

Effects of Peanut Stunt Virus, *Meloidogyne incognita*, and Drought on Growth and Persistence of White Clover

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ABSTRACT

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Productivity and persistence of white clover (*Trifolium repens*) in the southeastern United States are limited by a complex of pathogens and environmental stresses including peanut stunt virus (PSV), root-knot nematodes (*Meloidogyne incognita*), and drought. Determining the separate and combined effects of these factors has been hampered by the rapid natural spread of PSV to noninoculated plants. Half-sib white clover plants with and without hypersensitive resistance to PSV were used to overcome this problem. Effects of PSV, *M. incognita*, and drought on clover growth and persistence in the field were measured using a factorial arrangement of treatments in a split-plot design in which half of the plots were irrigated to eliminate drought stress. Data were collected in 2 consecutive years on dry weight herbage yield, stolon density, leaf area, petiole length, seed production, nematode population density, rainfall, and soil moisture. Virus infection reduced cumulative herbage yield 14% in the first year and 24% in the second year. In the first year, irrigation increased cumulative herbage yield 5 and 13% in the presence and absence of *M. incognita*, respectively, compared with nonirrigated control

plots without *M. incognita*. Cumulative herbage yield in drought-stressed plots was reduced 9% in the presence of *M. incognita* compared with nonirrigated control plots without *M. incognita*. In the second year, cumulative herbage yield in the absence of *M. incognita* was 54% higher in irrigated plots than in nonirrigated plots, while yield in nonirrigated plots was 17% lower in the presence of *M. incognita*. Clover persistence, as measured by stolon density, was reduced by PSV, *M. incognita*, and drought, but no interactions occurred. Reductions in persistence were most severe in *M. incognita* treatments, resulting in nearly complete loss of stands by the end of the second year. Virus infection reduced leaf area and petiole length in all treatments, but reductions were proportionately greater in irrigated plots. Nematode infestation generally reduced leaf area and petiole length, but the effect was less than that of PSV. Mean seed yields from 100 seed heads per plot were lower in nematode-infested plots, but slightly higher in nonirrigated plots and PSV-treated plots. Drought stress, *M. incognita*, and PSV acted independently in reducing forage productivity and persistence.

Additional keywords: diseases losses, forage legumes, soilborne pathogens.

White clover (*Trifolium repens* L.) is one of the most widely grown pasture legumes in the southern United States. It is an excellent forage legume crop that, through association with symbiotic Rhizobia, provides a natural source of nitrogen to companion plants. It may also be used as a cover crop (9). The productivity and persistence of white clover stands in the southeastern United States frequently decline within 2 to 3 years after establishment. Decline is believed to be due to a complex of diseases and environmental stresses (8,13). Diseases caused by viruses and nematodes have been commonly associated with white clover decline (2,3). Peanut stunt cucumovirus (PSV) and the root-knot nematode *Meloidogyne incognita* (Kofoid & White) Chitwood have been identified as primary pathogens in this complex (16,23). Among environmental stresses, drought has been suggested as another important component of the decline complex (12,14).

M. incognita is the most important nematode pest of white clover in the southeastern United States (3,23). Many white clover cultivars and germ plasms are very susceptible to root-knot nematodes (24). Clover roots infected with root-knot nematodes are stunted, may be heavily galled, and may deteriorate rapidly leading to plant death (25). *Meloidogyne* spp. are particularly damaging following summer droughts and limit clover growth in the fall and, subsequently, reduce clover overwintering ability (23). *Meloidogyne* spp. have reduced white clover persistence by up to 79% (3).

PSV is widely distributed in the Southeast and high incidences of PSV infection of white clover plants in the field have been reported (13,16). Infected plants are often weakened and stunted, causing significant yield losses in greenhouse and field chamber studies (6,8). Documenting the effects of PSV in the field has been hampered because of the rapid natural spread of the virus by aphids from inoculated to noninoculated plants (18). The best estimates of PSV effects on white clover forage yield and persistence in the field have been obtained from a study by Taylor et al. (22) that compared performance of germ plasms of varying levels of PSV resistance with that of the susceptible cultivar Regal. Conclusions from that study were that "factors other than virus" affected persistence of white clover and that "isogenic lines with and

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without virus resistance' would provide a better comparison of PSV effects (22). Half-sib plants of white clover with and without hypersensitive resistance to PSV (15) made that comparison possible in the present study. Using these half-sib plants, we were able for the first time to measure effects of PSV on growth and persistence of white clover in an open field experiment without confounding effects from natural spread and varying levels of infection of PSV among plots of the same treatment.

No information is available from field studies on the effect of concurrent infections of white clover by *M. incognita* and PSV. Most studies on interactions of nematodes and viruses have focused mainly on ectoparasitic nematodes that serve as virus vectors. The effects of drought stress on white clover have not been determined and possible interactions with PSV and *M. incognita* have not been studied.

The objectives of this study were to determine the effects and possible interactions of PSV, *M. incognita*, and drought on growth and persistence of white clover in the field. White clover plants with and without hypersensitive resistance to PSV were grown in small field plots treated in all possible combinations with PSV and *M. incognita*. Data on critical plant growth parameters were collected through 2 growing seasons that included periods of natural drought. Effects of natural drought stress were determined by comparison of data from naturally drought-stressed treatment plots with that from treatment plots that received supplemental irrigation.

MATERIALS AND METHODS

Half-sib white clover clones. All clones were derived from open-pollinated field crosses of a PSV-resistant clone, designated 22R, that possessed a heterozygous single dominant gene conferring hypersensitive resistance to PSV (16). Approximately equal numbers of hypersensitive and nonhypersensitive half-sib progeny plants occurred among seedlings grown from this open-pollinated seed. Seedlings were grown in the greenhouse and cloned by stolon cuttings. Responses to mechanical inoculation with PSV were determined on individual ramets of each clone. Clones were grouped as susceptible or hypersensitive resistant based on the development of systemic mosaic symptoms or necrotic local lesions, respectively (14,15). Ten clones of each group were selected. Virus-free plants of each clone were subsequently propagated in the greenhouse by stolon tip cuttings dipped in *Rhizobium* inoculant (Nitragin Co., Milwaukee, WI) and planted in Jiffymix (CASSCO, Montgomery, AL) in 3.8-cm-diameter conetainers (Stuewe & Sons, Inc., Corvallis, OR).

Plot establishment. Experimental plots were established in a Marietta fine sandy loam at the Leveck Animal Research Center, Mississippi State, MS. The plot area was followed by repeated discing in July and August of 1991, and then tilled on 5 September 1991 to a depth of 15 cm, covered with a transparent plastic tarp, and fumigated with 98 g of methyl bromide/m². Forty-eight 1 × 2-m plots separated by 1-m alleys were marked, and the alley and border areas were seeded with endophyte-infected KY31 tall fescue (*Festuca arundinacea* Schreber) on 13 September 1991. The plot area was irrigated twice by overhead sprinklers during the following week to induce germination and establishment of the fescue. Lime was surface-applied to each plot at 0.22 kg/m², according to soil test recommendations, and incorporated in the top 5 cm of soil on 17 September 1991. White clover plants propagated from stolon cuttings in the greenhouse were transplanted as spaced plants on 21 October 1991. Plots were irrigated as above to encourage establishment of the transplants. Plots consisted of one plant of each of 10 clones of either PSV-resistant or PSV-susceptible plants. Plants were equally spaced in two rows of five plants each, approximately 33 cm apart within and between rows and approximately 33 cm from the outside edges in each plot. Half of the plots were planted with PSV-

resistant clones and half with PSV-susceptible clones of the 20 half-sib plants selected.

Grassy weeds and fescue plants from seed washed into the plots during irrigations were removed from plots on 3 February 1992 by treatment with sethoxydim postemergence herbicide according to label directions. Volunteer clovers and other broadleaf weeds were removed from the plots in early March 1992 by careful hand-weeding. By mid-March 1992, the clover plants had grown together and outward within each plot to cover nearly the entire area within each plot. All plots were clipped to a height of 7 cm using a rotary mower on 16 March 1992. Herbage from the clipped plots was removed and discarded.

Micronutrient analyses of herbage samples from selected plots representing healthy control treatments were conducted in July 1992. Samples submitted to the Soil Testing and Plant Analysis Laboratory of the Mississippi Cooperative Extension Service at Mississippi State University were within or above recommended sufficiency levels for nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, iron, manganese, zinc, copper, and boron.

Virus inoculation and sampling. White clover plants susceptible to PSV were mechanically inoculated with PSV on 13 April 1992. Inoculum was prepared by homogenizing freshly harvested leaf tissue, from PSV-infected white clover stock culture plants maintained in the greenhouse, in 0.03 M sodium phosphate buffer (pH 7.3) containing 0.02 M 2-mercaptoethanol using a mortar and pestle. Carborundum (600 mesh) was added as an abrasive. Inoculum was rubbed over the upper leaf surfaces of the plants using a pestle. Three to four leaves were inoculated at each grid point on an approximately 15-cm-square grid pattern over the entire surface of each plot.

The presence or absence of PSV-infected plants in the treatment plots was confirmed by visual assessment of symptoms approximately 1 month after inoculation. Subsequently, in November of 1992 and 1993, leaf samples were collected from all plots for bioassay. Five samples of six leaves each were collected equal distances apart down the center line of each plot. Samples were, thus, collected midway between the two rows of plants and included leaves from each plant in each plot. Each six-leaf sample was prepared separately as for the inoculum described above and assayed for PSV by inoculation of the primary leaves of 10-day-old blackeye cowpea (*Vigna unguiculata* (L.) Walp. subsp. *unguiculata*) seedlings (5).

Nematode infestation and sampling. A race 4 population of *M. incognita* was increased on tomato (*Lycopersicon esculentum* Miller cv. Floradel) in the greenhouse. After 8 to 10 weeks, nematode eggs were collected from roots according to published methods using NaOCl (11). Plots were infested with *M. incognita* eggs on 18 May 1992. Nine holes spaced 5 cm apart and measuring 1.0 cm in diameter and 7.5 cm in depth were punched in irrigation-softened soil in an "X" pattern over each transplanted clover plant. A wooden nine-hole template placed over each plant positioned a complementary nine-peg dibble that was inserted into the template so that the sharpened dibble pegs penetrated the soil in a uniform and consistent manner around each plant. Twenty-five hundred *M. incognita* eggs suspended in water were dispensed into each hole using a technique similar to that of Rao-Arelli et al. (19) for a total of 22,500 eggs per plant and 225,000 eggs per infested plot.

M. incognita population densities were determined in July and October 1992 and in June, September, and December 1993. Eight soil cores 2.5 cm in diameter were taken 10 to 15 cm deep from each plot at each sampling date. Second-stage juveniles were extracted from 250-cm³ soil samples by a sieving-centrifugation method (1). Eggs were extracted from egg masses on roots with NaOCl (11).

Drought treatment. Half of the plots received supplemental irrigation during drought periods from 8 July 1992 to 5 November 1992 and from 18 June 1993 to 31 October 1993. Water was sup-

plied through a network of "soaker" hoses positioned over each row of plants in irrigated plot areas. Hoses were removed before forage harvests. Irrigated plots received irrigation water equivalent to 1.25 cm of rainfall twice weekly unless rainfall equaled or exceeded that amount. Surface runoff water was channeled away from nonirrigated (drought) plot areas by a network of 15-cm-deep channels cut into the fescue sod of the alleys midway between treatment areas. Soil moisture at a depth of 15 cm was monitored daily using "Jet-Fill" Model 2725 ARL tensiometers (Forestry Suppliers, Jackson, MS) positioned at one end of a treatment plot near the center of each of three irrigated and each of three nonirrigated areas. Rainfall was recorded daily during these periods in 1992 and 1993. Daily tensiometer and rainfall data were combined and reported for weekly intervals as means and cumulative totals, respectively.

Clover growth and persistence. Herbage was harvested from individual plots with a rotary mower and catch baskets. Plants were cut to a 7-cm height at each harvest. Herbage was harvested on 10 April, 14 May, 5 June, 1 July, 13 August, 9 September, and 28 October in 1992 and 12 April, 10 May, 15 June, 10 August, 22 September, and 24 November in 1993. Harvests were taken when clover height reached 20 to 25 cm. Herbage was collected in cloth bags and placed in a drying oven at 65°C for 48 h, weighed, and the amount of forage dry matter recorded.

White clover stand density, a measure of persistence, was determined by counting the number of stolons intersecting a 2-m-long stick placed lengthwise down the center line of each plot, midway between the two rows of plants. Thus, all plants had equal opportunity for representation in the counts. Stolon densities were determined in each plot immediately following each harvest beginning with the 1 July 1992 harvest. Stolon counts were converted to stolons/meter for analysis.

Leaf area and petiole length measurements were made on 27 July in 1992 and 1993. Twenty leaves were collected from each plot. Leaves were collected at 10-cm intervals on the 2-m-long center line midway between the two rows of plants. Only fully expanded leaves 3 to 5 nodes distal of the stolon tip were collected. Leaves were transported on ice to the laboratory where individual leaf measurements were made. Leaflets were excised with a razor blade and their combined trifoliolate leaf area (square centimeters) measured with a LI-COR LI-3000A portable area meter (LI-COR, Lincoln, NE). Petiole lengths (centimeters) were measured to the nearest millimeter.

Measurements of seed production were made in August of 1992 and 1993. One hundred mature seed heads were collected at random from each plot and air-dried in brown paper bags in the laboratory. Dried seed heads were hand-threshed, the seed from each plot were weighed (grams), and the results were recorded.

Experimental design and statistical analyses. A split-plot design was used in which half of the plots were irrigated during drought periods. Eight treatments comprised with and without PSV (subplot), *M. incognita* (subplot), and irrigation (main plot) were imposed in a factorial arrangement with six replications. All treatments were randomized. Data were subjected to analysis of variance (ANOVA) with a SAS general linear models procedure (SAS Institute, Cary, NC) to test for interactions among and between treatments and, in the absence of interactions, to test treatment effects. Significant differences between treatment means were determined by the ANOVA *F* test (only differences with *P* < 0.05 were reported). Herbage yields of individual harvests were initially analyzed separately. Subsequently, regression analyses were used to compare cumulative herbage yields over time as affected by the virus, nematode, and irrigation treatments.

RESULTS

Half-sib white clover plants and PSV. Bioassays of white clover leaf samples in 1992 and 1993 were negative for PSV in all

plots containing plants with hypersensitive resistance and were positive for PSV in all plots containing plants without hypersensitive resistance.

Drought measurements. Cumulative weekly rainfall amounts during the recording periods varied from zero to nearly 12 cm in 1992 and from zero to 7 cm in 1993 (Fig. 1). Mean tensiometer values in nonirrigated plot areas fluctuated widely from relatively low values of 10 to 20 centibars (no drought stress) during periods of rainfall to relatively high values of 70 to 80 centibars (high drought stress) during extended periods of little or no rainfall (Fig. 1). During periods of high drought stress, the surface of the ground cracked in nonirrigated plot areas and the white clover plants wilted daily in the afternoon sun. Periods of relative drought lasting from 3 to 10 weeks occurred in each growing season, but stress was greater in 1993 when mean weekly tensiometer values in nonirrigated plot areas exceeded 80 centibars in July, September, and October. Tensiometer values in irrigated plots were usually maintained between 5 to 15 centibars and seldom exceeded 20 centibars.

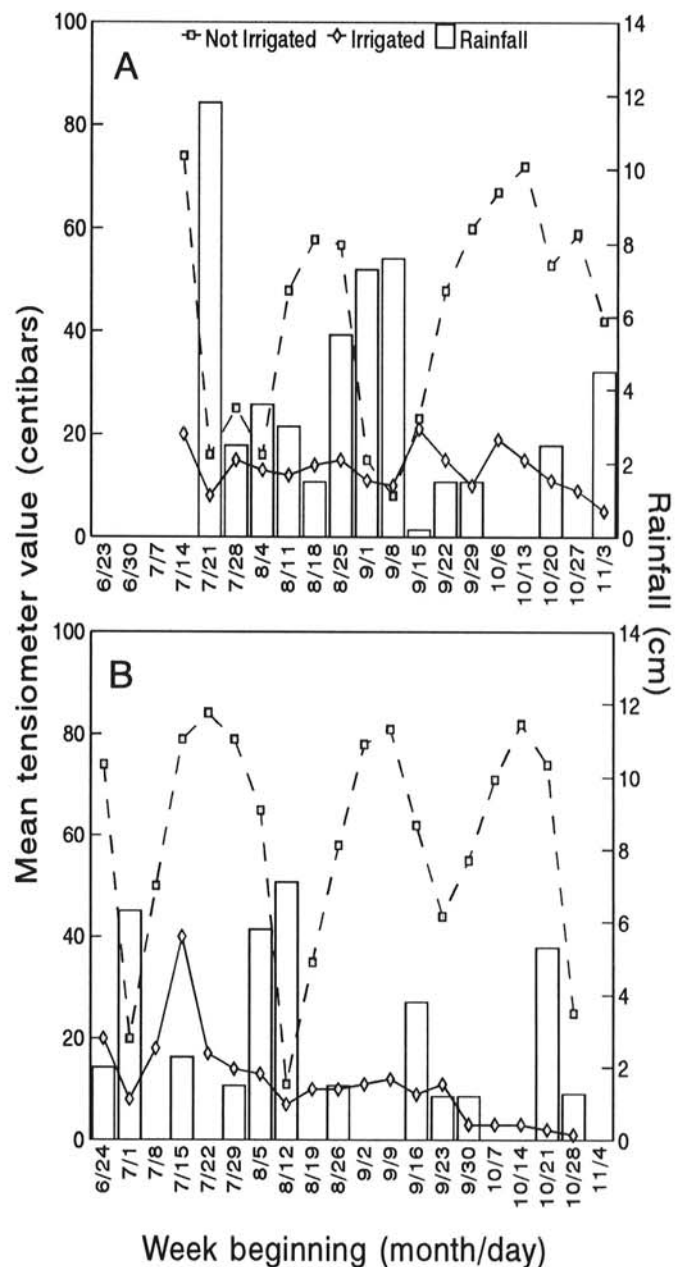


Fig. 1. Weekly rainfall accumulation and soil moisture in plots with and without supplemental irrigation during the summer/fall growing seasons of A, 1992 and B, 1993.

Differences in soil moisture between irrigated and nonirrigated plot areas were greatest in late summer and early fall in both years.

Effects on herbage yields. There were no interactions between PSV and *M. incognita* or irrigation for herbage yields in 1992 or 1993. However, there was an interaction between *M. incognita* and irrigation in 1992 and 1993.

Quadratic models best described the relationship between days after harvest and cumulative herbage yields for plots with and without PSV (Fig. 2). In 1992, PSV significantly suppressed yields beginning at the third harvest and continuing through the last harvest. Herbage yield losses caused by PSV were 11, 17, 17, 25, and 25% for harvests 3 through 7, respectively, in 1992 (Fig. 2A). Cumulative total herbage production was reduced 14% in plots with PSV. In 1993, herbage yields were reduced by PSV in all six harvests with losses of 22, 20, 16, 33, 47, and 65%, respectively. Cumulative total herbage production was reduced in PSV plots by 24% in 1993 (Fig. 2B).

In 1992, the relationship between days after nematode inoculation and cumulative herbage yields was best described by quad-

ratric models for treatments with and without irrigation and *M. incognita* (Fig. 3A). No differences in herbage yields were observed until the last two harvests in 1992. Irrigation increased cumulative total yields 5 and 13% in plots with and without *M. incognita*, respectively, over plots with no irrigation or nematodes.

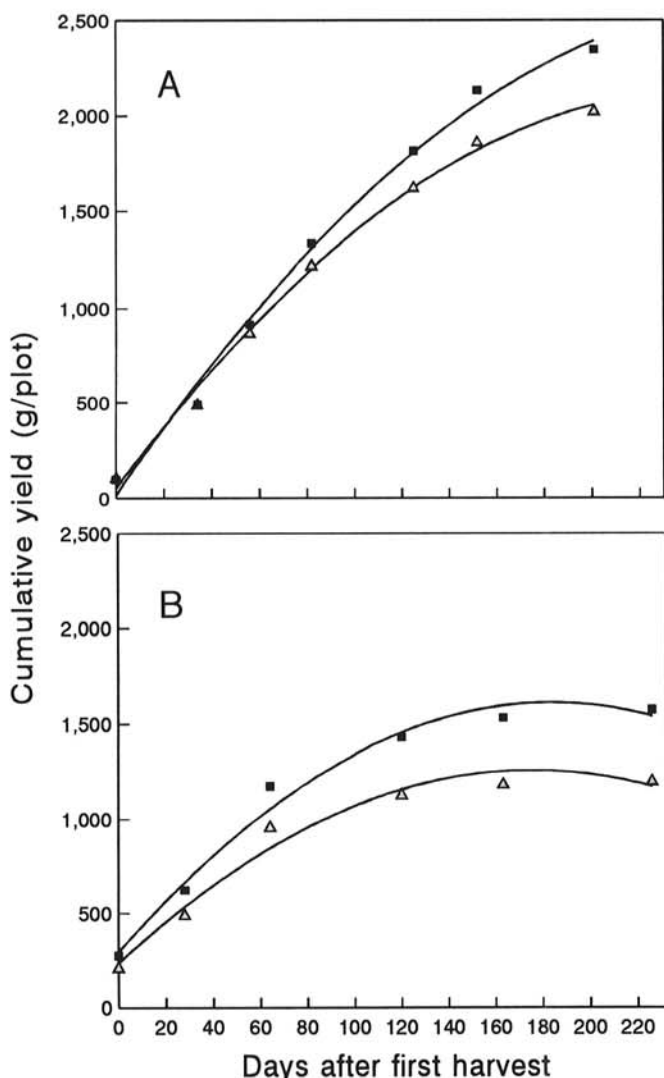


Fig. 2. Relationship between cumulative herbage yield of white clover and days after first harvest in plots with peanut stunt virus (PSV)-infected plants (Δ) and PSV-free plants (\blacksquare) in **A**, 1992 and **B**, 1993. The first harvest dates were 10 April 1992 and 13 April 1993. x = days after first harvest, quadratic regression equation for PSV-free plants in 1992 ($y = 16.7 + 18.4x - 0.03x^2$, $r^2 = 0.99$); quadratic regression equation for PSV-infected plants in 1992 ($y = 52.1 + 16.7x - 0.03x^2$, $r^2 = 0.99$); quadratic regression equation for PSV-free plants in 1993 ($y = 292.2 + 14.4x - 0.03x^2$, $r^2 = 0.98$); and quadratic regression equation for PSV-infected plants in 1993 ($y = 232.8 + 11.7x - 0.03x^2$, $r^2 = 0.97$).

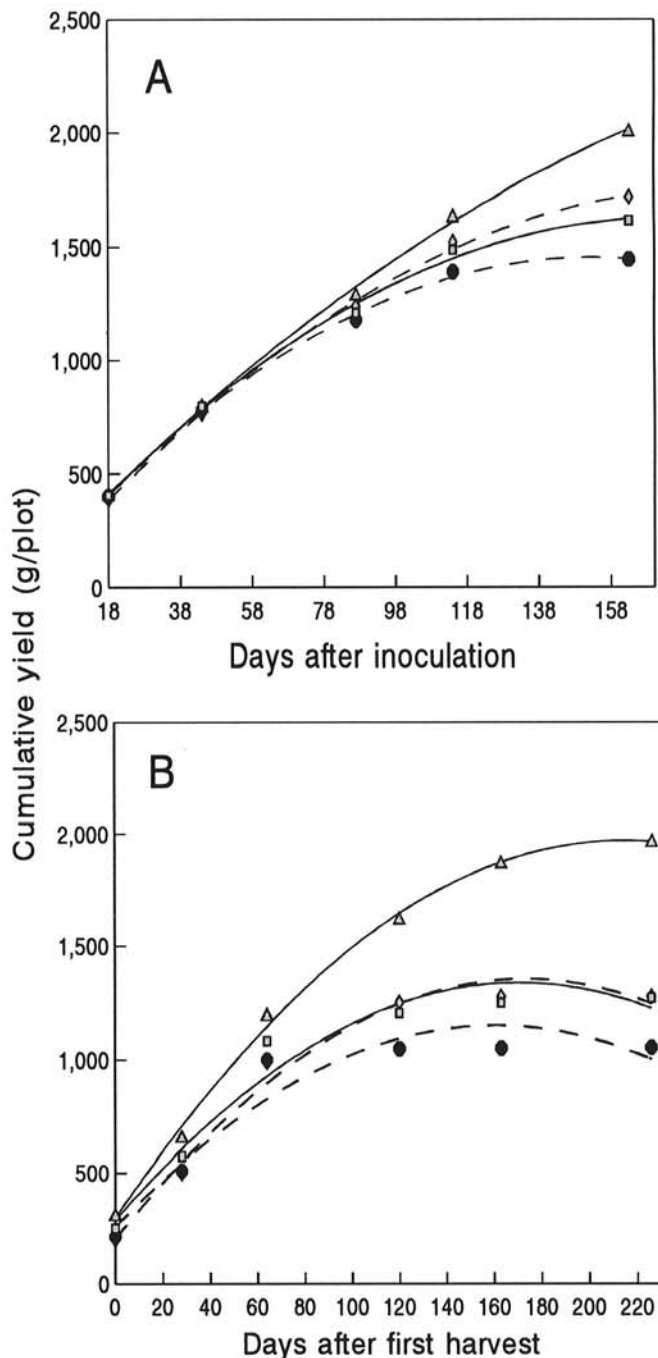


Fig. 3. Quadratic regression equations describing the effects of supplemental irrigation and *Meloidogyne incognita* on white clover herbage yield in **A**, 1992 and **B**, 1993. For 1992, x = days after inoculation with *M. incognita*, unfested plots without irrigation (\square - \square) ($y = 109 + 17.5x - 0.05x^2$, $r^2 = 0.99$); unfested plots with irrigation (Δ - Δ) ($y = 129.7 + 16.2x - 0.02x^2$, $r^2 = 0.99$); *M. incognita*-infested plots without irrigation (\bullet - \bullet) ($y = 104.8 + 17.6x - 0.05x^2$, $r^2 = 0.99$); and *M. incognita*-infested plots with irrigation (\diamond - \diamond) ($y = 80.22 + 17.6x - 0.04x^2$, $r^2 = 0.99$). For 1993, x = days after first harvest, unfested plots without irrigation (\square - \square) ($y = 288.9 + 12.3x - 0.03x^2$, $r^2 = 0.95$); unfested plots with irrigation (Δ - Δ) ($y = 299.7 + 15.5x - 0.03x^2$, $r^2 = 0.99$); *M. incognita*-infested plots without irrigation (\bullet - \bullet) ($y = 257.9 + 11.1x - 0.03x^2$, $r^2 = 0.98$); and *M. incognita*-infested plots with irrigation (\diamond - \diamond) ($y = 203.9 + 13.3x - 0.03x^2$, $r^2 = 0.98$). Plots were infested with *M. incognita* on 18 May 1992, and the first harvest date in 1993 was 13 April.

Cumulative total yield in plots with no irrigation and infested with *M. incognita* was reduced 9% from that of plots without irrigation and nematodes.

In 1993, quadratic models were the best fit for the relationship between days after first harvest and cumulative clover yields in plots with and without irrigation and *M. incognita* (Fig. 3B).

Yields were lower in plots with *M. incognita* and no irrigation in five of the six harvests when compared with yields from plots without irrigation or nematodes. Irrigated plots infested with *M. incognita* had yields comparable with plots with no irrigation and no nematodes. Irrigated plots with no nematodes had 54% higher cumulative total yield than plots with no irrigation and no nematodes. Without irrigation, plots infested with *M. incognita* had a 17% reduction in cumulative total yield compared with non-infested plots. Although irrigation had a positive effect on yields in *M. incognita*-infested plots, the clover stands in these plots declined rapidly and steadily through the summer and fall of 1993, and nearly all clover plants were dead by the last harvest, regardless of the irrigation treatment (Fig. 4A).

Effects on clover persistence. There were no interactions between PSV, *M. incognita*, or irrigation affecting white clover stolon density in 1992 or 1993, but individual treatment effects were significant. Treatments did not significantly affect stolon counts until October 1992 when all three treatments produced significant effects. Stolon densities in plots with either PSV or *M. incognita* were lower in October 1992 than in plots without these treatments, and stolon densities in nonirrigated plots were lower than in irrigated plots (Fig. 4). In 1993, stolon counts were numerically lower in PSV-treated plots throughout the growing season, and the reductions were significant for five of six counting dates (Fig. 4B). *M. incognita* reduced stolon density in all counts in 1993, and large differences were recorded in September and November (Fig. 4A). In September, plots with *M. incognita* averaged 2 stolons/m, while those without *M. incognita* averaged 36 stolons/m. Comparable values for November were 1 and 25 stolons/m, respectively. Irrigation increased stolon density throughout 1993, with significant increases recorded in four of six counts (Fig. 4C).

Effects on leaf area and petiole length. The effects of PSV, *M. incognita*, and irrigation on mean leaf area were similar in both years; so, 1992 and 1993 data were combined for further analysis. There was an interaction between PSV and irrigation; clover plants in irrigated plots had greater mean leaf area than in nonirrigated plots, but PSV reduced mean leaf area more in irrigated plots (Table 1). The presence of *M. incognita* affected leaf area in

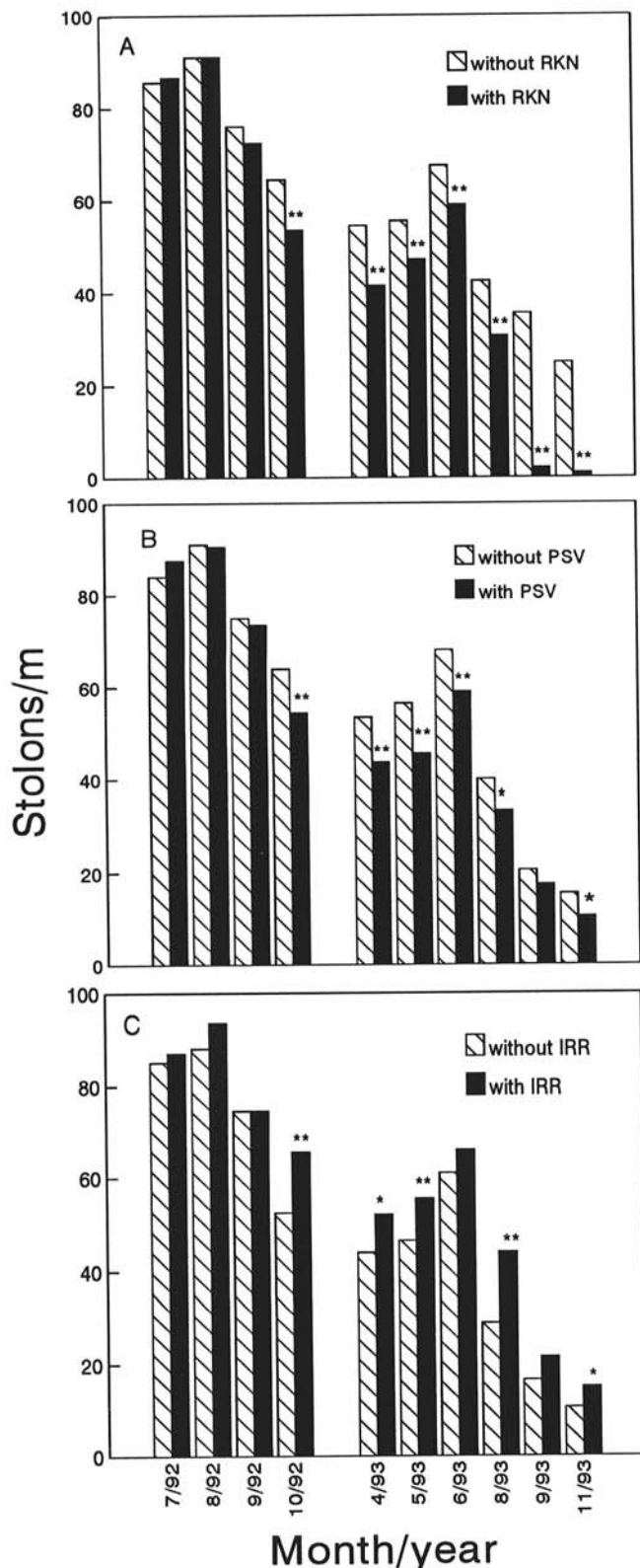


Fig. 4. Stolon density of white clover with and without A, *Meloidogyne incognita* (RKN); B, peanut stunt virus (PSV); and C, irrigation. * = different from the control at the 0.05 level by *F* test; ** = different from the control at the 0.01 level by *F* test.

TABLE 1. Supplemental irrigation interacted with peanut stunt virus (PSV), but not with *Meloidogyne incognita* (RKN) to affect the surface area of white clover leaves^a

	Mean leaf area (cm ²)	
	+ Irrigation	- Irrigation
- PSV	11.4	6.3
+ PSV	7.7	4.9
- RKN	9.8	5.9
+ RKN	9.3	5.3

^a Analysis of variance *F* test at *P* = 0.01 for combined data from 1992 and 1993.

TABLE 2. Interactions of supplemental irrigation with peanut stunt virus (PSV) and *Meloidogyne incognita* (RKN) on length of white clover petioles in 1992 and 1993^a

		Mean petiole length (cm)	
		+ Irrigation	- Irrigation
1992	- PSV	19.2	11.9
	+ PSV	14.3	9.4
1993	- PSV	16.8	6.1
	+ PSV	13.2	4.9
1992	- RKN	16.8	11.3
	+ RKN	16.8	10.0
1993	- RKN	16.4	6.1
	+ RKN	13.6	4.9

^a Analysis of variance *F* test significant at *P* = 0.01.

irrigated and nonirrigated plots similarly and no interaction was observed; leaf area decreased slightly in nematode-infested plots (Table 1).

There were interactions between irrigation and PSV and irrigation and *M. incognita* for mean petiole length. Plants in irrigated plots had longer petioles than plants from nonirrigated plots in 1992 and 1993 (Table 2). Plants infected with PSV had shorter petioles than plants without PSV (Table 2). Differences between years were also observed; petiole lengths were shorter in 1993. Petiole lengths were shorter in plots with *M. incognita* than in plots without *M. incognita*, with the exception of irrigated plots in 1992 (Table 2).

Effects on seed production. No significant differences in 100-head seed yields occurred among treatments in 1992, but in 1993, all treatments resulted in significant differences. Irrigation and nematode treatments reduced 100-head seed yields, while PSV treatment increased 100-head seed yield. The mean 100-head seed yields in 1993 were 5.2 and 6.1 g per plot (least significant difference [LSD] = 0.3, $P = 0.0001$) with and without irrigation, respectively; 5.9 and 5.3 g per plot (LSD = 0.3, $P = 0.0002$) with and without PSV, respectively; and 5.2 and 6.1 g per plot (LSD = 0.3, $P = 0.0001$) with and without nematodes, respectively.

***M. incognita* population dynamics.** *M. incognita* numbers (second-stage juveniles + eggs) were low in nematode-treated plots in July 1992, but had increased dramatically by October 1992 (Fig. 5). Nematode numbers ranged from 9,343 to 26,114 per 500 cm³ of soil. *M. incognita* numbers dropped sharply by June 1993 and stayed at low levels in September. By December 1993, *M. incognita* were almost undetectable in all nematode treatment plots. Although samples were not regularly collected from untreated plots, random spot sampling of these plots showed no *M. incognita*.

DISCUSSION

This study has provided the first definitive open-field measurement of the effects of PSV on the growth of white clover. Earlier studies of PSV effects on white clover growth were confined to potted plants in growth chambers (8), potted plants in open-top chambers in the field (10), and plants grown in small plots inside chambers in the field (6). In all of these situations, conditions for

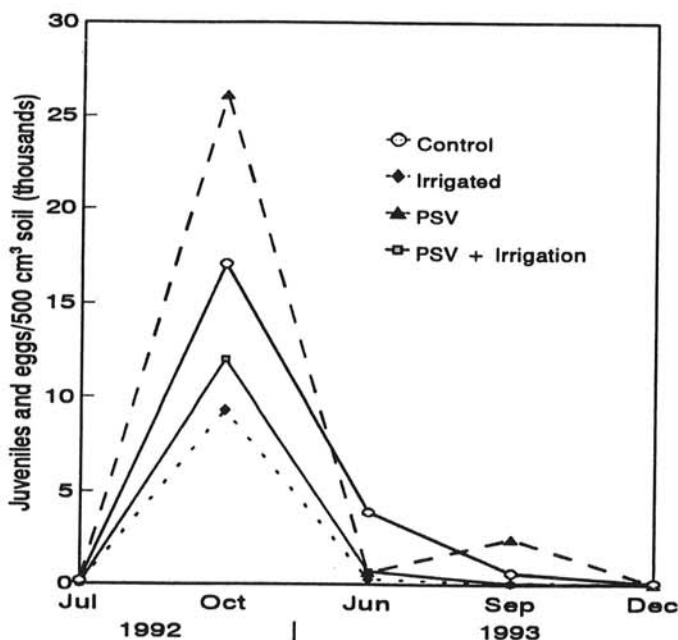


Fig. 5. *Meloidogyne incognita* population densities (juveniles + eggs) in white clover plots with and without irrigation and peanut stunt virus (PSV) in 1992 and 1993.

clover growth were necessarily artificial and carefully controlled; exclusion of insects and great care in handling plants were required to maintain PSV-free plants and avoid mechanical transmission. In the present study, use of half-sib white clover plants with and without hypersensitive resistance to PSV allowed unambiguous assessment of these effects under more natural field conditions. The accuracy of PSV treatments was maintained throughout the study. Herbage yields and stolon counts early in the study, before and immediately after PSV treatments were imposed, were not significantly different between plots with and without hypersensitive resistant plants, demonstrating that these half-sib plants have similar growth potential in the absence of PSV infection.

By the end of the 1992 growing season and throughout the 1993 growing season, significant reductions due to PSV were measured in herbage yield, leaf area, petiole length, and stolon density. Somewhat surprisingly, although losses due to PSV were significant, PSV-infected plants survived even in nonirrigated plots. The only interactions between PSV and drought appeared in leaf area and petiole length. Although leaves from irrigated plots in both PSV treatments had larger leaf areas and longer petioles than those from nonirrigated plots, virus infection caused relatively greater reductions in leaf area and petiole length in the irrigated plots than in the nonirrigated plots.

Since the white clover clones in this study were derived from a common maternal parent (clone 22R) with demonstrated drought tolerance (14), we propose that drought tolerance was inherited independently of hypersensitive resistance. We suggest that if half-sib plants with and without hypersensitive resistance had been developed from a drought-susceptible parent, the damaging effects of drought, *M. incognita*, and virus infection would have been even greater.

The damaging effects of *M. incognita* alone were significant, and, together with PSV during drought, caused literally a total loss of production and stand by the end of the second growing season. Nematode-infested plants were weakened as nematode numbers increased through the 1992 growing season, partially recovered during a mild winter and favorable spring, and then declined steadily through the summer and fall of 1993 until most finally died. These results were consistent with those from our earlier greenhouse study of PSV and *M. incognita* on white clover in which root systems were reduced more in plants with PSV and *M. incognita* than in plants with *M. incognita* alone (17). Most earlier studies of plant virus and nematode interactions dealt with nematodes as vectors of plant viruses. Few studies have examined non-vector relationships. Among these, most report additive effects (4,21) and few report synergistic effects (20). In the present study, PSV acted independently of drought and *M. incognita* in reducing productivity and persistence of white clover. The combined effect of PSV with either other factor appeared to be additive.

Results of the present study clearly demonstrate the need to include resistance to PSV, *M. incognita*, and drought in germ plasm and cultivars of white clover developed for use in the southeastern United States. We are currently selecting for *M. incognita* resistance within the PSV-resistant southern regional virus resistant white clover germ plasm (7) and are continuing selection and breeding with drought-tolerant PSV-resistant white clover clones from this and other studies.

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