

# Additional Hosts of Alfalfa Mosaic Virus and Its Seed Transmission in Tumble Pigweed and Bean

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## ABSTRACT

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Alfalfa mosaic virus (AMV) was isolated from naturally infected tumbleweed (*Amaranthus albus*) (AMV-P) and chickpea (*Cicer arietinum*) (AMV-CP) at Pullman, WA, and from *A. albus* (CF) at Central Ferry, WA. Infected tumble pigweed plants at both locations were stunted and the foliage showed yellow mosaic symptoms. Incidence of virus-infected *A. albus* was <1%. The host ranges of the two AMV isolates from tumble pigweed differed significantly, particularly in pathogenicity and symptomatology in different legumes, including bean (*Phaseolus vulgaris*) and cowpea (*Vigna unguiculata*). In experimentally inoculated plants, AMV-CF was seedborne in *A. albus* (15.5%) and in three of 12 bean lines (0.7–4.9%). This isolate, when seedborne, induced yellow mosaic symptoms in *A. albus* seedlings but was symptomless in infected bean plants. AMV-P was seedborne in 1.9% of the seeds of naturally infected *A. albus*. Transmission in seed of different plant species was confirmed by back-inoculation to sensitive indicator hosts and by immunodiffusion serology tests. Seed transmission of AMV undoubtedly aids in spread and survival of some isolates of the virus, particularly in the absence of such perennial reservoir hosts as alfalfa (*Medicago sativa*). Seed yields of chickpea mechanically inoculated in the field with isolates AMV-CF and AMV-CP at prebloom and full bloom were reduced by 100 and 78.8%, respectively. Most plants artificially inoculated at prebloom with both virus isolates were killed within 2–4 wk of inoculation.

The USDA plant germ plasm collections for bean (*Phaseolus vulgaris* L.), chickpea (*Cicer arietinum* L.), and lentil (*Lens culinaris* Medik.) are maintained by the Western Regional Plant Introduction Station at Pullman, WA. Annually, nurseries of different plant introduction accessions are increased in plots near Pullman or at an isolated area along the Snake River near Central Ferry, WA. Alfalfa mosaic virus (AMV) is one of several viruses that has been isolated from naturally infected chickpeas and lentils at both locations. Other viruses isolated from these hosts include bean yellow mosaic, pea enation mosaic, and pea streak. Alfalfa (*Medicago sativa* L.) appears to be one of the most important reservoirs and overwintering hosts of AMV and other viruses in the Pacific Northwest (2,6,10,16). However, we isolated AMV from additional crop and weed species at Pullman and Central Ferry, where the nearest alfalfa fields were 3 km away. These hosts included

*Amaranthus albus* L., *Chenopodium album* L., *Nepeta cataria* L., *Phaseolus vulgaris*, and *Vigna unguiculata* (L.) Walp. The two most prevalent weeds at both locations were *A. albus* and *C. album*. *A. albus*, known commonly as tumble pigweed, or white pigweed, is an important annual weed species widely distributed in the Pacific Northwest (3) and elsewhere (4).

The objectives of this study were to determine the importance of tumble pigweed as a host of AMV at Central Ferry, to study the host range and symptomatology of two isolates of the virus from tumble pigweed, to investigate the seedborne nature of a tumble pigweed isolate of AMV in different plant species, and to observe the effects of infection by a tumble pigweed and a chickpea isolate of AMV on yield and mortality of chickpea.

## MATERIALS AND METHODS

Three AMV isolates were investigated. They were found in naturally infected *A. albus* from Central Ferry (AMV-CF) and Pullman (AMV-P) and in *C. arietinum* (AMV-CP) from Pullman. AMV-CF and AMV-CP were maintained in *V. unguiculata* 'California Blackeye' and AMV-P in *C. quinoa* Willd. and *Nicotiana clevelandii* A. Gray. One to 2 g of tissue from AMV-infected plants was triturated in a mortar and pestle in 10–15 ml of 0.06 M K<sub>2</sub>HPO<sub>4</sub>, pH 7.0, to which was added a small amount of 0.22- $\mu$ m (600-mesh) Carborundum. The triturate was rubbed onto the leaves of test plants and immediately rinsed off with tap water. After 2–4 wk, test plants were

checked for systemic virus infection by mechanical inoculation to healthy cowpea (AMV-CF) or *C. quinoa* (AMV-P) seedlings. Plants were grown in a greenhouse at 15–25 C and received periodic applications of pesticides at recommended rates to control insects and mites.

As many as 500 seeds were collected from plant species inoculated with AMV-CF and from *A. albus* naturally infected with AMV-P. Seeds were sown in moist vermiculite and emerged seedlings were transplanted to sterile soil in 10-cm plastic pots (five plants per pot). Within 2–4 wk, one or two leaflets from each plant in a pot were triturated together and the sap was rubbed on the foliage of California Blackeye cowpea (AMV-CF) or *C. quinoa* (AMV-P) test plants. If test plants developed symptoms of AMV, each seedling in the pot was assayed individually to determine frequency of seed transmission. Observations were made on the effects of AMV-CF on growth of *A. albus* infected from seed. Measurements of height and weight of 15 healthy and 15 virus-infected plants were made 45 days after seedling emergence.

Two aphid species, *Acyrtosiphon pisum* (Harris) and *Myzus persicae* (Sulzer), were frequently found colonizing food legumes in the Palouse region. These species were used in vector-transmission studies with AMV-CF. Nonviruliferous aphid colonies were reared in screened cages on healthy Chinese cabbage (*Brassica campestris* L. subsp. *pekinensis* (Lour.) Olsson) (*M. persicae*) and *Vicia faba* L. 'Herz Freya' (*A. pisum*). Aphids were starved for 1–2 hr before being given acquisition feeding periods of less than 1 min (duration of a single probe) to 10 min on California Blackeye cowpea infected with AMV-CF and were then transferred to healthy California Blackeye cowpea test seedlings in groups of one to five for a 3-day inoculation access period, at which time plants were sprayed with the aphicide pirimicarb (Pirimor).

Gel-immunodiffusion tests were used to study antigenic relationships among AMV isolates in conjunction with other tests to detect and identify AMV in naturally infected or greenhouse-inoculated plants used in the host-range and seed-transmission studies. Immunodiffusion tests were conducted in 0.8% Difco agar gels containing 0.85% sodium chloride and 0.02% sodium azide (8).

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Antisera to selected spherical viruses were also included in some of the tests to ensure that our AMV source plants were free of other viruses.

AMV isolates CF and CP were included in a field trial at Central Ferry to study the effects of virus infection on yields and survival of a large, cream-seeded chickpea line (PI 458870, USA). Replicated plots were manually inoculated with both AMV isolates at prebloom (35 days after planting) and full bloom (63 days after planting) stages of plant growth. The inoculum of each virus isolate (systemically infected California Blackeye cowpea) was rubbed on the leaflets of chickpea plants with the thumb and forefinger. Twenty to 25 days after inoculation, plants showing virus symptoms were tagged and dead plants were counted 77–84 days after seeding. At harvest, seed yields were determined from 40 tagged plants in each plot.

## RESULTS

### Incidence and symptoms of disease.

AMV was isolated from *A. albus* plants in chickpea plantings at Central Ferry and Pullman. Tumble pigweed plants naturally infected with AMV showed yellow mosaic (Fig. 1) and stunting symptoms (Fig. 2). AMV was not isolated from *A. powellii* Wats. and *A. retroflexus* L., which are also found at both locations and elsewhere in the Palouse region. AMV-infected *A. albus* plants were scattered through and around chickpea plantings and disease incidence was usually <1%. For example, of 500 *A. albus* plants surveyed for AMV infection at Central Ferry in 1980, four plants were infected with the virus.

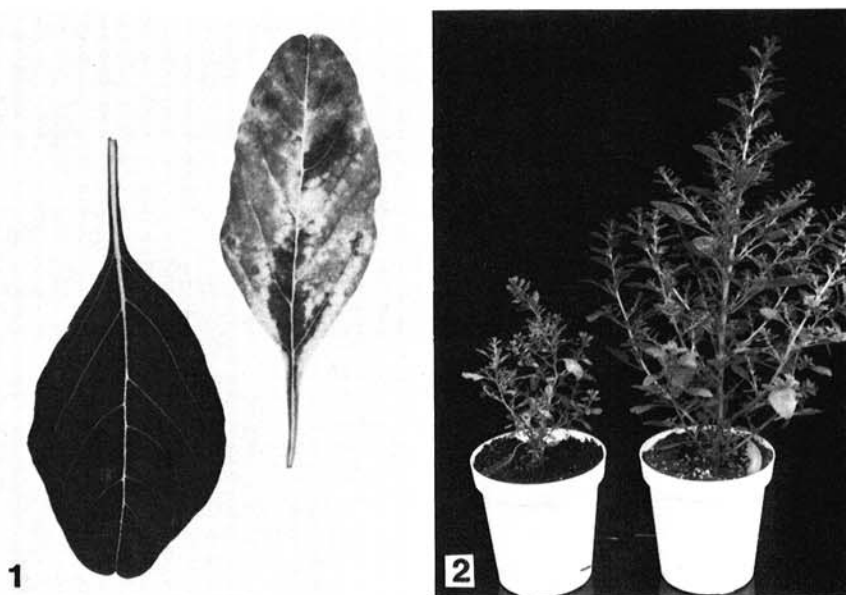
**Host-range studies.** Both AMV isolates from *A. albus* differed markedly in pathogenicity and symptomatology (Table 1). These isolates were easily differentiated in inoculated bean and cowpea test plants. AMV-CF produced systemic yellow mosaic symptoms in bean and cowpea, whereas AMV-P failed to infect these two hosts systemically. Both AMV isolates produced systemic yellow mosaic symptoms in two of the three *Amaranthus* species found in eastern Washington and also systemically infected other important wild species occurring in the Palouse region, including *C. album*, *Melilotus alba* Medik., and *Solanum nigrum* L.

In greenhouse inoculation tests, AMV-CF produced systemic symptoms in several food legumes maintained in the Western Regional PI collection; these included bean, broadbean, chickpea, and lentil. Of 38 bean and nine chickpea PI lines tested for resistance to AMV-CF, all were systemically infected by AMV. Some of the infected bean lines appeared to recover and eventually resume normal growth, but the virus could still be isolated from these plants when back-inoculated to California Blackeye cowpea.

**Seed transmission.** AMV-CF was seedborne in 0.7–15.5% of the seedlings in two of seven plant species inoculated with the virus. Seed transmission was detected in *A. albus* (23 seedlings infected/148 tested) and three bean lines, including Stringless Green Refugee (3/142), PI 419095 (3/61), and PI 152326 (1/152). All *A. albus* seedlings from infected seed were stunted and showed yellow mosaic symptoms (Fig. 3). The height of *A. albus* plants from infected seed was reduced by 69% and dry weight by 90% compared with healthy plants of the same age. Bean plants from infected seed were symptomless, but the virus could be detected in

bean by assays on indicator hosts and by immunodiffusion serology tests. Seed transmission was not detected in *A. retroflexus* (0/50), *C. album* (0/100), *Phaseolus lunatus* (0/54), *S. nigrum* (0/115), *V. unguiculata* (0/168), or the following bean lines: Aurora (0/197), Bountiful (0/89), Long Tom (0/76), Red Kidney (0/38), Sanilac (0/53), PI 197687 (0/167), PI 416127 (0/39), PI 416299 (0/26), and PI 416426 (0/62).

Transmission of AMV also was determined in two of 107 (1.9%) germinating seeds of *A. albus* naturally infected with AMV-P. Infected seedlings were stunted and the foliage showed



**Figs. 1 and 2.** (1) (Right) Yellow mosaic symptoms in *Amaranthus albus* infected with an isolate of alfalfa mosaic virus from *A. albus* from Central Ferry, WA (AMV-CF); (left) healthy leaf. (2) (Left) Stunted *A. albus* plant infected from seed with isolate AMV-CF; (right) healthy plant.

**Table 1.** Host range and symptoms of two isolates of alfalfa mosaic virus from *Amaranthus albus* from Central Ferry (AMV-CF) and Pullman (AMV-P), WA

Plant species	Host reaction to virus isolates	
	AMV-CF <sup>a</sup>	AMV-P
<i>Amaranthus albus</i>	SYM, St	SYM, St
<i>A. powellii</i>	NS <sup>b</sup>	NS
<i>A. retroflexus</i>	M, St	M, St
<i>Chenopodium album</i>	CLL, LD, M	LD, M, St
<i>C. quinoa</i>	CLL, LD, M	CLL, LD, M, St
<i>Cicer arietinum</i> PI 458870, USA	TN, W, St	TN, W, St
<i>Glycine max</i> 'Bragg'	M	M, St
<i>Gomphrena globosa</i> 'NSSL 93612'	RLL, SYM	RLL, SI
<i>Lens culinaris</i> 'Benewah'	LC, St	SI
<i>Medicago sativa</i> 'Hairy Peruvian'	LC, M	NS
<i>Melilotus alba</i>	LC, M	LC, SI
<i>Nicotiana clevelandii</i>	M	M
<i>Phaseolus vulgaris</i> 'Bountiful'	CLL, VN, SYM	NLL
<i>P. vulgaris</i> 'Stringless Green Refugee'	CLL, VN, SYM	NLL
<i>Pisum sativum</i> 'Dark Skin Perfection'	M	NS
<i>Solanum nigrum</i>	M	NS
<i>Vicia faba</i> 'Long Pod Fava'	NLL, M	VN, M, St
<i>Vigna unguiculata</i> 'California Blackeye'	CLL, SYM, LD, St	NLL

<sup>a</sup>Symptom abbreviations: CLL = chlorotic local lesions, LC = leaf curling, LD = leaf deformation, M = mosaic, NS = not susceptible, NLL = necrotic local lesions, RLL = red local lesions, SI = symptomless systemic infection, St = stunting, SYM = systemic yellow mosaic, TN = tip necrosis, VN = vein necrosis, and W = wilting.

<sup>b</sup>Symptomless test plants were assayed by back-inoculation to *V. unguiculata* 'California Blackeye' (AMV-CF) and *C. quinoa* (AMV-P).

yellow mosaic symptoms similar to those infected from seed with AMV-CF. AMV was not isolated from any of the 105 seedlings not showing symptoms of yellow mosaic and stunting.

**Serology tests.** Gel-immunodiffusion tests were used to confirm results of other tests on the identity of AMV from naturally and artificially inoculated plant species. Serology was used to detect AMV in *A. albus* naturally infected with AMV-CF and P and in seedlings of *A. albus* and bean infected from seed with AMV-CF. No serological distinctions were observed among the three AMV isolates used in this study (AMV-CF, P, and CP). None of the AMV source plants reacted with antisera to bean pod mottle, cucumber mosaic, cowpea chlorotic mottle, southern bean mosaic, and tobacco ringspot viruses.

**Insect transmission.** *M. persicae* and *A. pisum* proved to be vectors of AMV-CF. Both aphid species transmitted AMV-CF from virus-infected California Blackeye cowpea to healthy cowpea test plants in a nonpersistent (styletborne) manner. Virus transmission incidence ranged from 20 to 30% with both aphid species.

**Field trials.** Seed yields of chickpea PI 458870 mechanically inoculated in the field with AMV-CF and AMV-CP at prebloom and full bloom were reduced by 100 and 79%, respectively. All plants inoculated at prebloom with either AMV isolate were killed before harvest. Mortality of plants inoculated at full bloom with either isolate was <15%. Plants infected at full bloom were stunted and chlorotic and there was discoloration of the phloem tissues. Seeds from virus-infected plants were smaller than those from healthy control plants. No transmission of AMV-CF or CP was observed

in >100 germinating chickpea seeds collected from plants inoculated at full bloom with either virus isolate.

## DISCUSSION

Alfalfa mosaic virus is seedborne in several plant species (5,7,9). In this study, we have identified tumble pigweed (*A. albus*) and bean (*P. vulgaris*) as new hosts in which AMV is seedborne. Two isolates of AMV differing in host range and symptomatology (AMV-CF and P) infected tumble pigweed naturally and were seedborne in this host. Wild species frequently serve as important reservoir hosts of different viruses (1). Tumble pigweed is one of most important weeds of cultivated areas and wastelands in the Palouse region. Transmission of AMV in tumble pigweed seed undoubtedly plays an important role in spread and survival of some isolates of the virus, particularly in the absence of perennial reservoir hosts like alfalfa. Some *Amaranthus* species produce numerous seeds that are long-lived in soil (12). In the Palouse, this appears to be the situation with *A. albus*, which would tend to ensure survival and perpetuation of isolates of AMV that are seedborne in this host.

Virus surveys were conducted between 1979 and 1982 of wild species growing in and around our seed-multiplication plots near Central Ferry. Although white sweet clover (*Melilotus alba*) is the primary reservoir of several viruses in this area (8), our attempts to isolate AMV from this host were unsuccessful. Alfalfa is not cultivated in the vicinity of these plots. AMV was isolated from tumble pigweed and only once from catnip (*Nepeta cataria*), which is not a prevalent weed. Tumble pigweed appears to be the primary source of inoculum for some AMV isolates near the Central Ferry

station. Migrating aphids are probably responsible for spread of AMV from infected tumbleweed plants to nearby seed-increase plots of chickpea, lentil, and cowpea.

One of the isolates (AMV-CF) was transmitted in 0.7–4.9% of the seed in three of 12 bean lines. Thomas (14,15) and Zaumeyer (17) failed to demonstrate seed transmission of AMV in bean with different isolates of the virus from the Pacific Northwest. They may have overlooked seed-infected bean plants if they relied only on symptomatology to establish seed transmission of AMV or they may have worked with isolates of AMV that were not seed-transmitted in beans. To ascertain seed transmission of AMV in other plant species, it may be necessary to assay seedlings by back-inoculation to sensitive indicator hosts or use appropriate serological tests.

Several isolates of AMV differing in host range have been derived from various food, forage, and weed species in the Pacific Northwest (2,6,10,14–17). Some were similar to the AMV isolates used in this study (AMV-CF and CP) in that they infected beans and cowpeas systemically (15–17). From 1979 to 1982, only five of 20 AMV isolates we collected from different plants in the Palouse region produced systemic yellow mosaic symptoms in bean and/or cowpea. However, all Palouse isolates of AMV were highly pathogenic to chickpea, which usually resulted in severe stunting, yellowing, and premature death of infected plants. Lentil was also susceptible to most AMV isolates.

In greenhouse studies, isolates of AMV have been reported to infect *A. retroflexus* in Germany (13), *A. hybridus* L. subsp. *incurvatus* (Gren. & Godr.) Brenan in Kenya (7), and *A. caudatus* in the United States (11). However, this appears to be the first record of natural infection of this genus by AMV. Although incidence of AMV-infected *A. albus* at Central Ferry and Pullman was low, this annual species appears to serve as a reservoir host of seedborne isolates of the virus and possibly of its aphid vectors.

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Fig. 3. Two *Amaranthus albus* seedlings infected from seed with an isolate of alfalfa mosaic virus (AMV-CF) from *A. albus* showing stunting and yellow mosaic symptoms; (lower right) healthy seedling.

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