Influence of Seeding Rate and Interplanting with Tall Fescue on Virus Infection of White Clover

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ABSTRACT

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The effects of white clover seeding rate and tall fescue seeding method on virus infection of white clover were determined in field plots during 1986 and 1987. Incidence of peanut stunt virus (PSV) in the cultivar Regal in July and November 1986 was greater where clover had been sown at 0.56 kg/ha than where it had been sown at 1.68 or 3.36 kg/ha. Clover yield measured after virus sampling of plots sown at the two higher rates was similar and was greater than that of plots sown at the lowest rate. Although clover yield in July 1987 was similar across clover seeding rates, incidence of PSV decreased as seeding rates increased. Incidence of white clover mosaic virus (WCMV) was lower and that of alfalfa mosaic virus (AMV) was higher in 1987 at successively greater seeding rates. The method of seeding tall fescue (broadcast vs. drilled) did not influence the incidence of virus infection. Reduced incidence of WCMV and AMV infection of white clover sown with tall fescue compared with monoculture was a benefit of this forage combination.

White clover (*Trifolium repens* L.) is a perennial forage legume commonly established in perennial grass pastures in the southeastern United States to improve forage productivity and quality. Stands of white clover often begin to decline in the second year after establishment (3). This decline in yield and persistence has been associated with a number of factors, among them virus infections (7,19). Several viruses, including alfalfa mosaic virus (AMV), clover yellow vein virus (CYVV), peanut stunt virus (PSV), and white clover mosaic virus (WCMV), are known to infect white clover in the southeastern United States (1,18). Studies documenting the significant negative effects of single and multiple infections by these viruses on clover yield, quality, and persistence have been thoroughly reviewed (4).

Although persistence of white clover has been improved under experimental conditions by pesticide application (3,8,11), broad use of pesticides in pastures may be uneconomical and ecologically undesirable. Lewis et al (14) and Scott (22) reported that initial incidence and rate of spread, respectively, of WCMV in red clover (*T. pratense* L.) were reduced when the clover was interplanted with ryegrass (*Lolium multiflorum* Lam.). Other management factors influencing virus incidence include the number of years a pasture remains planted to perennial clover and whether the clover persists from year to year as a perennial plant or by annual reseeding (1).

In the present study, we examined two management practices that can be employed to alter the ecology of grass-clover pastures, i.e., white clover seeding rate and grass seeding method. The effects of reducing white clover seeding rate and drilled vs. broadcast seeding of tall fescue (*Festuca arundinacea* Schreb.) were compared in terms of clover virus incidence. Grass drilled in rows may act as a barrier against the spread of viruses across the sward, similar to the manner in which corn planted between susceptible soybean crops prevents the spread of peanut mottle virus (5). The primary objective of this study was to determine the effects of these management practices on the incidence of PSV. Although PSV is considered the most prevalent and damaging virus disease of white clover in the Southeast (6,18,19), the incidences of other viruses known to infect white clover in this region were also measured. These were CYVV and AMV (aphid-transmitted) and WCMV (mechanically transmitted). The reported incidence of CYVV among white clover bait plants in the region is significantly higher than that of AMV and WCMV (18). AMV and WCMV were included, however, because they are reported to increase in incidence among experimental plants subjected to repeated cutting (10,18) and because our study used periodic harvesting practices.

MATERIALS AND METHODS

Seed of tall fescue cultivar Kentucky 31 free of endophyte (*Acremonium coenophialum* Morgan-Jones & Gams) infection was broadcast or drilled in rows 25 cm apart on 3 × 5 m plots at 16.8 kg/ha, and white clover cultivar Regal was oversown at 0.56, 1.68, and 3.36 kg/ha, the recommended rate (13), in late August of 1985 at Mississippi State, Mississippi. White clover was also sown in monoculture at 3.36 kg/ha. White clover seedling density was determined 14 days after seeding by counting seedlings within two randomly placed 0.25-m^2 quadrats. The experiment was a three-by-two factorial (three white clover seeding rates and two tall fescue seeding methods) with one control treatment (monoculture white clover sown at 3.36 kg/ha) arranged in a randomized complete block design with four replications. Lime, phosphorus, and potassium were added to the Catalpa silty clay soil (fine, montmorillonitic, thermic Fluvaquentic Hapludoll) according to soil test recommendations and were maintained at recommended levels throughout the experiment.

Whenever plot sward height reached approximately 25 cm in 1986 and 1987, forage yield was measured by cutting two 0.5-m swaths at a 70-mm stubble height through each plot with a rotary mower. A 600- to 800-g subsample was taken from each yield sample and dried at 65 C for 48 hr to determine forage dry matter. Botanical composition of each plot was determined by harvesting duplicate 0.125-m^2 quadrats at a 70-mm stubble height outside the harvest swaths and manually separating the white clover and tall fescue components.

The incidence of PSV, CYVV, AMV, and WCMV in white clover was assessed before harvest on 1 July and 10 November 1986 and on 2 July 1987. On each sampling date, 10 samples, each consisting of six to eight intact clover leaves, were collected at 1-m intervals on a square grid pattern 1 m from the long edge and 0.5 m from the short edge of each plot. Samples were held between layers of moist paper towels and stored at 5 C until processed for virus testing. Within 24–48 hr of collection, samples

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were homogenized with a motorized sap extractor in 0.02 M sodium phosphate buffer, pH 7.3, containing 0.05% (v/v) Tween 20 and 0.02 M sodium diethyldithiocarbamate. Homogenates were tested for the presence of viruses by the double antibody sandwich enzyme-linked immunosorbent assay (ELISA) (17), using antisera to AMV, CYVV, WCMV, and PSV in 1986 and to AMV, CYVV, and WCMV in 1987 (16). Absorbance measurements for ELISA were made at 405 nm on a BioTek Model 307 EIA Reader (BioTek Instruments, Inc., Burlington, VT) in 1986 and a BioTek Model EL 309 EIA Reader in 1987. Positive and negative control wells were included with all tests. Test wells were rated positive if their absorbance exceeded the mean absorbance plus two standard deviations of negative control values. Tests for PSV in 1987 were conducted by mechanically inoculating the primary leaves of 8-day-old cowpea seedlings (*Vigna unguiculata* (L.) Walp. 'California Blackeye') with aliquots of the sap homogenates prepared for ELISA, using 600-mesh Carborundum and cotton-tipped applicators. Cowpea plants infected by PSV developed chlorotic local lesions on primary leaves and systemic veinclearing and mosaic symptoms in trifoliolate leaves. Plants were evaluated for characteristic PSV symptoms 14 days after inoculation.

Data were analyzed by analysis of variance (ANOVA) using the Statistical Analysis System (21). Comparisons between treatments were made using Fisher's least significant difference (LSD) test ($P = 0.05$), except where noted.

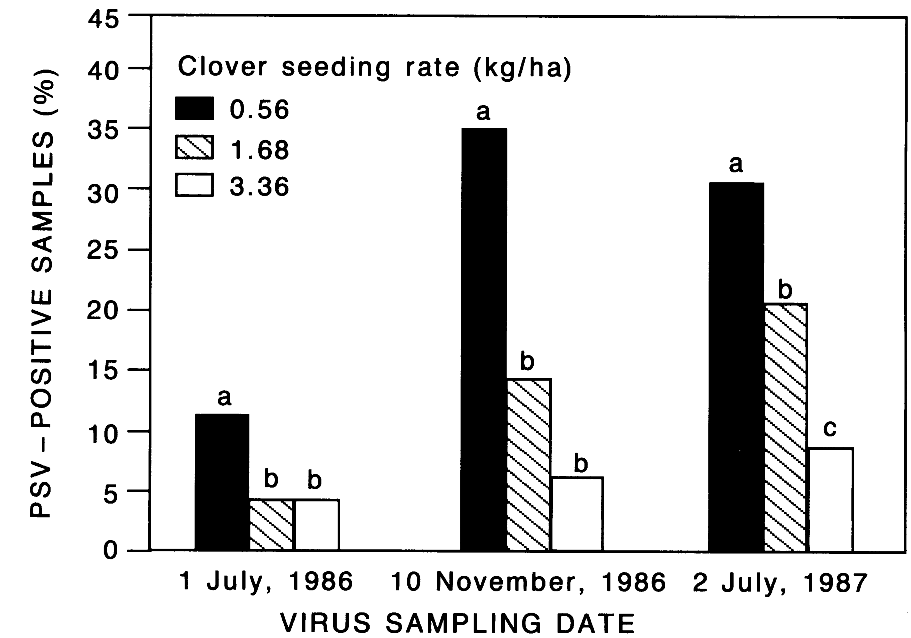

Fig. 1. Effect of white clover seeding rate on peanut stunt virus (PSV) incidence in white clover on three dates. Percentages within a sampling date having the same letter are not different at $P = 0.05$.

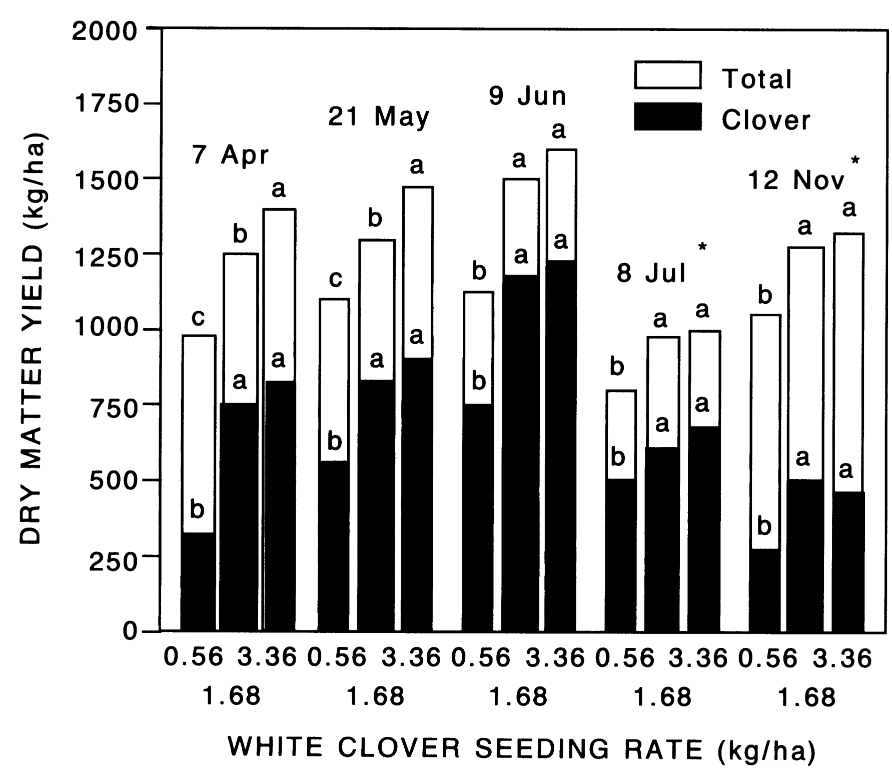

Fig. 2. Effect of white clover seeding rate on total and clover dry matter yield at five harvest dates during 1986. Total or clover dry matter yields within a harvest date having the same letter are not different at $P = 0.05$. * = Harvest date immediately following virus sampling.

RESULTS AND DISCUSSION

Average densities of white clover seedlings 14 days after seeding at 0.56, 1.68, and 3.36 kg/ha were, respectively, 71, 238, and 448 seedlings per square meter. There was no significant difference in seedling density where white clover had been sown at a similar rate in monoculture or with broadcast or drilled tall fescue.

Incidence of PSV infection of white clover in the first year (sampled on 1 July and 10 November 1986) was greater where white clover had been sown at 0.56 kg/ha than where it had been sown at 1.68 and 3.36 kg/ha (Fig. 1). In the second year, PSV incidence was lower at successively greater seeding rates. A similar trend was observed by Heathcote (9), who found that incidence of beet mild yellow virus of sugar beet at 17,500 plants per hectare was 51%, compared with 15% when at 126,500 plants per hectare. The increase in PSV incidence with successive sampling dates was likely due to the combined initial movement of virus into the plots from external sources of primary inoculum and subsequent secondary movement of virus within plots.

Unlike the sugar beet plants utilized by Heathcote (9), white clover plants increase in size by lateral growth of rooting stolons, particularly where competition from grass is minimal, thus compensating for initial differences due to density of seedlings. Marshall and James (15) reported that stolon density of white clover cultivar Olwen established at 48 and 100 plants per square meter was similar (99 vs. 84 m/m^2) but was greater than that established at nine plants per square meter (50.3 m/m^2). On the 8 July and 12 November 1986 harvests after virus sampling, clover yields among seeding rate treatments were greater at 1.68 and 3.36 kg/ha than at 0.56 kg/ha (Fig. 2). Thus, the quantity of leaf and stem tissue available for virus infection was similar at the two higher seeding rates but greater than that available at the lower rate. Although there were significant differences in PSV incidence among the seeding rate treatments on 2 July 1987

(Fig. 1), there were no differences in clover yield among the three seeding rates at the 14 July 1987 harvest (Fig. 3). Severe drought may have been a major factor influencing clover yield at this time in the growing season.

Overall incidence of CYVV was very low (means of 1, 2, and 13%, respectively, on 1 July and 10 November 1986 and 2 July 1987) and precluded detection of effects of seeding on CYVV incidence on all three sampling dates. Incidence of AMV and WCMV were affected by seeding rate only on the final sampling date (2 July 1987). Similar to PSV incidence, WCMV incidence was lower at successively greater seeding rates (43, 36, and 29% at 0.56, 1.68, and 3.36 kg/ha, respectively). In contrast, AMV incidence was greater ($P = 0.10$) at successively greater seeding rates (18, 28, and 35% at 0.56, 1.68, and 3.36 kg/ha, respectively). These opposing trends indicate that different epidemiological factors likely influenced movement and buildup of these viruses. The spread of WCMV, a monopartite genome potexvirus with no known insect vector (2), was presumably due to mechanical transmission by the rotary mower used in harvesting. Spread of PSV, a multipartite genome cucumovirus (20), and AMV, a multipartite genome virus (12), both of which may be mechanically or nonpersistently aphid-transmitted, was presumably due to the rotary mower and aphids. Thus, differences in viral genome encapsidation and in mechanism of transmission (mechanical vs. aphid) cannot readily account for the difference in response to seeding rate for AMV compared with PSV and WCMV.

Tall fescue seeding method (broadcast vs. drilled) did not influence white clover virus incidence at any of the three sampling dates. An agronomic advantage that drilling had over broadcasting tall fescue was to increase annual clover yield both years. Annual white clover yield was less at the 1.68 kg/ha seeding rate than at the 3.36 kg/ha rate when tall fescue was broadcast (2-yr mean of 3,161 vs. 3,362 kg/ha, C.V. = 10%) but was equivalent at the two seeding rates when tall fescue was drilled (3,585 vs. 3,464 kg/ha, C.V. = 8%).

Similar to results observed by Lewis et al (14) for red clover-ryegrass mixtures, seeding white clover with tall fescue (drilled or broadcast) compared with white clover in monoculture reduced AMV incidence at the last two sampling dates from 35 to 10% and from 80 to 35%, respectively, whereas incidence of WCMV was reduced from 95 to 30% at the last sampling date. Thus, although tall fescue often vigorously competes with clover in a pasture for other limiting resources, interplanting these species is beneficial to clover production in terms of reduced virus incidence.

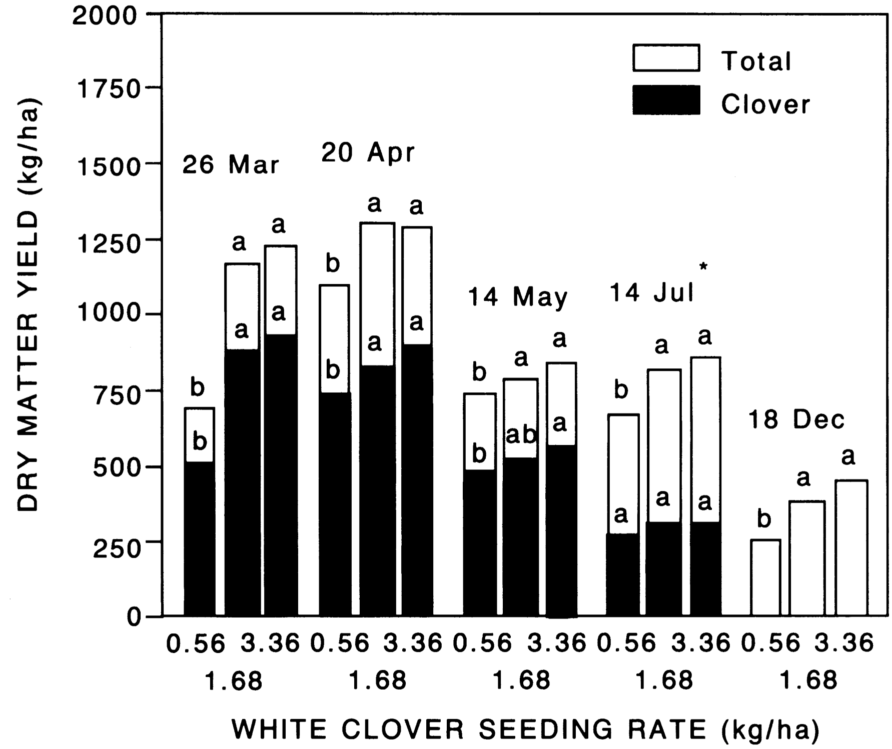

Fig. 3. Effect of white clover seeding rate on total and clover dry matter yield at five harvest dates during 1987. Total or clover dry matter yields within a harvest date having the same letter are not different at $P = 0.05$. * = Harvest date immediately following virus sampling.

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. Bercks, R. 1971. White clover mosaic virus. No. 41 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, England.
3. Blake, C. T., Chamblee, D. S., and Woodhouse, W. W., Jr. 1966. Influence of some environmental and management factors on the persistence of ladino clover in association with orchardgrass. Agron. J. 58:487-489.
4. Campbell, C. L. 1986. Quantifying clover yield losses due to virus diseases. Pages 736-742 in: Forage Legume Viruses. Vol. 3. Monogr. 14. J. R. Edwardson and R. G. Christie, eds. Agric. Exp. Stn., Inst. Food Agric. Sci., University of Florida, Gainesville.
5. Demski, J. W., and Kuhn, C. W. 1977. A soybean disease caused by peanut mottle virus. Ga. Agric. Exp. Stn. Res. Bull. 196. 36 pp.
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., and Hollowell, E. A. 1966. White clover. U.S. Dep. Agric. Handb. 314. 33 pp.
8. Graham, J. H., Sprague, V. G., and Zeiders, K. E. 1961. Effect of management and pesticides on persistence of Ladino white clover. Agron. J. 53:356-357.
9. Heathcote, G. D. 1974. The effect of plant spacing, nitrogen fertilizer and irrigation on the appearance of symptoms and spread of virus yellows in sugarbeet crops. J. Agric. Sci. 82:53-60.
10. Hiruki, C., and Miczynski, K. A. 1987. Severe isolate of alfalfa mosaic virus and its impact on alfalfa cultivars grown in Alberta. Plant Dis. 71:1014-1018.
11. 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.
12. Jaspars, E. M. J., and Bos, L. 1980. Alfalfa mosaic virus. No. 229 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, England.
13. Kimbrough, E. L., Watson, V. H., and Knight, W. E. 1984. Forage types, varieties, planting dates, and rates. Miss. Coop. Ext. Serv. Inf. Sheet 1168.
14. Lewis, G. C., Heard, A. J., Gutteridge, R. A., Plumb, R. T., and Gibson, R. W. 1985. The effects of mixing Italian ryegrass (*Lolium multiflorum*) with perennial ryegrass (*L. perenne*) or red clover (*Trifolium pratense*) on the incidence of viruses. Ann. Appl. Biol. 106:483-488.
15. Marshall, A. H., and James, I. R. 1988. Effect of plant density on stolon growth and development of contrasting white clover (*Trifolium repens*) varieties and its influence on the components of seed yield. Grass Forage Sci. 43:313-318.
16. McLaughlin, M. R., Barnett, O. W., Burrows, P. M., and Baum, R. H. 1981. Improved ELISA conditions for detection of plant viruses. J. Virol. Methods 3:12-25.
17. McLaughlin, M. R., Barnett, O. W., Gibson, P. B., and Burrows, P. M. 1984. Enzyme-linked immunosorbent assay of viruses infecting forage legumes. Phytopathology 74:965-969.
18. McLaughlin, M. R., and Boykin, D. L. 1988. Virus diseases of seven species of forage legumes in the southeastern United States. Plant Dis. 72:539-542.
19. McLaughlin, M. R., and Pederson, G. A. 1985. Coincidence of virus diseases and decline of white clover in a Mississippi pasture. (Abstr.) Phytopathology 75:1359.
20. Mink, G. I. 1972. Peanut stunt virus. No. 92 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, England.
21. SAS Institute, Inc. 1985. SAS User's Guide: Statistics. SAS Institute, Inc., Cary, NC. 956 pp.
22. Scott, S. W. 1982. The effects of white clover mosaic virus infection on the yield of red clover (*Trifolium pratense* L.) in mixtures and in pure stands. J. Agric. Sci. 98:455-460.