rooting nodes per stolon, and these two factors, temperature and virus treatment, interacted ($P < 0.01$) for number of stolons per plant, length per stolon, and number of nodes per centimeter of stolon (Table 5). Number of stolons per plant was greatest for each virus treatment at 20 C and was greatest at all temperatures in the virus-free treatment; number of stolons per plant varied as did dry weight measurements with respect to virus treatments as determined by linear contrasts. In general, length per stolon was greatest at 20 C, and the relationship among virus treatments as determined by linear contrasts was the same as for root and shoot dry weight; the interaction is apparent in that

TABLE 1. Dry weight (grams) of plants of two clover clones for field plots harvested three times in the summer of 1982

	Harvest 1 (29 June)			Harvest 2 (19 July)			Harvest 3 (19 August)		
Treatment[x]	T7[y]	T17[y]	Mean	T7	T17	Mean	T7	T17	Mean
Virus-free	9.2	11.8	10.5 a[z]	11.3	13.3	12.3 a	10.6	13.5	12.1 a
CYVV	8.3	8.3	8.3 a	7.5	8.2	7.8 b	9.1	9.8	9.5 ab
PSV	1.8	4.8	3.1 b	2.3	5.9	3.7 c	3.6	9.3	5.9 bc
CYVV + PSV	1.4	2.7	2.3 b	1.4	3.6	2.5 c	2.6	5.1	4.3 c
Mean	5.2 a[z]	6.9 b		5.6 a	7.7 b		6.5 a	9.4 a	

[x]Virus-free = free of viruses detectable in *Chenopodium quinoa*, CYVV = infected by clover yellow vein virus, PSV = infected by peanut stunt virus, and PSV + CYVV = infected by both PSV and CYVV.
[y]T7 and T17 are clover clones.
[z]Means in a column followed by the same letter are not significantly different, $P = 0.05$, according to Duncan's new multiple range test. Means in a row followed by a different letter are significantly different, $P = 0.05$, according to ANOVA and an F test. No significant clone by treatment interaction was found.

TABLE 2. Dry weight (grams) of plants of two clover clones for field plots harvested three times in the summer of 1983

	Harvest 1 (6 May)			Harvest 2 (7 July)			Harvest 3 (8 August)		
Treatment[x]	T7[y]	T17[y]	Mean	T7	T17	Mean	T7	T17	Mean
Virus-free	4.2	4.2	4.2 a[z]	41.0	40.9	40.9 a	10.3	13.4	11.8 a
CYVV	0.8	0.4	0.5 b	17.8	9.8	13.7 b	0.0	2.7	1.4 b
PSV	0.4	0.2	0.4 b	5.9	9.3	7.6 b	0.2	1.0	0.5 b
CYVV + PSV	0.0	0.0	0.0 b	3.6	0.4	2.0 b	0.7	0.0	0.3 b
Mean	1.4 a[z]	1.2 a		17.1 a	15.1 a		2.8 a	4.3 a	

[x]Virus-free = free of viruses detectable in *Chenopodium quinoa*, CYVV = infected by clover yellow vein virus, PSV = infected by peanut stunt virus, and PSV + CYVV = infected by both PSV and CYVV.
[y]T7 and T17 are clover clones.
[z]Means in a column followed by the same letter are not significantly different, $P = 0.05$, according to Duncan's new multiple range test. Means in a row followed by the same letter are not significantly different, $P = 0.05$, according to ANOVA and an F test.

length per stolon was similar at 20 and 30 C for virus-free and CYVV-infected plants but was different for PSV and PSV + CYVV-infected plants. Number of nodes per centimeter of stolon was greatest in all virus treatments at 10 C and least at 20 C. Considered over all temperatures, virus-free plants had a greater number ($P = 0.05$) of nodes per centimeter of stolon than did virus-infected plants. Number of rooting nodes per stolon was generally greater at 20 and 30 C than at 10 C. The pattern of results for number of rooting nodes per stolon with regard to virus treatments was similar to that for nodes per centimeter of stolon.

TABLE 3. Dry weight (grams) of fescue harvested in the summer of 1983

Clover treatment[y]	Harvest 1 (6 May)	Harvest 2 (7 July)	Harvest 3 (8 August)
Virus-free	34.4 a[z]	20.6 c	36.1 c
CYVV	38.5 a	36.3 ab	54.3 ab
PSV	32.4 a	21.1 bc	44.4 bc
CYVV + PSV	36.9 a	44.2 a	59.1 a

[y]Virus-free = free of viruses detectable in *Chenopodium quinoa*, CYVV = infected by clover yellow vein virus, PSV = infected by peanut stunt virus, and PSV + CYVV = infected by both PSV and CYVV.
[z]Means in a column followed by the same letter are not significantly different, $P = 0.05$, according to Duncan's new multiple range test.

TABLE 4. Final mean shoot and root dry weights[x] for ladino clover plants of clone T17 grown for 83–85 days under controlled-environment conditions

Treatment[y]	Temperature (C) 10	20	30	Mean
Shoot dry weight[z] (g)				
Virus-free	0.19	1.52	1.25	0.99
PSV	0.08	0.79	0.62	0.50
CYVV	0.21	1.12	1.10	0.81
PSV + CYVV	0.04	0.60	0.35	0.33
Root dry weight[z] (g)				
Virus-free	0.21	1.00	0.53	0.58
PSV	0.06	0.46	0.23	0.25
CYVV	0.17	0.73	0.44	0.45
PSV + CYVV	0.03	0.29	0.11	0.14

[x]Values are the mean of three runs (replications in time) with eight pots per treatment per run. Temperatures were randomly assigned to chambers at the initiation of each run.
[y]Virus-free = free of viruses detectable in *Chenopodium quinoa*, CYVV = infected by clover yellow vein virus, PSV = infected by peanut stunt virus, and PSV + CYVV = infected by both PSV and CYVV.
[z]The temperature by virus treatment interaction was significant at $P = 0.05$.

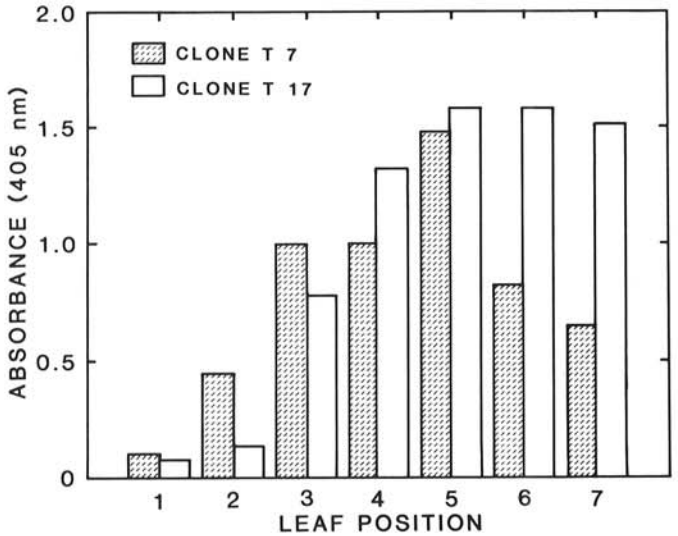

Fig. 1. Absorbance readings at 405 nm from enzyme-linked immunosorbent assay of plants of ladino clover clones T7 and T17 at seven leaf positions. Leaf position 1 was the youngest, fully expanded trifoliolate leaf on the stolon; leaf positions 2–7 represent increasing age of leaves in succession along the stolon.

Virus distribution studies. Virus distribution for plants grown in the greenhouse was measured in this study by determining the amount of coat protein antigen detectable by ELISA within plant tissue. Amount of antigen within each leaflet of a trifoliolate leaf had been determined in preliminary studies to be similar for each clover clone; however, leaf position among the 10 stolons assayed per clone was a determining factor ($P = 0.05$) for CYVV concentration for each clone (Fig. 1). CYVV was not detected in either clone at leaf position 1 but was detectable in clone T7 at leaf position 2 and in leaf positions 3–7 in both clover clones. Concentration of CYVV reached a maximum at leaf position 5 of plants for clone T7 and exhibited a decrease in detectable level of virus at leaf positions 6 and 7, which was not observed for plants of clone T17. Differences in level of virus titer existed ($P = 0.05$) between clones of leaf positions 6 and 7.

The concentration of CYVV was lower in the petioles and stolons of the two clones than in the leaf tissue for each clone. The same relative trends in CYVV concentration with regard to clones were measured in the stolons and petioles as were detected in leaves at the same position; however, the differences in virus titer between plants of clones T7 and T17 were not significant at $P = 0.05$. The virus concentration ranged from 0.144 and 0.053 in the youngest petioles to 0.579 and 0.701 in the oldest petioles of clover T7 and T17, respectively. The maximum virus titer was reached at the middle stolon position for plants of both clones, T7 and T17. While the titer in plants of clone T17 was only slightly higher in the oldest stolon section, the titer as indicated by $A_{405nm} = 0.450$ was approximately twice that at the oldest position for clover T7 ($A_{405nm} = 0.231$).

Position and time of sampling had no significant effect on virus levels in the roots of either clone. Plants of clone T17 ($A_{405nm} = 1.105$) had a higher level ($P = 0.05$) of CYVV in the roots than did plants of clone T7 ($A_{405nm} = 0.253$).

TABLE 5. Components of growth[w] for ladino clover plants of clone T17 grown for 83–85 days under controlled-environment conditions

Treatment[x]	Temperature (C)[z] 10	20	30	Mean
Stolons/plant[y] (no.)				
Virus-free	4.29	6.04	5.29	5.21
PSV	2.56	5.06	3.60	3.71
CYVV	4.00	4.75	4.75	4.51
PSV + CYVV	1.76	4.81	2.47	3.02
Length/stolon[y] (cm)				
Virus-free	1.31	5.94	5.63	4.29
PSV	1.16	4.84	3.62	3.18
CYVV	1.30	5.63	5.67	4.24
PSV + CYVV	1.07	5.24	3.66	3.32
Nodes/cm stolon[y] (no.)				
Virus-free	4.03	1.70	2.07	2.60
PSV	3.81	1.72	3.16	2.92
CYVV	4.39	1.83	2.13	2.76
PSV + CYVV	4.55	1.62	3.17	3.12
Rooting nodes/stolon[z] (cm)				
Virus-free	2.13	5.88	6.14	4.72
PSV	1.74	4.25	4.76	3.57
CYVV	2.41	5.67	4.79	4.31
PSV + CYVV	1.07	4.39	5.66	3.71

[w]Values are the means of three runs (replications in time) with eight pots per treatment per run. Temperatures were randomly assigned to chambers at the initiation of each run.
[x]Virus-free = free of viruses detectable in *Chenopodium quinoa*, CYVV = infected by clover yellow vein virus, PSV = infected by peanut stunt virus, and PSV + CYVV = infected by both PSV and CYVV.
[y]The temperature by virus treatment interaction was significant at $P = 0.05$.
[z]The temperature by virus treatment interaction was not significant at $P = 0.05$.

DISCUSSION

Initial infection of clover plants by CYVV, PSV, or CYVV + PSV reduced yield of clover compared to plants that were initially virus-free in a transplanted clover-fescue system in the field. Differences in treatments persisted with respect to yield over a growing season even though many virus-free plants became virus-infected and some of these became doubly infected. A proportion of the yield reductions observed may have resulted from stress placed on plants due to virus infection prior to transplanting into field plots. Thus, yield reductions observed in this study indicate only the potential for losses due to virus infection in clones grown in a clover-fescue system under field conditions.

Data from 1982 were more consistent over all three harvests than data from the 1983 study. Plants were severely stressed by persistent, unusually high temperatures from June to August 1983. Although irrigation was applied, growth of clover in all treatments was less at the 8 August harvest than the 7 July 1983 harvest.

Virus infection can severely reduce yield of white clover in a system similar to a pasture under normal environmental conditions, and viruses play a significant role in persistence of clover in a clover-fescue system. Although no effect on fescue yield was found in the 1982 experiment, in 1983 field plots, infection with both viruses resulted in a significant increase in yield of fescue over that of fescue in the uninfected clover treatments. In the 1983 study, plants were in the field for a longer period of time (12 mo) than in the 1982 study (4 mo). Thus, if a competition effect similar to that found by Scott (17) is involved (i.e., the grass has increased growth with less competition from clover), this effect would be more likely to be seen in the second study.

Infection of white clover by PSV is much more severe, in terms of yield reduction, than infection by CYVV. This confirms results obtained by previous researchers (10,11) and is in agreement with our results in the controlled-environment studies. In addition, we have demonstrated clonal differences with respect to the relative sensitivity to virus infection as measured by growth characteristics and yield. Thus, in general, plants of clone T17 were more tolerant to PSV-infection, and those of clone T7 were more tolerant of CYVV-infection. This is consistent with a previous report of greater tolerance of plants of clone T7 than plants of clone T17 to CYVV infection (4). These clonal differences are not unexpected since there is a wide genetic diversity of the cultivar Tillman white clover populations (12) and *Trifolium repens* populations (2,3). Loss levels were similar for PSV and PSV + CYVV-infected plants in the field, and severity of PSV infection appears to be the dominant factor. In controlled-environment studies, however, reductions in final dry shoot and root weights appeared to be additive in the PSV + CYVV treatment compared to the PSV and CYVV treatments alone.

Controlled-environment studies demonstrated which components of growth were responsible for the yield reduction seen in the field due to virus infection. With reduction in stolon per plant and length per stolon, especially in PSV and PSV + CYVV treatments, a reduction in yield would be anticipated. Also, the reduction in numbers of rooting nodes per stolon in these treatments could account, in part, for reduced persistence of similarly virus-infected plants in the field.

The data presented suggest that clone T7 and clone T17 differ in ability to support or maintain CYVV multiplication and in the distribution of CYVV within plant organs. The hypothesis that the pattern of CYVV distribution was genetically controlled rather than environmentally controlled is supported, since time of sampling over a 2.5-mo period had no effect on distribution. This difference in clonal ability to support varying levels of virus multiplication could explain how CYVV-infection of clone T7 results in lower yield losses than does CYVV infection of clone T17. Another significant factor affecting yield reduction could be the presence of considerably more detectable virus in the roots of clone T17.

The yield loss observed with these white clover clones and viruses is consistent with previous research (1,4–6,8–11,14,15) using an experimental design which mimics a pasture system under natural environmental conditions by incorporating tall fescue grass and clover in field experiments. The yield reductions can be linked to specific components of growth as indicated in this study and in previous work (11). Further, the identification of differences between clones of an existing cultivar with respect to virus distribution and the apparent relationship of these differences to yield sensitivity to virus infection provides an intriguing possibility for future efforts to reduce yield losses due to virus infection in white clover. This work also emphasizes the need for additional breeding efforts to increase clover resistance to viruses.

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