Resistance and Tolerance to Alfalfa Mosaic Virus in Alfalfa

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ABSTRACT

Resistance and tolerance to alfalfa mosaic virus (AMV) in alfalfa (*Medicago sativa* L.) were observed in commonly grown cultivars. Resistance to AMV strain 1234-5670 was shown to be controlled by a recessive gene. Susceptible clones varied in reaction to AMV, but one clone was shown to be highly tolerant to strain 1234-5670. Phytopathology 61:369-371.

Additional key words: alfalfa clones, *am-1* gene controls resistance.

Alfalfa (*Medicago sativa* L.) is the major forage legume grown in the Great Lakes States, and recent studies (6, 7, 8, 10) have shown that alfalfa mosaic virus (AMV) greatly reduces yield of alfalfa forage. Development of cultivars resistant to AMV appears to be the most feasible means of disease control (5, 11, 12). Since no cultivars have been developed which are resistant to AMV and no information is available concerning the genetics of resistance to AMV in alfalfa, the objectives of this research were to locate sources of resistance and tolerance to AMV and to determine the genetic control of resistance to AMV in alfalfa. In this paper, "resistance" refers to the condition of the host plant which prevents the pathogen from becoming established under a gamut of environmental conditions, "susceptibility" is the condition of the host which allows the pathogen to become established, and "tolerance" is the ability of a susceptible plant to produce a normal or near-normal yield while infected by the pathogen.

MATERIALS AND METHODS.—Currently grown alfalfa cultivars are composed of germplasm of diverse origin and nature (4), and were selected as possible sources of resistance. Twenty clones from each of 20 cultivars (Table 1) were evaluated for both resistance and tolerance to 26 isolates of AMV. The 26 isolates were later shown to be composed of 18 different strains (6). Procedures for vegetatively increasing the clones and method of inoculation have been described (7, 8).

Tolerance to isolate 514 of AMV strain 1234-5670 was studied in the field, using Vernal alfalfa clones V4, V6, and V8. These clones were selected because they were symptomless when inoculated and grown in the greenhouse, even though relative infectivity and serological tests indicated that AMV was present in a relatively high concn. Stem cuttings of the three clones were produced (7, 8); half were inoculated with isolate 514 and half maintained virus-free. Cuttings were 35 days old when inoculated, and were transplanted to the field 2 days later. The noninoculated cuttings were randomly assayed for AMV, and none were found to be infected; the inoculated plants were assayed at random and all were found to contain virus. Treatments were inoculated vs. noninoculated plots arranged in a randomized complete block design with five replications. Each treatment contained five rows, 1 foot apart, with 12 plants/row spaced 9 inches apart. Each treatment was surrounded by three rows of Vernal alfalfa with plants spaced 9 inches apart. Plot size was 1/968 acre. Cuttings were transplanted on 19 July 1968. Retransplanting was done on 2 August 1968 to obtain 100% stands. One cutting of hay was obtained on 3 October 1968.

Inheritance of resistance to isolate 514 of AMV strain 1234-5670 was studied in the greenhouse by crossing resistant and susceptible clones. Clones M8 and R7 were derived, respectively, from the cultivars Pioneer 525 and Saranac, and were selected as resistant parents on the basis that virus could not be recovered from them after repeated inoculations with isolate 514. AMV was readily recovered from the susceptible clones which were designated G4, H4, O5, and O7, and were derived, respectively, from the cultivars Glacier, Grimm, Ranger, and Ranger. Isolate 514 was maintained in *Melilotus alba* L. and periodically inoculated on differential hosts to insure that it continued as a genetically stable strain.

Crosses among clones were made using standard hand pollination techniques. Pods containing the F_1 alfalfa seeds were harvested individually, and seed of all identical crosses later combined. The F_1 seeds were scarified, planted in flats of steam sterilized compost soil. Seedlings were maintained in the flats in the greenhouse until about 3 days prior to anthesis when each flat was placed in a cage with one nucleus of about 1,500 bees. The F_2 seed was obtained from the bee-pollinated plants and handled in the same manner as the F_1 seed. When the F_2 seedlings were about 20 cm tall, they were inoculated by rubbing Carborundum-dusted leaves with expressed sap from *Pisum sativum* L. 'Perfected Wales' which had been infected with AMV isolate 514 for 14 days. Twenty-one days after inoculation, the tops of each seedling were removed, triturated in a mortar with a pestle, diluted 1:2 (w:v) in distilled water, and rubbed onto Carborundum-dusted primary leaves of at least three 10-day old

TABLE 1. Per cent plants of 20 clones from each of 20 alfalfa varieties developing symptoms when inoculated with the least (545), intermediate (514), and most (532) virulent isolates of alfalfa mosaic virus as compared to the mean reaction of 26 isolates on these clones

Variety	% Infection by selected AMV isolate			Mean % infection by all 26 AMV isolates
	545	514	532	
WL 202	0		39	9
Rhizoma	0	5	67	11
Arnim	0	5	44	14
Atlantic	0	10	33	14
Saranac	5	11	11	15
Vernal	0	0	53	15
Buffalo	0	12	70	16
Glacier	0	25	25	16
Caliverde	0	6	45	16
DuPuits	0	15	32	17
Ranger	0	5	29	17
New Gold	0	21	40	17
Grimm	11	15	50	19
Pioneer 525	16	11	35	19
Dairyman	0	25	75	21
Pioneer X-583	0	15	50	21
Pioneer 522	6	25	24	22
Narraganset	11	17	100	24
Terraverde	0	14	80	26
Milfeuil	0	20	78	28
Mean	2.7	12.9	47.7	

Phaseolus vulgaris L. 'Bountiful' plants. If local lesions formed on any of the bean leaves, the F_2 alfalfa seedling was considered to be infected and, therefore, susceptible; if no lesions formed, it was considered to be resistant. The sensitivity of this method was tested by crossing two susceptible clones and simultaneously studying them with the other crosses. The method for detection of plants susceptible to AMV was considered to be quite accurate, since only 1.3% of the known 150 susceptible F_2 seedlings actually escaped infection. Susceptibility and resistance were not measured in the F_1 due to difficulties involved in producing sufficient quantities of seed.

RESULTS.—Eight thousand and four plants, representing 400 clones from 20 cultivars, were inoculated with 26 isolates of AMV; 17.9% developed symptoms; the remainder were symptomless. Virulence of the isolates varied considerably. The least virulent isolate (No. 545) produced symptoms on only 2.7% of the clones, while the most virulent (No. 532) produced symptoms on 47.7% of the clones (Table 1). The average infection for each clone is summarized in Table 1. The cultivar in which the fewest plants with symptoms developed was WL-202 (9%), while Milfeuil had the most plants with symptoms (28%).

The tolerance reaction when studied in the field supported observations which had been made in the greenhouse. Plant vigor varied considerably among clones V4, V6, and V8, but variation did not occur within clones in the greenhouse and did not appear to be influenced by infection with AMV. When tolerant clones were studied in the field, clone V8 yielded almost twice as much forage as clones V4 or V6, whether infected with AMV or not (Table 2). Inoculated plants

TABLE 2. The reaction of tolerant clones of Vernal alfalfa to inoculation with alfalfa mosaic virus isolate 514 in the field

Clone no.	Inoculated	Dry wt (g/plot)	% Dry matter	% Yield reduction by AMV
V4	Yes	339	23	−4.2
	No	354	22	
V6	Yes	267	21	−7.8
	No	289	20	
V8	Yes	674	22	+3.3
	No	652	23	

of clone V4 yielded considerably less forage than noninoculated ones, indicating a low tolerance to AMV. Clone V6 exhibited tolerance similar to V4, while V8 was most tolerant. Differences between inoculated and noninoculated were significant at the .05 level.

Results of the study on inheritance of resistance to AMV (Table 3) indicate that a single recessive gene controls resistance to isolate 514 of strain 1234-5670. The data show good fits for F_2 3:1 and 7:9 (susceptible:resistant) ratios, with susceptibility being dominant. The 7:9 ratios were expected for an F_2 developed by crossing homozygous resistant with a heterozygous susceptible when resistance is recessive. Dominance also appears to be complete, since no intermediate reactions were detected. No indication of extrachromosomal inheritance was evident when reciprocal crosses were assayed separately.

DISCUSSION.—Two distinct reactions to AMV in alfalfa were observed: (i) resistance to the pathogen; and (ii) tolerance of the pathogen by susceptible plants. Although alfalfa is generally tetraploid in nature, some marker genes have been shown to be inherited as diploid rather than tetraploid (1, 3). The simplest explanation of the data reported in this paper necessitates a diploid explanation; consequently, the remainder of the discussion is based upon this assumption.

In a previous report (9), it was suggested that the gene conditioning resistance be designated *amv-1*. The symbol *am-1* is now recommended to designate the recessive gene controlling resistance to this strain of AMV, since these symbols conform to the rules for naming genes established by the Alfalfa Improvement Conference. The symbol, am, refers to alfalfa mosaic virus, and the No. 1 to the first gene described which confers resistance to AMV in alfalfa. It is assumed that more genes will be found which will confer resistance to other strains of AMV, and these can be numbered consecutively and chronologically. This system should avoid much of the confusion that has developed with other host:pathogen gene systems of nomenclature. When this symbolism is applied to the parental material used in this study, their genotypes can be written as follows:

M8 = homozygous resistant	*am-1*	*am-1*
R7 = homozygous resistant	*am-1*	*am-1*
O5 = heterozygous susceptible	*AM-1*	*am-1*
O7 = heterozygous susceptible	*AM-1*	*am-1*
G4 = homozygous susceptible	*AM-1*	*AM-1*

TABLE 3. F_2 segregations and X^2 values for three alfalfa crosses to study inheritance of resistance to alfalfa mosaic virus

Cross and class	Expected ratio (susceptible:resistant)	No. observed	X^2	P value
M8 × G4 and G4 × M8	3:1			
($am_1am_1 \times AM_1AM_1$ and $AM_1AM_1 \times am_1am_1$)				
Susceptible		111	0.0447	
Resistant		40	0.1341	
Total		151	0.1788	.50-.70
07 × R7 and R7 × 07	7:9			
($AM_1am_1 \times am_1am_1$ and $am_1am_1 \times AM_1am_1$)				
Susceptible		73	1.728	
Resistant		70	1.345	
Total		143	3.073	.05-.30
R7 × 05	7:9			
($am_1am_1 \times AM_1am_1$)				
Susceptible		44	0.1241	
Resistant		62	0.0966	
Total		106	0.2207	.50-.70

The authors are fully cognizant that the diploid segregations may, in fact, be artifacts (3, 4), and the inheritance of resistance to AMV using other parental material may be of a tetraploid nature; however, further speculation is not warranted since the actual chromosome number of the material used in this study was not determined. Other genes in alfalfa have been shown to be of a diploid nature (2, 4); therefore, resistance to AMV may be inherited as in a diploid.

The development of a technique to accurately measure the per cent of escapes is applicable to all host: pathogen systems which are subjected to genetic analysis. The system described above was 98.7% efficient, since only 1.3% escapes occurred. The F_2 data were not corrected for this error, as it was insignificant, but, when a high per cent of disease escape occurs, the technique would provide a method of analysis by correction which would be otherwise impossible.

Resistance to at least one strain of AMV can probably be easily incorporated into alfalfa cultivars which are currently in use commercially. The possibility of using cultivars with tolerance to AMV needs to be further investigated. Since alfalfa has been cultivated as a forage crop from antiquity (5), selection pressure may have resulted in the evolution of a very efficient tolerance mechanism.

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