Filtered-Air Enclosures Exclude Vectors and Enable Measurement of Effects of Viruses on White Clover in the Field

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ABSTRACT

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Circular, clear plastic enclosures, with air introduced through insect-proof filters as a means of excluding vectors, were evaluated in a study of the effects of three aphid-transmitted viruses on white clover growing in field plots. Successful use of these enclosures requires a level test area with access to electricity and irrigation water. Peanut stunt virus caused a greater reduction in forage yield than clover yellow vein virus. In a single test with duplicate plots, alfalfa mosaic virus caused reductions in yield intermediate between those caused by peanut stunt virus and clover yellow vein virus.

Additional key words: *Trifolium repens*

Viruses transmitted by aphids occur in most areas where white clover (*Trifolium repens* L.) is grown. The almost ubiquitous presence of aphids, lack of a chemical control to prevent virus transmission by viruliferous winged aphids, and possible transmission by harvesting procedures, have prevented accurate assessment of effects of viruses on growth of white clover in the field. Most measurements of virus damage to clover have been made in greenhouses or controlled environment chambers (3,9,10) because of difficulties in controlling aphids in the field.

Enclosures with filtered air offer a means to exclude aphids from plants growing in the field. Circular enclosures have been used to study the effect of air pollution on plant growth (7), and rectangular enclosures have been used to exclude vectors in virus research (6).

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Our research evaluated the use of circular, clear plastic enclosures, with air introduced through filters to exclude vectors, in a study of the effects of alfalfa mosaic virus (AMV), clover yellow vein virus (CYVV), and peanut stunt virus (PSV) on forage yields of white clover growing in field plots. Circular enclosures were chosen because filtered air can be introduced around the entire perimeter to provide a reasonably uniform environment.

MATERIALS AND METHODS

Enclosures. Three filtered-air enclosures with walls and tops of clear plastic (Glass-clear EE-S, 0.25 mm thick; Renolit, West Germany) were constructed following plans of Heagle et al (7) with two minor modifications (Fig. 1). Walls were 1.2 rather than 2.4 m high, and a top with holes 5 cm in diameter spaced 25 cm between centers was added to exclude flying insects. Fans replaced the air in the enclosures four to five times per minute and resulted in air exiting through the 5-cm holes at a velocity of about 2.8 m/sec. Fans were controlled by a thermostat set for continuous operation at temperatures above 0 C; they were not run at temperatures below 0 C to avoid clogging of filters with frost and ice. Removal of the top and use of an A-frame ladder provided access and eliminated the need for doors in walls. Fiberglass, residential-type furnace air filters coated with Tack-Trap (polyisobutylene; Animal Repellants, Inc., Griffin, GA) were used to filter vectors from air blown into enclosures. Filters were replaced as needed to maintain airflow. Air temperatures at plant canopy height inside and outside enclosures were measured at 15-min intervals during a representative part of the summer with thermocouples and a recorder.

Viruses. Ladino white clover was used because this cultivar has a broad gene base and is representative of the large-type cultivars of white clover. Sources of viruses, virus identifications, and inoculation techniques were the same as published previously (1).

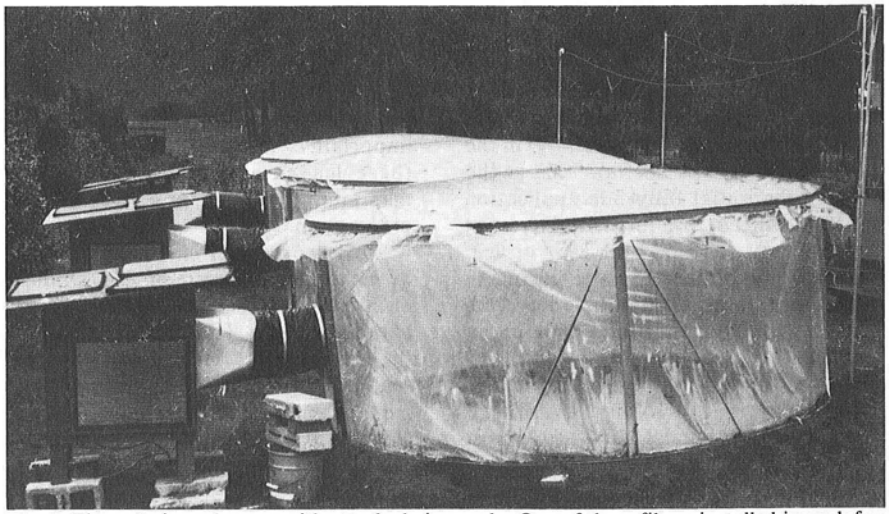

Fig. 1. Filtered-air enclosures with attached air supply. One of three filters installed in each fan housing is visible. Three new replacement filters are on top of the housing. The dark color of the installed filter indicates that it is partially clogged by foreign matter and should be replaced.

Eight triangular plots, each with a base of 67 cm and sides of 85 cm, were arranged in each enclosure (Fig. 2). A trickle-type irrigation system of porous-wall tubing was installed in the soil at a depth of about 10 cm. Plots were surrounded with boards measuring 2 × 9 cm, with 4 cm in the soil to confine plant growth to the plots and to serve as a guide for harvesting at a uniform height of 5 cm. Lime, fertilizers, and irrigation water were applied in amounts adequate for vigorous growth of clover. Soil at the test site was fumigated with methyl bromide before the enclosures were installed and the clover planted.

Plants for four virus conditions—virus-free or singly infected with AMV, CYVV, or PSV—were grown and inoculated in the greenhouse. Plants for one enclosure were vegetatively propagated from those that had been verified for virus infections previously. Seedlings were inoculated with the respective viruses for the other enclosures. Plots were established in the fall of 1978 by transplanting 10 plants to each plot as shown in Fig. 2. Two plots of each virus condition were placed at random in each enclosure.

The first harvest was taken on 13 December 1978, the second on 16 March 1979, and later ones at approximately 4-wk intervals thereafter, for a total of 13. At each harvest, all forage above the board borders was taken, dried in ovens at 70 C, and weighed. Shears and hands were sanitized between plots. The plots and space inside each enclosure were sprayed with a pesticide after each harvest, and a systemic pesticide was added biweekly in irrigation water. After the 12th harvest, stands were evaluated by determining the number of live stolons per meter (4). Enclosures were removed after the 13th harvest on 13 December 1979, and plots were fenced to exclude grazing animals. Observations were continued and one harvest was made in the spring of 1980.

Analysis. Enclosures formed independent replicates. Two randomized plots of each of the four virus conditions per enclosure were intended. However, the development of an obvious moisture gradient caused by the slope of the site necessitated adjustments of yields for upper and lower positions on the slopes within enclosures. This adjustment was achieved by least-squares analysis of yields from CYVV-infected, PSV-infected, and virus-free plots in all three enclosures.

When enclosures were removed, assays of five plants per plot verified that all plants inoculated with CYVV and PSV were infected with these viruses. Only vegetatively propagated plants were uniformly infected with AMV. Therefore, the effects of CYVV and PSV were evaluated in three enclosures and AMV in only one.

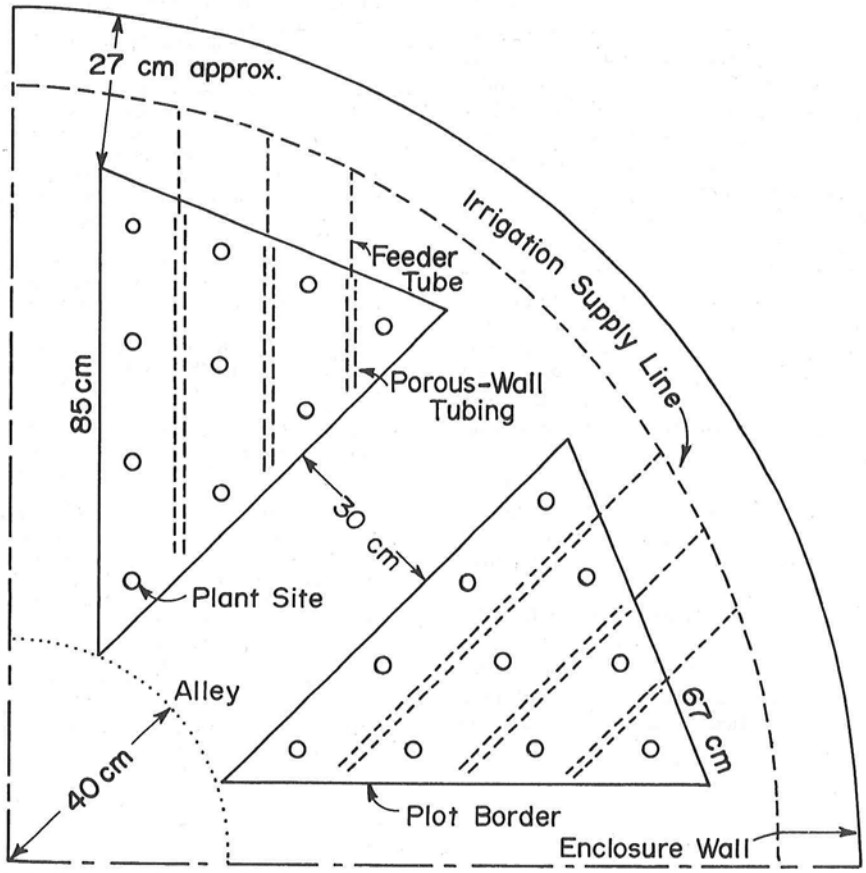

Fig. 2. Quadrant of area enclosed by a filtered-air enclosure, showing plan of plots and irrigation system.

RESULTS

Enclosures. All but one plant assayed were in the same virus condition in December 1979 as when planted in the fall of 1978. The exception was a plant from a control plot, adjacent to an AMV-infected plot, found infected with AMV.

The walls and tops of enclosures slowly became less transparent and less flexible during the test period. Transparency was reduced as the plastic developed a pale brown color, and by growth of an unidentified yeast on inner surfaces. A few cracks developed late in the test period. The average temperature difference between the inside and the outside of the enclosures, for the 6-mo period of July–December, was less than 0.5 C; daily averages of these differences never exceeded 2 C. Continuous replacement of air, except when the temperature was below 0 C, caused a rapid loss of soil moisture, and this loss aggravated a moisture gradient that was caused by a 2% slope of the test area.

Viruses. Vegetatively propagated and seedling plants had similar yields and growth patterns. The total mean yield per plot for control plots established with seedlings was 617.0 g. The corresponding yield for control plots established with vegetatively propagated plants was 602.1 g. The similarity of the two kinds of plants was further demonstrated by the absence of any detectable difference in their response to treatments. Therefore, data from the two kinds of plants were combined.

Over the 12-mo experiment, white clover yield losses amounted to 14% when infected with CYVV and 28% when infected with PSV. These reductions, displayed in the cumulative adjusted yield curves (Fig. 3), increased progressively with duration of infection.

Yields were compared by season—

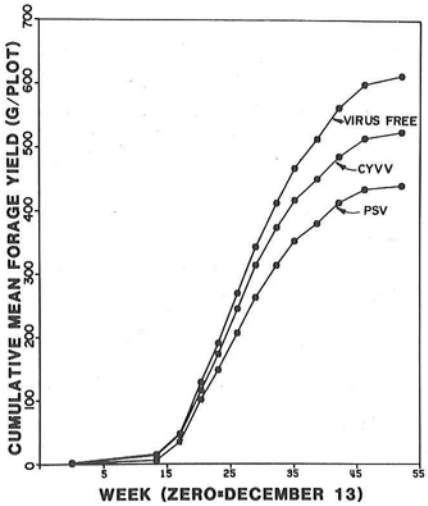

Fig. 3. Cumulative yields (dry weight) of white clover infected with clover yellow vein virus (CYVV) and peanut stunt virus (PSV) and of virus-free clover grown in filtered-air enclosures in the field for 1 yr.

Table 1. Forage yields of virus-free and virus-infected white clover grown in filtered-air enclosures in the field for 1 yr[a]

Virus condition[b]	Winter-spring (161 days)		Summer (84 days)		Fall (119 days)	
	Yield, g/day	% of control	Yield, g/day	% of control	Yield, g/day	% of control
Three enclosures, six plots per condition[c]						
Virus-free	1.185	100	3.282	100	1.219	100
CYVV	1.081	91	2.887	88	0.900	74
PSV	0.924	78	2.424	74	0.732	60
± SE[d]	± 0.036		± 0.045		± 0.042	
One enclosure, two plots per condition[e]						
Virus-free	1.236	100	3.291	100	1.284	100
CYVV	1.082	88	2.954	90	0.921	72
AMV	0.972	79	2.543	77	0.916	71
PSV	0.872	71	2.357	72	0.647	50

[a] Mean forage yields are in grams of dry weight. Harvests were made on 13 December 1978, 16 March 1979, and 3- to 4-wk intervals thereafter for a total of 13 harvests. The last harvest was made on 12 December 1979.
[b] AMV = alfalfa mosaic virus, CYVV = clover yellow vein virus, PSV = peanut stunt virus.
[c] Seedling and vegetatively propagated plants.
[d] Standard error.
[e] Vegetatively propagated plants.

winter-spring, summer, and fall—using estimates of average daily growth rates (Table 1). Clover produced more growth in the summer, when virus infected, while relative yield losses due to virus infection were larger in the fall. In the enclosure supplying results for AMV infection, yields from healthy plots and from those infected with CYVV or PSV were similar to corresponding yields in other enclosures. Over the 12-mo period, yield losses due to AMV were 24%, intermediate to losses due to CYVV (15%) and PSV (34%).

The clover grew well throughout the year, producing a canopy that completely covered the ground of all plots on most harvest dates. Average stand thickness at the 12th harvest, based on stolon counts, varied from 50 to 52 live stolons per meter, but this variation could not be attributed to virus conditions.

After the enclosures were removed on 13 December 1979, the growth that accumulated on all plots during the winter was remarkably similar. This growth was removed and discarded on 1 May 1980. The clover was harvested again on 30 May when differences in growth were evident. Plots that were initially virus-free yielded an average of 40.0 g of oven-dry forage per plot, those initially infected with CYVV yielded 26.7 g, and those initially infected with PSV yielded 27.2 g. The two plots initially infected with AMV yielded an average of 38.1 g, and the corresponding control plot yielded 47.8 g.

DISCUSSION

Enclosures. Control of viruses transmitted by vectors is essential in most research conducted to measure virus effects in the field. Virus-free and virus-infected white clover plants can be grown in adjacent plots in the field for 1 yr inside these filtered-air enclosures. The one uncontrolled infection detected could have resulted from root anastomosis.

The plastic walls of these enclosures need to be replaced annually because of weathering, including some cracks and a small reduction in transparency. White clover, which grows well in partial shade, was not affected adversely by the loss in transparency; other species may not be so tolerant. We found it necessary to adjust for a soil moisture gradient in analyses of yield results. This might be avoided by choosing a level site or an improved irrigation technique. Plant growth in the enclosures was generally similar to that observed outside, despite the combined effects of light quality and intensity, increased transpiration, and elimination of most rain and dew.

Viruses. As we found in earlier work (5), seedling and vegetatively propagated plants performed similarly. It is desirable to use both types because clover pastures are often a mixture of plants from seed and from asexual propagation (8). All three viruses reduced productivity of white clover plants in the enclosures, which corresponded to results obtained from controlled environment chambers (3).

Although viruses are known to affect stolon development (3), the numbers of live stolons were similar in virus-infected and control plots. Yield reductions in similar stands indicate that leaf development was the primary component of growth affected by virus infection.

Forage yields from virus-free and virus-infected white clover in enclosures, observations of growth in the spring after enclosures were removed, and results of previous research (2) indicate that virus diseases reduce the productivity of white clover in pastures. The magnitude of damage in pastures depends on duration of infection, number of viruses infecting a plant, number of plants infected, and such environmental and management stress factors as temperature (11), moisture level, insects, other diseases, defoliation, and plant communities. The net effect of virus infection indicates the need for resistant cultivars.

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