

Effects of Three Viruses on Growth of White Clover

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ABSTRACT

GIBSON, P. B., O. W. BARNETT, H. D. SKIPPER, and M. R. McLAUGHLIN. 1981. Effects of three viruses on growth of white clover. *Plant Disease* 65:50-51.

The effects of single infections by alfalfa mosaic, clover yellow vein, and peanut stunt viruses on components of vegetative growth, neutral-detergent fiber and nitrogen contents, and nodulation of white clover plants were studied for 1 mo in a controlled environment chamber. Leaf dry weight, nodulation, and seven other components of growth were reduced by all three viruses. Other growth components studied, such as leaves per plant, petiole length, and rooting nodes in primary stolon, were reduced by one or more of the viruses. No effects of viruses on neutral-detergent fiber and nitrogen contents were detected. The detrimental effects of virus infections clearly indicate a need for virus control in white clover.

Additional key words: *Rhizobium trifolii*, *Trifolium repens* L.

White clover, *Trifolium repens* L., is used widely as a pasture legume in most humid temperate regions. It contributes high quality forage and biologically fixed nitrogen (5). Viruses that infect white clover are widely distributed in the United States (1). Although reductions in forage and seed yields caused by virus infections have been estimated (2,5-7), little information is available on the effects of individual viruses on components of plant growth and on nitrogen fixation. Such information is needed to establish priorities and provide guidance for research.

The objective of our research was to determine the effects of three widely distributed viruses, alfalfa mosaic (AMV), clover yellow vein (CYVV), and peanut stunt (PSV), on components of vegetative growth, neutral detergent fiber and nitrogen contents, and nodulation of white clover.

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Contribution of Agricultural Research, SEA, USDA, in cooperation with the South Carolina Agricultural Experiment Station. Funded in part by cooperative agreement 12-14-7001-1124 and in support of Regional Project S-127, Forage Legume Viruses. Published with the approval of the Director of the South Carolina Agricultural Experiment Station as Paper 1750.

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MATERIALS AND METHODS

Ladino, a white clover cultivar that has a wide gene base and is representative of the large type of *T. repens*, was used in the experiment. Ten seedlings were selected at random and propagated vegetatively as clonal lines. One plant of each clone was maintained free of viruses, and three were inoculated individually with AMV, CYVV, and PSV by using sources of viruses and inoculation techniques previously reported (1). A stolon tip from each plant was rooted in sand and transplanted into a 20 × 28 × 10-cm stainless steel flat containing an amended soil growth medium. At transplanting, the stolon tips were pruned to uniform length and positioned at the ends of the flats so that the apical buds would grow toward the opposite ends.

The growth medium for each flat was prepared by compositing equal parts by volume of Davidson clay soil, sand, and a potting mixture that consisted of equal parts of sphagnum moss and vermiculite. Phosphorus and potassium fertilizers and lime were added as needed, as indicated by a soil test, to support vigorous growth of clover. On the day before transplanting the stolons, the medium in each flat was mixed with about 1.5 g of a commercial *Rhizobium trifolii* inoculum for white clover (courtesy Nitragin Co., Milwaukee, WI) and was moistened with water.

Throughout the test, the plants were held in a controlled environment chamber at a continuous temperature of 25 C and illuminated 12 hr/day with 10.8 lx supplied by 32 6-ft fluorescent and four 40-W incandescent lamps. The environment was selected to minimize flowering and to support vigorous vegetative growth with pronounced expression of virus symptoms (8,12).

After each transplanted stolon produced one or more leaves, all unfolded leaves except the apical leaf were pruned and the position of each apical bud was marked by placing a wire staple over it. The marked stolon is referred to hereafter as the primary stolon. We pruned new buds behind the staple as they appeared, thereby restricting growth to that of the marked bud. All data were collected from the new growth. The tips of primary stolons of several plants grew to the ends of their respective flats in 1 mo at which time we terminated the experiment and made appropriate counts, measurements, and analyses (4,15).

Nodulation was rated visually on a 0-10 scale with 0 for no nodules and 10 for well-nodulated plants. Size, shape, color, and number of nodules were considered. The small amount of plant material provided by 1-mo growth limited chemical analyses to determinations of nitrogen and neutral detergent fiber contents. The total soluble carbohydrates and fats were estimated by subtracting the sum of neutral detergent fiber and protein ($6.25 \times N$) from 100.

Plants were arranged in 10 blocks, each containing four plants of a clone, arranged at random. At harvest, three leaves from each inoculated plant were tested for virus by enzyme-linked immunosorbent assay (9). Virus was not detected in 10 plants that had been inoculated; therefore, data from these plants were not included in the analysis. Because of these missing values, data were analyzed by the least squares method.

RESULTS

Virus infections reduced forage yields and other growth components but did not affect composition of plant material (composite of leaves and stolons) as indicated by percent neutral detergent fiber and nitrogen (Table 1). All three viruses reduced nine of the growth components. AMV and PSV reduced growth at the nodes, as indicated by reductions in the numbers of rooting nodes, secondary stolons, and leaves. Infections with PSV also resulted in reductions in number of nodes on the primary stolon, petiole length, root length, and total nitrogen. These effects are generally associated with stunting of plants.

All three viruses severely inhibited

Table 1. Effect of alfalfa mosaic (AMV), clover yellow vein (CYVV), and peanut stunt (PSV) viruses on growth components, nodulation, and percent nitrogen and neutral-detergent fiber of white clover^a

Component	Virus-free control	AMV	CYVV	PSV
Affected by AMV, CYVV, and PSV				
Primary stolon length (cm)	21.8	16.8**	19.6**	12.2**
Secondary stolons, total length (cm)	36.0	15.8**	26.2**	8.2**
Nodes in secondary stolons (no.)	32.0	21.3**	26.3*	16.6**
Rooting nodes in secondary stolons (no.)	12.1	4.5**	7.1**	0.7**
Nodulation on roots at oldest node ^c	6.9	3.4**	5.4**	3.8**
Nodulation, total of scores on roots at next five nodes ^c	25.1	5.7**	19.8*	9.2**
Leaves, total dry weight (g)	1.1	0.7**	0.9*	0.5**
Stolons, total dry weight (g)	0.4	0.3**	0.4*	0.2**
Neutral detergent fiber per plant (mg)	0.4	0.3*	0.3*	0.2**
Affected by AMV and PSV				
Rooting nodes in primary stolon (no.)	10.5	8.4*	10.8	8.1**
Secondary stolons (no.)	8.1	6.4*	7.1	6.4*
Leaves per plant (no.)	37.8	26.1**	35.7	25.0**
Affected by PSV				
Nodes in primary stolon (no.)	12.5	11.4	12.5	10.8**
Petiole length (cm)	16.4	16.5	15.6	13.0**
Longest roots on plant (cm)	65.3	55.6	60.1	48.2**
Nitrogen per plant (mg)	0.06	0.05	0.05	0.04*
Not affected by any of these viruses				
Neutral detergent fiber in composite of leaves and stolons (%)	27.8	27.6	27.8	25.9
Nitrogen in composite of leaves and stolons (%)	3.8	4.0	4.0	4.0

^a Plants of each of 10 clones were inoculated with the three viruses, grown in a controlled environment chamber at 25 C, with 12-hr daylength, and harvested after 1 mo.

^b * Differs from control at $P = 0.05$. ** Differs from control at $P = 0.01$.

^c Nodulation rated on 0-10 scale: 0 = no nodules, 10 = well nodulated.

nodulation over the entire root system, thereby reducing the plant's potential for nitrogen fixation. Although the viruses did not alter the nitrogen concentration in stolons and leaves, the total nitrogen content per plant was reduced by PSV. Similar effects of virus infections on nodulation and nitrogen fixation have been reported in other legumes (10,11,13,14). AMV had the greatest effect on nodulation, whereas PSV had the greatest effect on total nitrogen and total growth.

In general, the conditions chosen for the experiment were highly satisfactory. The soil medium supported vigorous growth and separated easily from the roots, thereby facilitating the rating of nodulation. Only seven flowers were produced, and all of these were on infected plants. Larger flats that would permit a longer growth period would provide more plant material for chemical analysis and would permit more advanced development of nodules.

DISCUSSION

Evaluation of virus effects and their magnitude indicated that PSV damaged white clover the most, followed in order of decreasing damage by AMV and

CYVV. Regardless of the differences among effects of individual viruses, the adverse effect of each virus was severe; therefore, in choosing priorities for control, differences in rates of spread and incidence of infections in pastures are probably more important than the small differences in severity of damage.

Although the results indicate the detrimental effects of the viruses on the nitrogen fixation process, more research is needed to explain the action of viruses. The mechanism probably is related to an impeding of photosynthesis and translocation of photosynthate thereby restricting the energy available to bacteria in the nodules.

The importance of virus effects on yield are obvious; the effects on longevity and continued production are less obvious. Stolon branching and root development at nodes particularly influence future growth (3). We believe reductions in frequency of rooting nodes and stolon branching as well as reductions in nodulation and length of roots indicate that virus infection weakens white clover plants and consequently could render them more susceptible to damage by other stresses.

Our results indicate that although white clover plants were not killed by

virus infections, their immediate and potential forage production was adversely affected. Furthermore, plants weakened by virus infection probably are more susceptible to damage by other diseases and environmental stresses than uninfected plants. The need for a virus disease control program in white clover is clearly indicated.

ACKNOWLEDGMENTS

We thank P. M. Burrows for assistance with data analyses, A. K. Torrence for chemical analyses, and R. B. Baker, J. L. Jackson, F. D. King, and V. J. McAlister for technical assistance.

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