

Response of *Trifolium hybridum* Germ Plasm to Mechanical Inoculation with Bean Yellow Mosaic and Clover Yellow Vein Viruses in the Greenhouse

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ABSTRACT

Alconero, R. 1983. Response of *Trifolium hybridum* germ plasm to mechanical inoculation with bean yellow mosaic and clover yellow vein viruses in the greenhouse. *Plant Disease* 67:1272-1273.

Multiple inoculations with either bean yellow mosaic virus or clover yellow vein virus under greenhouse conditions resulted in the relatively rapid development of symptoms within 2 wk of the first inoculation in 7% of the 129 accessions tested. Most of the accessions (87%) showed a delayed response in all inoculated plants after four inoculations and 13 wk of observation. In six plant introductions, at least 20% of the plants tested remained symptomless for 6 mo although serology and bioassays detected low virus titers in most plants.

Trifolium hybridum L. (alsike clover) is the clover species most adversely affected by viruses in germ plasm collections of perennial clovers at the Northeast Regional Plant Introduction Station. Field performance records indicate that with few exceptions, alsike clover accessions are not winterhardy and do not recover well in the spring following planting. There is considerable variation within each accession, as would be expected in a self-incompatible species, and some indication of possible winterhardiness and tolerance to viruses in some individuals. The aim of these studies was to identify individuals tolerant to bean yellow mosaic virus (BYMV) or clover yellow vein virus (CYVV) under controlled greenhouse inoculations and later expose them to field conditions for further evaluation.

MATERIALS AND METHODS

Tests were conducted during a period of 18 mo in a greenhouse at 27–33 C with sunlight supplemented daily by 2 hr of fluorescent light (2,000 lux) during the winter. Twenty plants per accession, four plants per 15-cm-diameter pot, were inoculated per virus when plants were about 1 mo old. Four inoculations of at least four leaves per plant were made at 2-wk intervals. Corundum-dusted leaves were mechanically inoculated with a 1:5 dilution of triturated *T. repens* L. leaves infected with CYVV or *T. hybridum* L. leaves infected with BYMV in phosphate-buffered saline, pH 7.5, with 0.001 M ethylene diamine tetraacetic acid and 1% polyvinylpyrrolidone. Symptomatic

plants were discarded as soon as symptoms were clearly expressed.

Possible latent infections were tested by enzyme-linked immunosorbent assay (5) 6 mo after the first inoculations. Bioassays for possible latent infections were also made by inoculating bean seedlings (*Phaseolus vulgaris* L. 'Black Turtle Soup'). Special care was taken to avoid aphids by regular application of insecticides. The virus isolates BYMV-Ky204-1 and CYVV-Pratt and their antisera with 1:8192 and 1:2048 micro-precipitin titers, respectively, were obtained from O. W. Barnett, Clemson University, Clemson, SC.

RESULTS AND DISCUSSION

Preliminary tests indicated the need for multiple inoculations and relatively high temperatures of 27–33 C for adequate symptom expression. Lower temperatures (22–26 C) usually resulted in more latent infections not unlike those observed by Hampton and Hanson in alsike clover with viruses that were seedborne in red clover (11). At 27–33 C, the response of individual plants within each accession varied from severe mosaic, necrosis, and stunting 14 days after inoculation to very mild mosaic or veinbanding that appeared only after four inoculations. In some cases, detectable symptoms were not observed after 6 mo of observation but systemic infections were detectable by serology or bean bioassay. Transfer of symptomless plants to cooler temperatures (22–26 C) did not result in symptom expression.

Relatively few accessions, nine of 129, responded with all individuals symptomatic within 2 wk of the first inoculation. After four inoculations and about 13 wk of observation, all plants in 87% of the accessions had been eliminated. In these accessions, most were equally affected by both viruses. Standard alsike clovers Alaska Common, Aurora, Canadian Common, Minnesota Common, and Oregon Common re-

sponded as most accessions did to BYMV and CYVV.

A degree of tolerance, as evidenced by only latent infections or a 6-mo delay in symptom expression to either virus in at least 20% of individuals tested, was found in six accessions (Table 1). Tolerance to CYVV was only found in 15 accessions and to BYMV, only in one. When tolerance was found to both viruses, as in plant introductions (PIs) 120080, 120081, 174390, 174391, 184559, and 251852, their previous records of field infection by unidentified viruses varied from low to high. Two accessions from Iran, PIs 228368 and 230274, had been reported from field evaluations to be free of virus symptoms and to be fair to good in winterhardiness and spring recovery. Under greenhouse conditions, they were found to have some tolerance to CYVV only.

Surveys of viruses in germ plasm field plantings indicate that white clover mosaic virus and red clover vein mosaic virus can be found in multiple infections with CYVV and BYMV in 1-yr-old alsike clover plants (1). The presence of these viruses in alsike and other clovers in the northeastern United States and adjacent areas has been recorded (7,12–14) and the

Table 1. Alsike clover introductions found tolerant to bean yellow mosaic virus (BYMV) and clover yellow vein virus (CYVV) under greenhouse conditions

PI no.	Origin	Virus	
		BYMV	CYVV
120080	Turkey	5/20 ^a	10/20
120081	Turkey	5/20	7/20
174390	Turkey	7/18	6/20
174391	Turkey	9/18	6/18
184343	Sweden	0/19	4/20
184552	Finland	0/20	5/20
184555	Finland	1/19	9/18
184559	Finland	4/20	4/18
184989	Sweden	0/19	5/20
204524	Turkey	1/19	5/19
205310	Turkey	4/15	1/20
205311	Turkey	1/19	3/17
206763	Turkey	0/20	5/19
228368	Iran	2/20	4/20
230274	Iran	2/18	4/18
251852	Italy	4/18	4/18
253198	Yugo	0/20	6/20
278792	USA	0/20	4/20
278797	USA	0/20	6/20
278812	USA	0/20	4/20
278826	USA	0/16	5/18
278828	USA	1/20	5/20

^a Plants with no symptoms/plants inoculated four times.

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presence of other viruses infecting clovers (2-4,6,8-10) has yet to be determined in field plantings of perennial clover germ plasm. The relatively high number of viruses to which alsike clover is susceptible and the various combinations that may be found under field conditions make it unlikely that good correlation will be observed between field infections and greenhouse evaluations with one virus. Nevertheless, the individuals found tolerant to either BYMV or CYVV under greenhouse conditions are being evaluated to determine their response to other strains of BYMV and CYVV as well as to other viruses.

Among accessions found tolerant to BYMV and/or CYVV, 36% originated in Turkey. These accessions represented about 77% of the representatives of that country and 10% of the PIs in the alsike germ plasm collection. Considering the generally poor performance and high susceptibility of the alsike clover

introductions to several viruses, new acquisitions from eastern Mediterranean countries, especially Turkey, may offer the multiple resistance needed.

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