Effects of Clover Yellow Vein Virus and *Codinaea fertilis* on Growth of White Clover

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ABSTRACT

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Clones T7 and T17 of the white clover cultivar Tillman with or without clover yellow vein virus (CYVV) infection were grown in soil either infested or not infested with *Codinaea fertilis* in closed Saran cages in the greenhouse for 20 wk. All growth components of plants stressed by clipping to 3–5 cm every 14 days were less than those of unclipped plants. Clone T7 was more tolerant of CYVV and *C. fertilis* infection than was clone T17. Root rot was generally greater in CYVV-infected than in CYVV-free plants. CYVV infection reduced stolon length and number and all plant dry weight measures. *C. fertilis* infection also reduced certain growth components, but not to the extent CYVV infection did. The overall effects of CYVV and *C. fertilis* on the growth of white clover appear to be additive.

Additional key words: reduced yield

White clover (*Trifolium repens* L.) is widely used in a grass-legume mixture for pastures in most humid temperate regions and is the principal grazing forage legume in North Carolina. Stands of clover usually decline rapidly 2–3 yr after seeding, however. A combination of diseases, insect damage, adverse environmental conditions, and management practices has been associated with this decline (10,11).

Virus infection commonly occurs in stands of white clover (1,16), reducing plant vigor (5) and decreasing forage and seed yields by as much as 55% (2,12,13). The growth-limiting effects of alfalfa mosaic (AMV), clover yellow vein (CYVV), and peanut stunt (PSV) viruses on components of white clover plant growth and nitrogen fixation have been determined under controlled-environment conditions (7).

Root-rotting fungi also commonly infect white clover plants (4,14). Species of *Fusarium, Codinaea*, and *Rhizoctonia* can be readily isolated from clover roots with and without root-rot symptoms throughout the life span of a clover plant (11,19; C. L. Campbell, *unpublished*), and root diseases caused by these fungi have been cited as significant in the decline of white clover in pastures (11,14,21).

The interrelationship of viruses and root-infecting fungi has been examined infrequently in clover systems (6,18), and information is lacking on the quantitative effects of root-rotting fungi alone or in combination with viruses on growth and yield of white clover. The objective of this research was to better understand the nature of these complex relationships. Two clover pathogens, CYVV and *Codinaea fertilis* Hughes & Kendr., that frequently infect white clover in North Carolina were chosen for investigation. The experiments were conducted under controlled greenhouse conditions to remove the influence of unmanageable stresses imposed under field conditions.

MATERIALS AND METHODS

Two seedlings, designated clones T7 and T17, were selected randomly from a population of the white clover cultivar Tillman and propagated by rooting 1.5-cm stolon sections in pasteurized riverbank sand for 2 wk. CYVV-infected and CYVV-free plants of T7 and T17 were maintained in Saran screen (32 × 32 mesh) cages (0.96 × 0.87 × 1.22 m) in the greenhouse. CYVV-infected plants were selected from naturally infected sources of each clone and propagated similarly to CYVV-free plants. Plants were routinely indexed for CYVV on *Chenopodium quinoa* Willd. and confirmed by enzyme-linked immunosorbent assay as CYVV-free or CYVV-infected by O. W. Barnett and R. Baum at Clemson University. This assay also confirmed the absence of AMV and PSV in all plants.

One trial was conducted in a mixture of pasteurized loam soil and sand (3:1, v/v) either uninfested or infested with 5% cornmeal-quartz sand inoculum of *C. fertilis* (CMS) (4) to give a 1:6 CMS:soil ratio (v/v). A pasteurized Cecil clay loam soil either uninfested or infested at a 1:6 CMS:soil ratio (v/v) was used in the second trial. In both trials, sufficient 5% CMS, autoclaved at 20 psi and 180 C for 20 min, was added to give a 1:6 CMS:soil ratio (v/v) in uninfested soil. All cuttings were trimmed to two expanded trifoliolate leaves when transplanted into 10.4-cm-diameter clay pots. CYVV-infected and CYVV-free plants were placed in separate

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Table 1. Effect of clover yellow vein virus (CYVV) and *Codinaea fertilis* on growth components of unclipped plants of white clover clones T7 (CT7) and T17 (CT17) grown in a loam soil-sand (3:1, v/v) mix for 20 wk in the greenhouse

	Treatment[a]									
	V × F[b]		CYVV-free		CYVV		*C. fertilis*-free		*C. fertilis*	
Growth component	CT7	CT17	CT7	CT17	CT7	CT17	CT7	CT17	CT7	CT17
Stolons										
No./plant	···	···	14.0	30.3	13.2	11.7*	14.7	21.5	12.5	20.5
Length (cm)	···	+	10.7	11.0	8.6*	8.6	10.3	9.9	9.1	9.7
Nodes/cm (no.)	···	···	1.5	1.3	1.5	1.9*	1.5	1.7	1.6	1.6
Rooting nodes/cm (no.)	···	···	1.0	1.0	1.0	1.4	1.1	1.2	0.9	1.2
Shoots										
Final dry weight (g)/plant	···	···	10.1	42.4	9.2	18.2*	11.2	35.3	8.1	25.3+
Roots										
Final dry weight (g)/plant	···	···	4.8	7.3	3.8	2.3**	5.3	5.1	3.3*	4.4
Root rot										
Percent/plant	**	···	15.0	11.7	31.7*	16.7	0.0	0.0	46.7*	28.3**
Flowers										
No./plant	···	···	0.0	16.7	0.0	5.8*	0.0	11.8	0.0	10.7

[a] Values are "marginal" means, ie, the mean of one treatment factor averaged over the two levels of the other treatment factors (eg, CYVV-free averaged over *C. fertilis*-free and *C. fertilis*). + = Difference between levels of a factor or an interaction significant at $P = 0.10$, * = difference or interaction significant at $P = 0.05$, and ** = difference or interaction significant at $P = 0.01$.
[b] Result of the test for a significant virus × fungus interaction.

Saran screen cages immediately after transplanting.

During the trial in the soil-sand mix, plants of clone T7 were either not clipped or clipped to 3–5 cm approximately every 14 days beginning 1 mo after transplanting. Fresh and dry weights of clipped foliage were taken and cumulative totals recorded. All plants were assayed on *C. quinoa* for CYVV during week 6 of the experiment.

During the trial in the pasteurized clay loam soil, all plants were clipped once a month, beginning 1 mo after transplanting. Supplemental lighting was provided daily at 0800–2000 hours by high-intensity fluorescent lights suspended 0.9 m above the plants.

The duration of each experiment was 20 wk after transplanting (26 February–14 July and 22 July–7 December 1981). Average monthly greenhouse temperatures were 26.3 C in February, 26.7 C in March, 28.2 C in April, 23.4 C in May, 25.7 C in June, 25.9 C in July, 24.7 C in August, 24.1 C in September, 25.1 C in October, 19.2 C in November, and 17.2 C in December. After 20 wk, soil was washed from the roots in running tap water and the following characteristics were determined: flowers per plant (when present), stolons per plant, stolon length, nodes per stolon, rooting nodes per stolon, and fresh and dry weights of roots and shoots. The percentage of root rot was estimated visually, using the Horsfall-Barratt rating scale (9).

A factorial design was used with three replicates per treatment in the soil-sand mix and six per treatment in the clay soil. Treatments were CYVV-infected or CYVV-free, *C. fertilis*-infested or *C. fertilis*-free, and clipped or unclipped (clone T7, soil-sand mix only). Data for each clone were analyzed separately. The Statistical Analysis System (8) was used, and data were analyzed using analysis of variance (fixed effects model) (20) and Duncan's new multiple range test for mean separations.

Table 2. Effect of clover yellow vein virus (CYVV) and *Codinaea fertilis* on growth components of white clover clone T7 grown in loam soil-sand (3:1, v/v) mix for 20 wk in the greenhouse and clipped to 3–5 cm every 14 days

	Treatment[a]				
Growth component	V × F[b]	CYVV-free	CYVV	*C. fertilis*-free	*C. fertilis*
Stolons					
No./plant	···	4.5	3.8	4.5	3.8
Length (cm)	···	4.1	3.0	3.4	3.8
Nodes/cm (no.)	···	2.6	3.1	3.5	2.1*
Rooting nodes/cm (no.)	···	1.7	2.0	2.2	1.6
Shoots					
Final dry weight (g)/plant	···	0.3	0.2	0.2	0.3
Cumulative clipped fresh weight (g)/plant	···	19.0	8.7**	15.0	12.8
Roots					
Final dry weight (g)/plant	···	0.3	0.2	0.2	0.2
Root rot					
Percent/plant	*	26.7	40.0*	0.8	65.0*

[a] Values are "marginal" means, ie, the mean of one treatment factor averaged over the two levels of the other treatment factors (eg, CYVV-free averaged over *C. fertilis*-free and *C. fertilis*). * = Difference between levels of a factor or an interaction significant at $P = 0.05$ and ** = difference or interaction significant at $P = 0.01$.
[b] Result of the test for a significant virus × fungus interaction.

RESULTS

Infection with CYVV and *C. fertilis* reduced specific growth components and generally reduced overall forage yield. The two clones reacted differently to the disease agents.

For clone T7 grown in the soil-sand mix, clipped plants had significantly ($P \leq 0.05$) lower values than unclipped plants in every category except root rot, where values were higher (Tables 1 and 2). Root rot was greater ($P = 0.05$) in CYVV-infected plants than in CYVV-free ones. A significant interaction occurred between virus infection and root rot in both clipped ($P = 0.01$) and unclipped ($P = 0.05$) plants. *C. fertilis* reduced the number of nodes per centimeter of stolon ($P = 0.05$) in clipped plants and the final root weight of unclipped ones. CYVV-infection reduced stolon length ($P = 0.05$) in unclipped plants. Cumulative fresh weight of clipped foliage was less ($P = 0.01$) in CYVV-infected plants than in virus-free plants.

For clone T17 grown in the soil-sand mix (Table 1), CYVV infection reduced stolon number ($P = 0.05$), internode length ($P = 0.05$), and shoot and root weights ($P = 0.01$) in unclipped plants. *C. fertilis* infection reduced ($P = 0.10$) shoot weight. An interaction ($P = 0.10$) between CYVV and *C. fertilis* occurred for stolon length. CYVV did not significantly increase root rot in unclipped plants.

For clone T7 grown in the clay loam soil, stolon length ($P = 0.05$) and final stolon ($P = 0.10$), root ($P = 0.05$), and total plant ($P = 0.10$) dry weights were significantly less in CYVV-infected plants than in CYVV-free plants (Table 3). *C. fertilis* infection induced a significant ($P = 0.01$) amount of root rot on clone T7 plants but growth components were not

Table 3. Effect of clover yellow vein virus (CYVV) and *Codinaea fertilis* on growth components of white clover clones T7 (CT7) and T17 (CT17) grown in pasteurized clay loam soil for 20 wk in the greenhouse and clipped to 3–5 cm monthly

	Treatment[b]							
	CYVV-free		CYVV		*C. fertilis*-free		*C. fertilis*	
Growth component[a]	CT7	CT17	CT7	CT17	CT7	CT17	CT7	CT17
Stolons								
No./plant	7.3	4.8	7.3	3.4+	7.8	4.8	6.8	3.4+
Length (cm)	5.1	5.4	3.5*	2.6**	4.5	4.8	4.1	3.2**
Nodes/cm (no.)	2.7	2.7	2.5	4.9**	2.5	3.3	2.7	4.4*
Rooting nodes/cm (no.)	2.0	2.0	1.7	4.3**	1.7	2.7	2.0	3.7+
Dry weights (g)								
Leaves, clip 1[c]	0.48	0.48	0.28	0.24+	0.40	0.48	0.37	0.24+
Leaves, clip 2	0.25	0.32	0.14	0.12*	0.20	0.27	0.19	0.17
Leaves, clip 3	0.49	0.57	0.28+	0.19**	0.38	0.45	0.38	0.30+
Leaves and petioles, clip 4	0.80	0.75	0.61	0.41**	0.75	0.69	0.66	0.47
Stolons	0.81	0.63	0.45+	0.27**	0.67	0.54	0.58	0.35+
Roots	0.89	0.89	0.51*	0.40**	0.74	0.68	0.66	0.61
Total	2.50	2.27	1.57+	1.07**	2.17	1.91	1.91	1.43+
Root rot								
Percent/plant	10.0	10.5	14.5	15.1	0	0	24.5**	25.5**

[a] Except for leaf clips 1–3, measurements or estimates were made at the termination of the experiment (8 December 1981). The experiment was begun 22 July 1981.

[b] Values are "marginal" means, ie, the mean of one treatment factor averaged over the two levels of the other treatment factors (eg, CYVV-free averaged over *C. fertilis*-free and *C. fertilis*). A significant virus × fungus interaction was not found for any growth component. + = Difference between levels of a factor at $P = 0.10$, * = difference at $P = 0.05$, and ** = difference at $P = 0.01$.

[c] Clip 1 on 8 September 1981, clip 2 on 8 October 1981, clip 3 on 9 November 1981, and clip 4 on 8 December 1981.

Table 4. Total dry weights for leaves and petioles, stolons, roots, and total plants of white clover clones T7 and T17 grown in pasteurized clay loam soil for 20 wk in the greenhouse and infected with clover yellow vein virus (CYVV) or *Codinaea fertilis*, or both

Treatment		Dry weight (g)			
CYVV	*C. fertilis*	Leaves and petioles	Stolons	Roots	Total plant
Clone T7					
–[a]	–	0.84[b]	0.85	0.91	2.61
–	+	0.75	0.76	0.88	2.39
+	–	0.66	0.49	0.57	1.72
+	+	0.56	0.41	0.45	1.42
Clone T17					
–	–	0.93	0.79	0.97	2.69
–	+	0.57	0.46	0.82	1.86
+	–	0.46	0.28	0.40	1.13
+	+	0.36	0.24	0.40	1.01

[a] – = Uninfected and + = infected.

[b] Use of a mean separation test was not appropriate because no significant interaction was found between the effects of CYVV and *C. fertilis*.

significantly different between infected and uninfected plants. Dry weight of clipped foliage was reduced ($P = 0.10$) by CYVV infection at clip 3.

For clone T17 grown in the clay loam soil, stolon number ($P = 0.10$) and length ($P = 0.01$) and all final plant dry weight measures ($P = 0.01$) were significantly lower in CYVV-infected plants than in CYVV-free plants (Tables 3 and 4). *C. fertilis* infection reduced number ($P = 0.10$), length ($P = 0.01$), and final dry weight of stolons ($P = 0.10$) and total plant weight ($P = 0.10$). CYVV and *C. fertilis* infection both significantly reduced internode length, indicated by increased numbers of nodes (CYVV $P = 0.01$, *C. fertilis* $P = 0.05$) and rooting nodes per centimeter of stolon (CYVV $P = 0.01$, *C. fertilis* $P = 0.10$) than in plants without either infection. Dry weight of clipped foliage was reduced at each clipping by CYVV infection ($P = 0.10$, 0.05, 0.01, and 0.01 for clips 1–4, respectively) but only at clips 1 ($P = 0.10$) and 3 ($P = 0.10$) by *C. fertilis* infection. Percentage of root rot was not significantly altered by virus infection.

Percentage of root rot was generally lower in the clay loam soil than in the soil-sand mix. Average root rot ratings were 33.4% (clone T7, clipped), 23.4% (clone T7, unclipped), and 14.2% (clone T17, unclipped) for the soil-sand mix and 12.3% (clone T7) and 11.4% (clone T17) for the clay loam soil.

DISCUSSION

CYVV and *C. fertilis* reduced plant growth components in white clover. The effects of these pathogens appeared to be additive, and the magnitude of the effects depended on inherent clonal differences and the degree of stress to which plants were subjected.

Frequent clipping of foliage severely stresses white clover plants and promotes development of root rot (17). This was manifest in reduction in every growth component in frequently clipped plants compared with unclipped plants (Tables 1 and 2). The more frequent clipping in the trial conducted in the soil-sand mix than in the trial in the clay loam soil may explain the detection of more differences in growth between CYVV-infected and CYVV-free plants in clay loam soil. Root rot severity also was increased by frequent clipping. Thus, frequent grazing of pastures or frequent harvesting of clover-grass hay may be an important predisposing factor in the decline of white clover in clover-grass pastures.

White clover clones T7 and T17 differed in degree of tolerance to CYVV and *C. fertilis*. Clone T7 was more tolerant to CYVV and *C. fertilis* than was clone T17. This difference in clones derived from Tillman white clover is not surprising, since Tillman has a wide gene base (7,15) and is a population of plants with a degree of genetic diversity.

The reduction in stolon growth, either number per plant or length per stolon, associated with CYVV and *C. fertilis* and the increase in number of nodes and rooting nodes per centimeter of stolon in clone T17 merit further investigation in view of the decline of white clover in pasture systems. After 12–24 mo, taproots of white clover are absent in the field (11) because of disease and insect damage or perhaps natural degeneration, and plant survival depends to a large extent on stolon roots. Because leaves are the harvested portion, total productivity depends on length and vigor of stolons producing leaves. Thus, if stolon number and/or length are reduced, clover yield and probability of plant survival may be proportionally reduced. The importance

of stolon branching and root development at nodes on future plant growth has been emphasized (3). The root-rotting effects of *C. fertilis* may contribute further to clover decline by reducing total root volume and thereby limiting nutrient and water uptake.

The overall effects of CYVV and *C. fertilis* were additive, although a significant CYVV × *C. fertilis* interaction was found in the soil-sand mix for clone T7. Effects of CYVV and *C. fertilis* alone or combined were evident in plant dry weight measurements (Table 4). From these results, we would postulate reduced clover yield and possibly reduced persistence in pastures in which clover is infected with CYVV and/or *C. fertilis*.

Gibson et al (7) found CYVV to be less damaging than AMV or PSV to clover; PSV damaged white clover the most. The potential yield-reducing effects of PSV alone or combined with CYVV or AMV and associated with root rot fungi such as *C. fertilis* should be determined, since of the three viruses, only PSV significantly affected root length. Future work should also attempt to quantify the effects of these viruses and root-infecting fungi on persistence and yield of white clover plants under pasture conditions.

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