Quantification of Yield Benefits from Incorporation of Virus-Resistant White Clover Germ Plasm into Grass-Legume Systems

N. L. Taylor, Department of Agronomy, and **S. A. Ghabrial,** Department of Plant Pathology, University of Kentucky, Lexington 40546; and **G. A. Pederson** and **M. R. McLaughlin,** USDA-ARS, Forage Research Unit, P.O. Box 5367, Mississippi State, Miss. 39762

ABSTRACT

Taylor, N. L., Ghabrial, S. A., Pederson, G. A., and McLaughlin, M. R. 1995. Quantification of yield benefits from incorporation of virus-resistant white clover germ plasm into grass-legume systems. Plant Dis. 79:1057-1061.

The performance of the virus-susceptible white clover (*Trifolium repens*) cultivar Regal was compared in broadcast and spaced-plant plots with three experimental breeding populations (Southern Regional Virus Resistant synthetic [SRVR], Brown Loam Synthetic No 2 [BL Syn. 2] and Florida Experimental No. 4 [FL Exp. 4]) expressing varying levels of resistance to viruses endemic to forage legumes in the southeastern United States. The experimental plots were established with and without grasses in fields at Lexington, Ky., and Mississippi State, Miss., with the objective of comparing the magnitude of yield losses from virus diseases in spaced-plant nurseries with those in broadcast plots that more closely resemble farm conditions. Data from virus incidence in spaced plants in both locations indicated that SRVR had significantly (least significant difference, $P = 0.05$) lower incidence of peanut stunt virus (PSV) and clover yellow vein virus (CYVV) than Regal. Although significantly lower estimates of PSV and/or CYVV incidence were also obtained with BL Syn. 2 and FL Exp. 4 at some testing dates, there were little or no differences at others. Furthermore, higher estimates of PSV and CYVV incidence were determined for spaced plants grown in plots without grass than those with grass. Unlike PSV and CYVV, incidence of alfalfa mosaic virus was similar among treatments. Data on yield, stand, and virus incidence in broadcast plots closely paralleled those of spaced plants. Percentage of virus incidence in broadcast plots in Kentucky, however, was generally lower than that in the spaced plants. The overall data indicated that the performance of SRVR, based on yield (14 to 55% higher than Regal) and resistance to PSV and CYVV, was superior to the other germ plasms and suggest that without virus resistance improved white clover yields and persistence cannot be obtained.

White clover (*Trifolium repens* L.) is an important forage legume that plays a vital role in the stabilization of soil, and replenishment of crop nutrients. It improves both the quantity and the quality of forage produced. White clover achieves its greatest importance as a pasture legume in the southeastern United States. In 1969, 70% of U.S. white clover hectarage was in the Southeast (15). In 1984, white clover was still the predominant perennial legume for the Southeast (13).

A major constraint on productivity and stand longevity of white clover has been identified as infection by viruses. Alfalfa mosaic virus (AMV), clover yellow vein virus (CYVV), peanut stunt virus (PSV), red clover vein mosaic virus (RCVMV), subterranean clover red leaf strain of soybean dwarf virus (SDV), and white clover mosaic virus (WCMV) have been reported to infect white clover in the southeastern United States (2,3,16–19). AMV, CYVV, PSV, and RCVMV are transmitted in a nonpersistent manner by aphids (8).

Studies of the detrimental effects of viruses have been conducted under greenhouse conditions and in the field with spaced plants or in broadcast-sown plots (9,11,19,22). While data from these environments aid our understanding of virus action, those from broadcast-sown plot studies come closest to reflecting actual farm conditions. One such study evaluated the effects of clover yellow mosaic virus (CYMV) and WCMV on yield of ladino white clover in broadcast-sown plots. Hay yields were approximately 33% less for plots of ladino white clover mechanically inoculated with either CYMV or WCMV than for control plots of noninoculated plants (22).

Several studies have furthered our understanding of the factors involved in yield losses in white clover (4–6,9,11). In greenhouse studies, PSV has been implicated as a weakening factor in white clover plants, by rendering them more susceptible to injury and death from environmental stresses and diseases (11). Alternatively, plants may be killed as a direct result of PSV infection. Both these situations contribute to the lack of persistence of white clover in pastures (19).

Although most available data (5,6,11, 19,22) show the detrimental effects of virus infection on yield and persistence of white clover, the highly artificial environmental conditions imposed in most studies make it difficult to relate to field conditions. Comparing the performance of isogenic lines with and without virus resistance under field conditions would be ideal, but no such isogenic lines exist. However, germ plasm with virus resistance that are otherwise similar to virus susceptible cultivars are now available for more definitive experiments. These include the Southern Regional Virus Resistant (SRVR) (10), Florida Experimental No. 4 (FL Exp. 4) (D. Baltensperger, *personal communication*), and Brown Loam Synthetic No. 2 (BL Syn. 2) (14) germ plasms.

Many of these new germ plasms were developed and evaluated under spaced plant conditions in weed-free soil, even though clover is usually sown with grasses or is grown after grass invasion. Whether spaced plantings are optimum for evaluation of virus resistance is unknown. For example, evidence with peanuts (*Arachis* sp.) suggests that aphid vectors are more attracted to widely spaced legume plants surrounded by bare soil than to those in a closed canopy (1).

The objectives of the present research were to quantify yield and other losses due to viruses by comparing virus incidence under spaced-plant conditions in the field with and without grass, and under broadcast-sown conditions with and without grass. The ultimate goal was to determine the effect of virus resistance on maintenance and yield of white clover grown under conditions that simulate farm pasture swards as closely as possible.

MATERIALS AND METHODS

The present research grew out of planning conducted by personnel of Regional Project S-228, Forage Legume Viruses: Identification and Genetic Resistance for Improved Productivity. Field trials were established at Lexington, Kentucky (KY), and Mississippi State, Mississippi (MS). Similar experimental conditions were used at the two locations (Table 1).

Paper 94-3-160 of the Journal Series of the Kentucky Agricultural Experiment Station.

This research was conducted as part of the S-228 regional project on Forage Legumes, supported by Hatch appropriations.

Accepted for publication 20 July 1995.

White clover germ plasms and grass cultivars. The virus-susceptible white clover cultivar used was Regal (12), developed by the Auburn University Agricultural Experiment Station and released in 1962. The three synthetics used were released with reported resistance to certain viruses: FL Exp. 4, which has a red "V" leaf mark (D. Baltensperger, *personal communication*); SRVR PI 520755, developed jointly in the Southern region by the USDA and several Agricultural Experiment Stations (10); and BL Syn. 2 PI 512040, released by the USDA and the Mississippi Agricultural and Forestry Experiment Station (14).

Grasses grown in both spaced-plant and broadcast plots were Kentucky bluegrass (*Poa pratensis* L.) at KY and tall fescue (*Festuca arundinaceae* Schreb.) cv. Kentucky 31 at MS.

At MS, white clover seedlings grown in the greenhouse during summer 1989 were transplanted to the field in October 1989. Tall fescue was sown in the field in September 1989. The Mississippi broadcast plots were sown to white clover at 3.4 kg ha^{-1} with and without tall fescue at 17.0 kg ha^{-1} in September 1989. At KY, white clover seedlings were grown in a greenhouse and were transplanted into methyl-bromide treated soil in the field in May 1990, and bluegrass seed (7.5 kg ha^{-1}) was sown with a cultipacker seeder immediately after transplanting was finished. The broadcast plots were sown in April 1990 with a "V" belt seeder to white clover at 2.24 kg ha^{-1} with and without bluegrass at 7.5 kg ha^{-1}.

Experimental design. Experimental design was the same at both locations for the spaced-plant and broadcast experiments. Both spaced-plant and broadcast plots were established in randomized complete block design with four replications and eight treatments (four germ plasms × two grass levels [absence and presence]) arranged at random within replications. At both sites, spaced-plant plot size was 4.75 × 4.75 m and plants were spaced 0.91 m apart in a square grid pattern in each plot making a total of 25 plants per plot. A 2.74-m-wide (KY) or 1.8-m-wide (MS) border between and surrounding the plots was sown to grass. Broadcast plot size was 0.91 × 3.0 m at KY and 1.50 × 3.7 m at MS. Borders seeded to grass were 0.91 m wide.

Data collection, KY. Data on plant survival, vigor and relative virus incidence were obtained on spaced plants at KY. Numbers of plants surviving in August 1990 and April 1991 were recorded. Vigor (an estimate of forage yield) was scored (scale of 1 to 9 with 1 being most vigorous) in October 1990 and in May 1991. The percentage of plants with viruslike symptoms was determined visually in May 1991. To relate visual observations on symptoms to incidence of PSV or CYVV, leaves from five randomly selected symptomatic plants per plot were collected and tested by enzyme-linked immunosorbent assay (ELISA) (2). In plots with less than five or no symptomatic plants, samples were collected at random. ELISAs were carried out twice, in October 1990 and in June 1991. A total of 160 out of 800 possible plants were sampled on each testing date (25 plants per plot × 4 replications × 2 grass levels × 4 clover populations) or 20% of the total. Plants sampled in 1990 were not sampled in 1991, so that a total of 10 different plants were sampled per plot over the 2 years. The spaced plants were kept from intermingling in 1991 by occasional pruning of stolons, but by early 1992 stolon elongation caused the plants to be intermingled and the experiment was discontinued.

Data on percent stand, virus incidence, and dry matter yield were collected on broadcast plots. Visual estimates of percent stand were made in April 1991, March 1992, and April 1992. Infection with PSV and CYVV was determined by collecting a bulk sample of leaves from five plants selected at random from each plot and extracts from the pooled samples were tested by ELISA. Data were recorded as percentage of plots having plants with positive virus tests. Plots were harvested with a Carter Flail Harvester (Carter Manufacturing Co., Brookston, Ind.) once in 1990 and three times in 1991. Stubble height was approximately 7 cm. Forage collected from the plots was dried at 71°C and weighed. All data were subjected to a randomized complete block analysis of variance. Because the interactions of clover populations with presence or absence of grasses were not significant, the data are presented as means of 4 germ plasms and 2 grass levels for simplicity.

Data collection, MS. Data on plant survival, vigor and virus incidence were obtained on spaced plants. Number of surviving plants and vigor were recorded

Table 1. Summary of experimental conditions

Locations	Spindletop, Kentucky Agricultural Experiment Station, Fayette County, Kentucky, N 38°05′ W 84°29′ (KY) Leveck Animal Research Center, Mississippi Agricultural and Forestry Experiment Station, Oktibbeha County, Mississippi, N 33°26′ W 88°48′ (MS)
Soil Type	KY: Maury silt loam (typic Palendalf) MS: Catalpa silty clay (fine, montmorillonitic, thermic Fluvaquentic Hapludoll)
Design (spaced plants)	MS and KY: randomized complete block, 4 replications
(broadcast)	MS and KY: randomized complete block, 4 replications
Treatments (spaced plants and broadcast studies)	Germ plasms: (1) Southern Regional Virus Resistant; (2) Brown Loam Syn. No. 2; (3) Regal; (4) Florida Exp. 4 Grasses: (1) Presence; (2) Absence
Grass species	KY: Kentucky bluegrass MS: Tall fescue (cv. Ky 31)
Seeding rates (kg ha^{-1})	MS: Tall fescue 17.0 MS: White clover 3.4 KY: Kentucky bluegrass 7.5 KY: White clover 2.24
Planting season	MS: Fall, 1989 KY: Spring, 1990

Table 2. Mean stand, percentage of alfalfa mosaic virus (AMV), clover yellow vein virus (CYVV), and peanut stunt virus (PSV) incidence and vigor of four white clover germ plasms spaced plants grown in Mississippi with and without tall fescue

	No. plants surviving[w]	Percentage of incidence[v]						Vigor[x]	
		AMV		CYVV		PSV			
Treatment	6/15/90	6/90	11/91	6/90	6/91	6/90	11/91	6/90	8/91
Germ plasm									
SRVR[y]	19.5 a[z]	5 a	18 a	2	0 b	0 c	8 b	4.0 a	6.6 a
BL Syn. 2	18.5 a	5 A	18 a	0	5 b	8 b	50 a	3.9 a	6.4 a
Regal	18.5 a	2 a	5 ab	0	22 a	22 a	40 a	4.3 a	7.3 b
FL Exp. 4	16.6 a	0 a	2 b	2	12 ab	0 c	15 b	5.4 b	7.3 b
Grass									
Tall fescue	19.7 a	0 a	10 a	0	5 b	4 b	20 b	4.8 b	7.2 b
No grass	16.9 a	6 a	11 a	2	15 a	11 a	36 a	4.0 a	6.6 a

[v] Values are percentages of infected plants based on infectivity assays and enzyme-linked immunosorbent assays of 40 plants per germ plasm (5 plants per plot × 4 replications × 2 grass levels; with or without grass). For the category "grass or no grass" values are based on testing 80 plants (4 germ plasms × 4 replications × 5 plants per plot). Incidence of CYVV in June 1990 was insufficient for statistical analysis.

[w] Twenty-five established.

[x] Score: 1 = most to 9 = least vigorous.

[y] SRVR = South Regional Virus Resistant; BL Syn. 2 = Brown Loam Synthetic No. 2; FL Exp. 4 = Florida Experimental No. 4.

[z] Means within a column (within a category; germplasm, grass) with the same letter are not significantly different by least significant difference test ($P = 0.05$).

in June 1990. Vigor was also scored (visual score of 1 to 9 with 1 being most vigorous) in August 1991. Percentage of plants with viruslike symptoms was not scored at MS. Instead, leaf samples from five plants in each plot were collected at random. Plants sampled in June 1990 and in November 1991 were tested by ELISA and indicator host assay for AMV, PSV, and CYVV (18). Due to random selection, some plants were sampled twice, so that an average of 8.4 different plants were sampled per plot over the 2 years. Indicator host assays, for AMV and PSV only, were conducted by mechanically inoculating the primary leaves of 7- to 10-day-old seedlings of cowpea (*Vigna unguiculata* L. Walp. subsp. *unguiculata* 'California Blackeye No. 5') with sap from test samples. Results were scored as positive for AMV when small (<1 mm diameter), red, necrotic, local lesions formed within 5 days postinoculation without systemic symptoms. Results were scored as positive for PSV when chlorotic spot or ringlike lesions (1 to 4 mm diameter) formed within 5 to 10 days postinoculation, followed by systemic vein clearing and mosaic of new trifoliolate leaves.

Data on percent stand, virus incidence, and dry matter yield were obtained on broadcast plots. A visual estimate of percent stand was made in March 1992. Plots were sampled and tested by ELISA for AMV, PSV, and CYVV, and by indicator host assay for AMV and PSV infection in November 1991, May 1992, and September 1992. Five samples were collected within each plot with each sample consisting of six leaves. Leaves composing an individual sample were collected from within a 100-cm^2 area, but not necessarily from the same plant. Samples were collected at 0.3-m intervals through the center of each plot. Broadcast plots were harvested for yield seven times in 1990, three times in 1991, and six times in 1992. Plots were harvested with a rotary mower and catch baskets to a 7-cm height. A subsample of 500 to 700 g of tissue was taken from plants harvested from each plot in one replicate and dried at 65°C for 48 h to determine forage dry matter. Data were analyzed by the same methods as at KY.

RESULTS

Spaced-plant evaluation: virus incidence. BL Syn. 2 and SRVR had significantly greater incidence of AMV (18, 18%) than did FL Exp. 4 (2%) at MS in November 1991, although none of the germ plasms were significantly different from Regal (Table 2). No differences in percent incidence of CYVV were found among germ plasms at KY (Table 3), but, at MS, Regal had more infected plants than SRVR and BL Syn. 2 in 1991 (22 versus 0 and 5%, respectively, Table 2). Significant differences in PSV incidence were shown at both locations and, as expected, Regal had one of the highest levels of infection (40 to 69%) and SRVR the lowest (8 to 12.5%). Interestingly, ELISA values of leaf extracts from PSV-infected SRVR plants were significantly lower than those from Regal, suggesting that the resistance to PSV in SRVR may be expressed as reduced virus titer (data not shown). Although sampled differently, FL Exp. 4 apparently showed a lower incidence of PSV infection at MS than at KY (15 versus 30% in 1991). Incidence of PSV was not significantly different between BL Syn. 2 and Regal at KY in 1990 and at MS in 1991 (Tables 2 and 3).

The differences in CYVV and PSV incidence between grass and no grass treatments (Tables 2 and 3) were significant at both locations. Whenever the difference was significant (6 of 8 dates), higher virus incidence occurred in the clover alone treatments than in the clover with grass treatments.

Symptom evaluations at KY. Estimates of the percentage of plants with viruslike symptoms, made only at KY (Table 3), generally agreed with the ELISA data, suggesting that evaluations based on visual examination of viruslike symptoms provided a realistic estimate of virus infec-

Table 3. Mean stand, vigor, percentage of plants with viruslike symptoms, and percent incidence of clover yellow vein virus (CYVV) and peanut stunt virus (PSV) in four white clover germ plasms spaced plants grown in Kentucky with and without bluegrass

Treatment	No. plants surviving[u] 4/25/91	Vigor[v] 5/91	Symptoms[w]	Virus incidence[x] CYVV 10/90	CYVV 6/91	PSV 10/90	PSV 6/91
Germplasm							
SRVR[y]	23.5 a[z]	5.0 a	24.6 b	7.5 a	5.0 a	15.0 c	12.5 d
BL Syn. 2	22.5 a	5.0 a	25.8 b	10.0 a	5.0 a	72.5 ab	53.8 b
Regal	18.6 b	7.3 b	46.1 a	17.5 a	11.5 a	95.0 a	68.8 a
FL Exp. 4	19.0 b	7.3 b	33.3 ab	17.5 a	8.8 a	52.5 b	30.0 c
Grass							
Bluegrass	23.6 a	5.2 a	18.8 b	5.0 b	3.7 a	45.0 b	32.5 b
No grass	18.0 b	7.1 a	46.1 a	21.5 a	11.3 a	72.5 a	50.0 a

[u] Twenty-five established.
[v] Score: 1 = most to 9 = least.
[w] Percentage of plants with viruslike symptoms scored in May 1991. Values shown for germ plasms are based on visual examination of 200 plants per germ plasm (25 plants per plot × 4 replications × 2 grass levels). For grass treatments, values are based on 400 plants per treatment (4 germ plasms × 4 replications × 25 plants per plot).
[x] Virus detection was by indirect enzyme-linked immunosorbent assay (ELISA) using antisera to PSV and CYVV. Forty plants per germ plasm (5 plants per plot × 4 replications × 2 grass levels) were tested by ELISA at each testing date. For grass treatments, values are based on 80 plants (4 germ plasms × 4 replications × 5 plants per plot) tested by ELISA at each testing date. Plants sampled in 1990 were not sampled in 1991.
[y] SRVR = South Regional Virus Resistant; BL Syn. 2 = Brown Loam Synthetic No. 2; FL Exp. 4 = Florida Experimental No. 4.
[z] Means within a column (in a category) with the same letter are not significantly different by least significant difference test ($P = 0.05$).

Table 4. Percentage of alfalfa mosaic virus (AMV), clover yellow vein virus (CYVV), and peanut stunt virus (PSV) incidence in four white clover germ plasms grown in broadcast plots in Mississippi with and without tall fescue

Treatment	Percentage of incidence[x] AMV 11/91	AMV 5/92	AMV 9/92	CYVV 11/91	CYVV 5/92	PSV 11/91	PSV 9/92
Germ plasm							
SRVR[y]	28 a[z]	78 a	30 a	0 b	0 b	5 b	18 b
BL Syn. 2	22 a	40 b	25 a	0 b	5 b	12 b	48 a
Regal	35 a	58 b	35 a	30 a	68 a	35 a	55 a
FL Exp. 4	25 a	32 b	15 a	10 ab	15 b	5 b	32 a
Grass							
Tall fescue	26 a	42 a	26 a	9 a	22 a	12 a	40 a
No grass	29 a	61 a	26 a	11 a	21 a	16 a	36 a

[x] Values are percentages of infected plants based on infectivity assays and enzyme-linked immunosorbent assays of 40 plants per germ plasm and 80 plants per grass treatment. The same plots but not the same plants were sampled at each testing date, resulting in a lack of relationship among dates of sampling.
[y] SRVR = South Regional Virus Resistant; BL Syn. 2 = Brown Loam Synthetic No. 2; FL Exp. 4 = Florida Experimental No. 4.
[z] Means within a column (in a category) with the same letter are not significantly different by least significant difference test ($P = 0.05$).

tion. Regal showed more plants with viruslike symptoms than SRVR and BL Syn. 2, (46.1, 24.6 and 25.8%, respectively), and white clover plants in bare ground had more symptomatic plants than those in association with bluegrass (18.8 versus 46.1%).

Vigor. Vigor data, obtained at both locations, was considered to be a visual estimate of yield. Regal (7.3) and FL Exp. 4 (7.3) were less vigorous than SRVR (6.6) and BL Syn. 2 (6.4) in 1991 at MS (Table 2). Regal (7.3) and FL Exp. 4 (7.3) also were less vigorous than SRVR and BL Syn 2 (both 5.0) at KY in 1991 (Table 3). In 1991, white clover plants associated with tall fescue were less vigorous than those grown on bare ground at MS (7.2 versus 6.6), but, at KY, plants grown with bluegrass were more vigorous than those grown alone (5.2 versus 7.1) (Tables 2 and 3).

Stands. Stands were counted once at MS (Table 2) and twice at KY (Table 3) to obtain a measure of stand loss as the experiment progressed. In August 1990 (KY, data not shown) and June 1990 (MS, Table 2), stands of the populations were not different. By April 1991 at KY (Table 3), Regal (18.6) and FL Exp. 4 (19.0) had lost more plants than SRVR (23.5) and BL Syn. 2 (22.5). Stands without grass had fewer plants remaining than those with grass at KY (23.6 versus 18.0).

Broadcast plot evaluation: Virus incidence. Incidence of AMV infection was similar among all the germ plasms at MS (Table 4) except in the May 1992 evaluation, when SRVR had higher virus incidence (78%). Incidence of CYVV infection was high in Regal on 1991 and 1992 testing dates (30 and 68%) with little or no virus incidence in SRVR and BL Syn. 2 (0 to 5%, Table 4). However, at KY, CYVV was detected in two of eight Regal plots in 1990 and in one of eight Regal plots and one of eight FL Exp. 4 plots in 1991. Other germ plasms in the KY test were not infected with any of the viruses included in this study (Table 5). Incidence of PSV infection was low at KY (Table 5), but high at MS (Table 4). Regal had significantly greater incidence of PSV infection in MS in 1991 (35%) but was significantly different only from SRVR in 1992 (55 versus 18%).

Grass sown in broadcast plots was not a significant factor in regard to virus incidence since clover tended to dominate stands. Virus infection was not different at MS (Table 4) and only a few plot samples were infected at KY (2 samples out of 32 in 1990 and 3 samples out of 32 in 1991), insufficient for statistical analyses (Table 5).

Dry matter yield. Total dry matter yields of clover and grass differed among germ plasms at KY only in 1991 when SRVR was higher yielding than the others (Table 6). At MS, FL Exp. 4 was consistently the lowest yielding except in 1992 when it was not significantly different from Regal (4,533 versus 4,646 kg ha^{-1}) (Table 5). Grass had little or no effect on yield at either location except in total yield in 1991 at MS when clover plots without tall fescue yielded significantly less than clover plots with tall fescue (2,054 versus 2,542 kg ha^{-1}) (Table 6).

Stands. Stands were estimated once at MS (Table 6) and three times at KY (Table 5). Initially, excellent stands were obtained at both locations and these data are not presented. At KY by April 1991, Regal had significantly less stand than SRVR (Table 5). By March 1992, stands had declined further but SRVR was still significantly greater than the FL Exp. 4 and Regal (53.0, 9.4, and 19.4%, respectively). After an early freeze all stands were reduced by April 1992 and the experiment was discontinued. At MS, SRVR and BL Syn. 2 had significantly greater stands than Regal and FL Exp. 4 (84, 96, 61, and 41%, respectively) (Table 6). Statistically greater white clover stands were obtained with no grass than with grass at both locations in March 1992.

Table 5. Percent stand, forage dry matter yield, ground cover, and percent incidence of clover yellow vein virus (CYVV) and peanut stunt virus (PSV) infection in four white clover germ plasms broadcast with and without bluegrass in Kentucky

	Yield (kg ha^{-1})		Stand (%)			Virus incidence (%)	
Treatment	**1990**	**1991**	**4/91**	**3/92**	**4/92**	**4/90**	**6/91**
Germplasm							
SRVR[u]	3,822 a[v]	10,094 a	98.0 a	53.0 a	10.6 a	0.0[w]	0.0
BL Syn. 2	3,269 a	8,920 b	95.3 ab	38.1 ab	11.9 a	0.0	0.0
Regal	3,578 a	8,847 b	88.3 b	19.4 bc	3.8 a	25.0[x]	25.0[y]
FL Exp. 4	3,431 a	8,774 b	92.3 ab	9.4 c	0.0 a	0.0	12.5[z]
Grass							
Bluegrass	3,578 a	9,265 a	92.0 a	24.7 b	4.3 a	0.0	6.2
No grass	3,093 a	9,120 a	93.5 a	37.2 a	8.4 a	12.5	12.5
No. of harvests	1	3					

[u] SRVR = South Regional Virus Resistant; BL Syn. 2 = Brown Loam Synthetic No. 2; FL Exp. 4 = Florida Experimental No. 4.
[v] Means within a column (in a category) with the same letter are not significantly different by least significant difference test ($P = 0.05$).
[w] Values are percentage of virus incidence in 8 composite samples per germ plasm (4 replications × 2 grass levels) or 16 samples per grass treatment (4 germ plasms × 4 replications). Each sample represents a plot and comprises 5 leaves collected at random.
[x] Infected with CYVV and PSV (2 plot samples doubly infected).
[y] Two plot samples infected, one with PSV and one with CYVV.
[z] One plot sample infected with CYVV.

Table 6. Forage dry matter yields and percent stand of four white clover germ plasms grown in broadcast plots in Mississippi with and without tall fescue

	Total yield (kg ha^{-1})			Stand (%)
Treatment	**1990**	**1991**	**1992**	**3/17/92**
Germplasm				
SRVR[y]	8,036 a[z]	2,501 a	7,198 a	84 a
BL Syn. 2	8,422 a	2,473 a	6,940 a	96 a
Regal	8,772 a	2,350 a	4,646 b	61 b
FL Exp. 4	6,538 b	1,866 b	4,533 b	41 c
Grass				
Tall fescue	7,910 a	2,542 a	5,531 a	63 b
No grass	7,974 a	2,054 b	6,127 a	78 a
No. of harvests	7	3	6	

[y] SRVR = South Regional Virus Resistant; BL Syn. 2 = Brown Loam Synthetic No. 2; FL Exp. 4 = Florida Experimental No. 4.
[z] Means within a column (in a category) with the same letter are not significantly different by least significant difference test ($P = 0.05$).

DISCUSSION

The spaced-plant data showed that the synthetics selected for virus resistance generally had less incidence of PSV and CYVV than the susceptible cultivar, Regal, but no differences in incidence of AMV infection from Regal were observed. Slight differences existed between locations, but most data confirmed that SRVR was superior, and that BL Syn. 2 and FL Exp. 4 were approximately equal in virus incidence. The high incidence of AMV infection in SRVR at MS was reported earlier (20) and confirms the need for additional selection and breeding for greater resistance to AMV in this germ plasm. Additive genetic effects were most important for breeding PSV and CYVV resistance into SRVR clones (21), but important non-

additive genetic and reciprocal effects for AMV resistance may complicate breeding for resistance to this virus (21).

As expected, the spaced-plant data showed that white clover growth on bare ground (without grass) resulted in more CYVV and PSV infection. This indicates that the more efficient method of evaluating white clover plants for virus resistance is in a spaced-plant nursery with no associated grass species. Apparently, as was suggested by A'Brook (1), viruliferous aphid vectors were attracted to clover plants above bare ground more often than to plants within a green background of grass.

If we assume that the level of virus incidence in spaced plants may serve as an indicator for host susceptibility/resistance we can quantify the yield benefits of resistance by comparing the performance of plant lines under spaced plant conditions with those under broadcast conditions that more closely approximate farmer use. In general, yield and stand data of entries in broadcast plots closely paralleled those of the spaced plant plots. This suggests that virus resistance can be a predominant factor in maintaining high yields and stands of white clover cultivars in the southern United States. The germ plasm with the greatest PSV and CYVV resistance was SRVR, and it maintained the highest yields and greatest stands in broadcast plots at both locations. Use of SRVR at MS resulted in no yield advantage in the first 2 years of broadcast plots, but gave a 55% increase over Regal in 1992. In a 4-year broadcast study at MS, SRVR was the highest yielding of 10 entries in the second, third, and fourth year, with yields 50, 124, and 11% greater, respectively, than Regal (7). These data were supported by two other broadcast studies at MS in which SRVR had 81 and 55% greater yields in a second and third year study, respectively, than Regal (20). At KY, SRVR gave a 14% increase over Regal in 1991, the last year of stand. At KY, little or no virus was detected in the broadcast plots (even though high virus incidence was found in adjacent spaced plants), and stands declined to a few plants in less than three seasons. The usual longevity of white clover at KY without re-establishment from volunteering plants is about three seasons. At MS, the BL Syn. 2 consistently produced high yields despite having low virus resistance. The broadcast experiment may have been ended prematurely at MS since BL Syn. 2 has been shown to yield equivalent to SRVR in the second and third year and then decline in the fourth year (7).

The data presented in this study suggest that virus resistance is important for improved white clover productivity and persistence but other factors also exist in the SRVR and BL Syn. 2 germ plasms that led to improved performance at both locations. We suspect that this improved performance is due to general adaptability of these germ plasms. Broadcast stands of virus-resistant germ plasm survived in sufficient quantity for only about 3 years, suggesting that many factors other than virus are involved in persistence of white clover.

Performance of isogenic lines with and without virus resistance would be a more valid comparison; nevertheless, these results show the importance of breeding for virus resistance, and suggest that without virus resistance, particularly to CYVV and PSV, persistence and high yields cannot be obtained in white clover.

LITERATURE CITED

1. A'Brook, J. 1968. The effect of plant spacing on the number of aphids trapped over the groundnut crop. Ann. Appl. Biol. 61:289-294.
2. Anderson, J. A., Ghabrial, S. A., and Taylor, N. L. 1991. Natural incidence of peanut stunt virus infection in hybrid populations of *Trifolium ambiguum* × *T. repens*. Plant Dis. 75: 156-159.
3. Barnett, O. W., and Diachun, S. 1986. Virus diseases of clovers: etiology and crop losses. Pages 625-676 in: Viruses Infecting Forage Legumes. J. R. Edwardson and R. G. Christie, eds. Monog. No. 14. Inst. Food Agric. Sci., Fla. Agric. Exp. Sta., Gainesville.
4. Barnett, O. W., and Gibson, P. B. 1975. Identification and prevalence of white clover viruses and the resistance of *Trifolium* species to those viruses. Crop Sci. 15:32-37.
5. Campbell, C. L. 1986. Quantifying clover yield losses due to virus diseases. Pages 735-742 in: Viruses Infecting Forage Legumes. J. R. Edwardson and R. G. Christie, eds. Monog. No. 14. Inst. Food Agric. Sci., Fla. Agric. Exp. Sta., Gainesville.
6. Campbell, C. L., and Moyer, J. W. 1983. Effects of clover yellow vein virus and *Codinaea fertilis* on growth of white clover. Plant Dis. 67:70-73.
7. Edwards, N. C., Jr., Askew, J., Boykin, F., Burdine, W. B., Jr., Cuomo, G., Elmore, R., Hovermale, C. H., Ingram, D. M., Ivy, R., Johnson, B., Lang., D., Pederson, G. A., Saunders, R., and Tomlinson, J. 1993. 1992-93 performance of forage crop varieties in Mississippi. Inf. Bull. 247, Miss. Agric. For. Exp. Sta., Mississippi State, Miss.
8. Ellsbury, M. M., McLaughlin, M. R., and Baer, R. B. 1988. Aphid flight activity in relation to seasonal establishment and growth periods of *Trifolium* species in Mississippi. Southwest. Entomol. 13:273-281.
9. 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.
10. Gibson, P. B., Barnett, O. W., Pederson, G. A., McLaughlin, M. R., Knight, W. E., Miller, J. D., Cope, W. A., and Tolin, S. A. 1989. Registration of Southern Regional Virus Resistant white clover germplasm. Crop Sci. 29:241-242.
11. 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.
12. Johnson, W. C., Donnelly, E. D., and Gibson. P. B. 1970. Registration of Regal white clover. Crop Sci. 10:208.
13. Knight, W. E. 1985. The distribution and use of forage legumes in the United States. Pages 34-39 in: Forage Legumes for Energy-Efficient Animal Production. R. F. Barnes, P. R. Ball, R. W. Brougham, G. C. Marten, and D. J. Minson, eds. Proc. Trilateral Worksh. Held in Palmerston North, N.Z., USDA, ARS, Washington, D.C.
14. Knight, W. E., McLaughlin, M. R., Rincker, C. M., Windham, G. L., Edwards, N. C., and Pederson, G. A. 1988. Registration of Brown Loam Synthetic No. 2 white clover germplasm. Crop Sci. 28:380.
15. Leffel, R. C., and Gibson, P. B. 1973. White clover. Pages 167-176. in: Forages: The Science of Grassland Agriculture, 3rd ed. M. E. Heath, D. S. Metcalfe, and R. F. Barnes, eds. The Iowa State University Press, Ames.
16. 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.
17. 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.
18. McLaughlin, M. R., Damsteegt, V. D., Duffus, J. E., and Hewings, A. D. 1988. Subterranean clover red leaf (soybean dwarf)-like luteovirus found in Mississippi. (Abstr.) Phytopathology 78:1584.
19. McLaughlin, M. R., Pederson, G. A., Evans, R. R., and Ivy, R. L. 1992. Virus diseases and stand decline in a white clover pasture. Plant Dis. 76:158-162.
20. Pederson, G. A., and McLaughlin, M. R. 1988. Performance of southern regional virus resistant (SRVR) white clover germplasm. Pages 57-58 in: Proc. *Trifolium* Conf., 10th, Texas AM University, Angleton, TX.
21. Pederson, G. A., and McLaughlin, M. R. 1994. Genetics of resistance to peanut stunt, clover yellow vein, and alfalfa mosaic viruses in white clover. Crop Sci. 34:896-900.
22. Pratt, M. J. 1967. Reduced winter survival and yield of clover infected with clover yellow mosaic virus. Can. J. Plant Sci. 47:289-294.