

Viruses Infecting Red Clover in Pennsylvania

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ABSTRACT

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Red clover of different ages in Centre, Crawford, Lancaster, Westmoreland, and Wyoming counties was surveyed for viruses in 1979; Centre and Lancaster counties were surveyed again in 1980. Enzyme-linked immunosorbent assay was used to detect specific viruses in randomly selected and symptomatic plants. Results for the two survey years were similar. Bean yellow mosaic virus, the most common in all fields, produced more than 80% of all positive assays in both years. Alfalfa mosaic, red clover vein mosaic, clover yellow vein, and peanut stunt viruses were detected at much lower frequencies. Clover yellow mosaic virus was not detected. Incidence of viral disease increased rapidly from year of seeding to the following year; it then stabilized at levels ranging from 30 to 54% of the plants infected, even in stands seeded 4 yr earlier.

Red clover (*Trifolium pratense* L.), an important forage legume in the northeastern United States, is grown more extensively than any other legume in Pennsylvania (J. E. Baylor, *personal communication*). It has a shorter life than alfalfa, presumably because of diseases (1,6) and other stresses (1). Viral symptoms are common in red clover in Pennsylvania, and viruses probably contribute to its poor persistence (1). The nature and extent of the virus problem in red clover have not been determined, partly because an expedient assay technique has not been available.

The Southern Regional Project, S-127, which was initiated in 1977, seeks to determine the prevalence of several viruses in forage legumes. Sensitized plates for enzyme-linked immunosorbent

assay (ELISA) were mailed to collaborators, who applied sap from survey plants to the plates and returned them to Clemson University for final processing and evaluation (2,3). The incidence of specific viruses in red clover stands of different ages and locations in Pennsylvania was determined in a similar manner. Results of the 1979 and 1980 surveys are reported here.

MATERIALS AND METHODS

ELISA plates were coated with antisera of alfalfa mosaic (AMV), bean yellow mosaic (BYMV), clover yellow mosaic, clover yellow vein, peanut stunt (PSV), and red clover vein mosaic (RCVMV). These viruses were selected because they were included in the S-127 survey, were suspected constituents in the red clover virus complex, because the northern range of peanut stunt virus was of interest, and because antisera were available.

We selected fields in Centre, Crawford, Lancaster, Westmoreland, and Wyoming counties to represent the corners and center of the state. All locations were sampled in 1979, but only those in Centre and Lancaster counties were sampled in 1980. Samples, collected in early September, consisted for each field of five leaves from each of 30 plants selected at

random at every 10 paces on a line walked across the fields. Fields planted in 1977, 1978, or 1979 were sampled at each location in 1979. Fields planted in 1977, 1978, 1979, or 1980 were sampled in 1980. Fields older than 3 or 4 yr were rare, and a 1977 field was not sampled in Crawford County.

Five additional plants with suspected viral symptoms were sampled from each field to detect viruses that might have been missed by the random sampling. Plants with symptoms were not found in two fields. Leaf samples were refrigerated at the field site and stored 1-3 days at 4 C before ELISA, according to procedures described previously (2). The published procedure was modified by adding 4.5 g of sodium diethyldithiocarbamate to the phosphate-buffered saline in which leaf samples were ground.

RESULTS

Viruses were detected at varying incidences in each of the counties (Table 1). Clover yellow mosaic virus was not detected in 1979 and was not assayed for in 1980. One plant in each of the 1979 Lancaster and Westmoreland samples contained PSV, and clover yellow vein virus was detected in a single plant in Westmoreland County in 1979 and again in one plant in Centre County in 1980. Because of problems with antisera, no results for PSV were obtained in 1980.

In 1979, 25% of the randomly sampled plants contained at least one of the viruses assayed. Incidences of virus in stands seeded in 1977 and 1978 were 30 and 35%, respectively. BYMV constituted 72% of all viral infections. Of the 75 symptomatic plants sampled, one had AMV, four had RCVMV, and 48 had BYMV.

The 1980 results were similar to those in 1979; 35% of the randomly sampled plants were positive for at least one virus. Incidences of virus in the stands seeded in

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Table 1. Incidence of three viruses in red clover of different ages sampled in five Pennsylvania counties in 1979 and two in 1980^a

County	AMV		RCVMV		BYMV		AMV + BYMV		Plants infected, %	
	1979	1980	1979	1980	1979	1980	1979	1980	1979	1980
Wyoming										
1977	0	... ^b	2	...	3	...	0	...	16	...
1978	0	...	0	...	2	...	0	...	6	...
1979	0	...	0	...	0	...	0	...	0	...
Lancaster										
1977	0	1	1	1	8	9	2	2	36	43
1978	1	1	1	1	4	11	1	1	23	46
1979	1	0	1	1	5	15	2	0	33	53
1980	...	0	...	0	...	0	...	0	...	0
Westmoreland										
1977	0	...	0	...	2	...	0	...	6	...
1978	1	...	0	...	8	...	1	...	36	...
1979	2	...	0	...	0	...	0	...	6	...
Crawford										
1978	0	...	8	...	9	...	0	...	56	...
1979	0	...	0	...	0	...	0	...	0	...
Centre										
1977	0	1	1	0	17	11	0	0	60	40
1978	0	0	0	1	16	8	0	0	53	33
1979	2	2	0	0	3	6	0	9	16	56
1980	...	0	...	0	...	2	...	0	...	6

^aNumber of infected plants in a sample of 30 randomly selected plants. AMV = alfalfa mosaic virus, RCVMV = red clover vein mosaic virus, and BYMV = bean yellow mosaic virus.

^bNot sampled.

1977, 1978, and 1979 were 42, 40, and 54%, respectively. BYMV made up 76% of all infections in Lancaster County and 65% of those in Centre County. Of the 30 symptomatic plants sampled, 2 had AMV and 10 had BYMV.

The largest increase in virus incidence occurred between the year of seeding and the second year; incidence then leveled off and changed little in subsequent years.

DISCUSSION

The survey established that BYMV was

the predominant virus in Pennsylvania red clover, with RCVMV and AMV much less prevalent. Virus incidence was comparable across the state except for Wyoming County in the northeast, where only 7% of the plants were infected. The reason for this exception is not known.

The 1979 and 1980 surveys indicated that BYMV incidence increased rapidly from year of seeding to the following year, and then plateaued at 30–50%. This plateau effect may result from several factors. Virus-infected red clover plants

are more susceptible to root rot (1,6) and winter injury (4), thus many virus-infected plants would be lost each year. Infection rates in older stands might fall below the death rate of virus-infected plants because aphid transmission of viruses would be reduced by the increased space between surviving clover plants. Further, the population would have higher proportions of nonhost weed species and resistant or unpreferred clover plants.

We can suggest several reasons for the failure of all symptomatic plants to assay positively with ELISA. When the frequency of obvious symptoms was low, as in the youngest fields, plants with questionable symptoms were selected, and viruses other than those assayed for could have caused some symptoms. Nonviral, foliar mottling also occurs in red clover (5).

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