Chickpea Filiform, a New Viral Disease of *Cicer arietinum*

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ABSTRACT

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A new viral disease of chickpea (*Cicer arietinum*) characterized by filiform leaves was detected in the USDA *Cicer* germ plasm collection at Central Ferry, WA. Incidence of virus-infected chickpeas was < 1%, and the virus, designated chickpea filiform virus (CFV), had a very restricted host range. All *Cicer* accessions tested (>30), including six wild species, were susceptible and developed filiform leaves. The only other plants infected systemically were lentil (*Lens culinaris*), fenugreek (*Trigonella foenum-graecum*), and *Nicotiana clevelandii*. CFV was transmitted in a styletborne (nonpersistent) manner from virus-infected chickpea to chickpea and fenugreek by *Myzus persicae* and *Acyrthosiphon pisum*. Seed yields of chickpea mechanically inoculated in the field with CFV at prebloom and full bloom were reduced 80 and 46%, respectively. Seed size was also adversely affected. No seed transmission of CFV was detected in chickpea or fenugreek. Virus particles typical of the potyvirus group were observed under the electron microscope. A serological relationship with bean yellow mosaic virus (BYMV), blackeye cowpea mosaic virus (BlCMV), and cowpea aphidborne mosaic virus (CAMV) was suggested in indirect enzyme-linked immunosorbent assay (ELISA) with BYMV, BlCMV, and CAMV antisera. A relationship with BYMV was confirmed by serologically specific electron microscopy. Ascitic fluid produced in mice against CFV, however, did not react in ELISA with BYMV, BlCMV, CAMV, or with three other potyviruses. Further research is needed to clarify the relationship of CFV to BYMV, BlCMV, and CAMV.

The USDA chickpea (*Cicer* spp.) germ plasm collection of 3,431 accessions is maintained at the Western Regional Plant Introduction Station, Pullman, WA. Chickpea germ plasm lines must be increased periodically in the greenhouse and/or field to replenish seed supplies. Diseases that affect the multiplication of this germ plasm are of utmost concern because it may be impossible to replace some of the chickpea accessions if they are lost. Most of the chickpea germ plasm is increased at an isolated research station along the Snake River near Central Ferry, WA. The major diseases affecting chickpeas at this location are caused by viruses, and these include alfalfa mosaic (AMV), bean yellow mosaic (BYMV), pea enation mosaic (PEMV), and pea streak (PSV) viruses (12,14). Virus infection can result in stunted growth, premature death, reduced yields, and poor-quality seeds.

Chickpea germ plasm plantings at Central Ferry are surveyed for virus diseases at periodic intervals from seedling emergence until harvest. Tissue from plants showing virus symptoms is indexed on indicator test plants in the greenhouse and used in serology tests. In 1981, a new virus disease was observed in the *Cicer* germ plasm collection that was characterized by filiform leaves. The authors had never observed similar symptoms before on chickpeas naturally infected with any of the four viruses mentioned above. The causal agent, designated chickpea filiform virus (CFV), is the subject of this report.

The purpose of this study was to examine the host range, symptomatology, and serological relationships of CFV isolated from cultivated chickpea (*C. arietinum* L.) at Central Ferry, to investigate vector and seed transmission of this virus in chickpea and other hosts, and to observe the effects of the disease on chickpea seed yields and quality.

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MATERIALS AND METHODS

Greenhouse studies. The isolate of CFV used in all studies was originally obtained from an infected plant of chickpea plant inventory (PI) 426163 (India) at Central Ferry. CFV was selected after serial single local lesion transfers on *Chenopodium quinoa* Willd. and was maintained in chickpea PI 458870 (United States). In host range studies, 1–2 g of tissue from systemically infected chickpea was triturated in a mortar and pestle with 10–15 ml of 0.06 M K_2HPO_4, pH 7.0, with a small amount of 0.22-μm (600-mesh) Carborundum. The triturate was rubbed onto the leaves of test plants with the thumb and forefinger and immediately rinsed off with tap water. After 2–4 wk, plants were assayed for systemic infection by mechanical inoculation to healthy *C. quinoa*. Plants were grown in a greenhouse at 15–25 C in steam-sterilized soil in 10-cm-diameter plastic pots and received periodic applications of fertilizer and pesticides.

Seeds of chickpea PI 458870 and fenugreek (*Trigonella foenum-graecum* L.) were harvested from plants infected in the seedling stage with CFV. The seeds were dusted with captan (Orthocide 50W) and planted in moist vermiculite. Seedlings were transplanted to sterile soil in 15-cm-diameter plastic pots with one to five seedlings per pot. Two to 3 wk after transplanting, leaf samples from one to five plants were combined and indexed on *C. quinoa* and chickpea and assayed by enzyme-linked immunosorbent assay (ELISA) in groups of five seedlings.

Vector transmission. Two aphid species, *Acyrthosiphon pisum* (Harris) and *Myzus persicae* (Sulzer), were used in vector transmission studies with CFV. Nonviruliferous aphid colonies of *M. persicae* were reared on healthy Chinese cabbage (*Brassica campestris* L. var. *pekinensis*) and *A. pisum* on Herz Freya faba bean (*Vicia faba* L.). Aphids were starved 1–2 hr in glass petri dishes and then given acquisition feeding periods of less than 1 min (duration of a single probe) to 1 hr on chickpea PI 458870 infected with CFV. After feeding, they were transferred in groups of one to five to healthy chickpea seedlings in aphid-proof cages for an 18-hr inoculation

access period. The test plants were then sprayed with an aphicide and held 3 wk in the greenhouse for symptom development.

Field studies. Seeds of chickpea PI 458870 were planted in single rows 5.3 m long with 1.5 m between rows in a randomized complete block design with four replicates of 50 seeds per row per replicate. Plots were manually inoculated with CFV at the prebloom (45 days after planting) and full bloom (79 days after planting) stages of plant growth. Virus inoculum was prepared and inoculations performed following the procedure outlined earlier. Twenty-five to 30 days after inoculation, plants showing virus symptoms were tagged, and counts were made of dead plants after 85 days. Plants were hand-harvested 140 days after planting, and seed yields were determined from 25 tagged plants in each plot. The seed size was determined by passing seeds through four metal sieves with round holes graduated in 0.8-mm increments.

Virus purification. Fifty to 100 g of infected chickpea and *C. quinoa* tissues were blended in two volumes (1 ml/g) of 0.1 M potassium phosphate buffer, pH 8.0, containing 0.01 M DIECA, and 0.02 M Na_2SO_3 and one volume of chloroform. The virus extract was clarified by centrifugation for 10 min at 6,000 × *g*. Virus was concentrated by precipitation using 4% polyethylene glycol (PEG-6000) and 0.5 M NaCl. The precipitate was collected by centrifugation at 6,000 × *g* for 10 min and resuspended by shaking for 0.5 hr in 0.01 M Tris buffer, pH 8.3, containing 0.5 M urea and 0.01 M EDTA. After removal of insoluble material by centrifugation at 6,000 × *g* for 10 min, virus was reprecipitated with 10% PEG-6000 and 0.5 M NaCl, collected by centrifugation, and slurried in 5 ml of 10% PEG-6000. The slurry was centrifuged on a reverse PEG solubility gradient following the method of Clark and Lister (5). Gradient centrifugation was for 20 min at 5,000 rpm in a SW 27 rotor. The virus band was collected and subjected to a second cycle of reverse PEG solubility gradient centrifugation and then dialyzed against 0.01 M Tris buffer, pH 8.3, containing 0.01 M EDTA.

Serology. Ascitic fluid was produced by injecting purified virus-Freund's complete adjuvant emulsion (1:1) into the peritoneal cavity of mice four times (0.2 ml per injection) at 10-day intervals (S. D. Wyatt, L. J. Seybert, and G. Mink, *unpublished*). Ascitic fluids were collected from the peritoneal cavity and were purified by affinity chromatography on Staphylococcal protein A sepharose (8). Host-specific antibodies were removed by absorption to acetone-precipitated healthy chickpea protein. The acetone powder was extracted with 80% ethanol at 50 C for 30 min and then washed with phosphate-buffered saline (PBS) (4). The washed protein was incubated with 2 ml of purified IgG at 37 C for 1 hr and then removed by centrifugation at 10,000 × *g* for 10 min. For indirect ELISA, virus samples were absorbed to Immulon 2 flat-bottomed plates (Dynatech Laboratories, Inc., Alexandria, VA) at 25 C for 1 hr. Tissue samples, ascitic fluids containing antibodies to CFV, and goat-antimouse alkaline phosphatase conjugate (Sigma) were diluted in PBS-Tween 20 (0.05%), ovalbumin (0.2%), and polyvinylpyrrolidone (2%). Primary and secondary antibody incubations were for 1 and 3 hr, respectively.

In ELISA with rabbit antisera of different potyviruses, tissues were triturated in either 0.05 M carbonate buffer, pH 9.6, or in pH 7.4 phosphate-buffered saline-Tween 20 (0.05%) containing ovalbumin (0.2%) and polyvinylpyrrolidone (2%). Samples processed at 1 g/10 ml were absorbed to microtiter plate wells during incubation for 1 hr at room temperature. Antisera were diluted 1:1,000 with the above buffer and incubated 1 hr. Antirabbit alkaline phosphatase conjugate was diluted 1:3,200 in the above buffer and incubated 3 hr at room temperature.

Sources of virus antisera. Antisera used in these studies are as follows: bean common mosaic virus (BCMV) (G. I. Mink, IAREC, Prosser, WA); blackeye cowpea mosaic virus (BlCMV) (C. W. Kuhn, University of Georgia, Athens; D. E. Purcifull, Gainesville, FL); bean yellow mosaic virus (BYMV) (R. O. Hampton, Oregon State University, Corvallis; R. Provvidenti, NYAES, Geneva, NY; and I. Uyeda, Sapporo, Japan); cowpea aphidborne mosaic virus (CAMV) (J. Uyemoto, University of California, Davis); pea seedborne mosaic virus (PSbMV) (G. I. Mink, IAREC, Prosser, WA); and peanut mottle virus (PMV) (K. R. Bock, Lilongwe, Malawi).

Electron microscopy. Healthy and virus-infected chickpea tissue extracts were examined under a Zeiss EM9-S2 electron microscope using serologically specific electron microscopy (6). Chickpea sap in 0.1 M PO_4 buffer, pH 7.0, was placed on Formvar-coated grids for 7 min, then washed with distilled water and negatively stained with a 2% solution of uranyl acetate. For antibody decoration, a drop of antiserum (1:10 dilution) was placed on virus-coated grids for 7–15 min, and the grids were then washed and negatively stained with 2% uranyl acetate. Antisera specific for BCMV, BlCMV, BYMV, CAMV, CYVV, PMV, and PSbMV were used.

RESULTS

Incidence and symptoms of disease. Chickpea plants infected with CFV were stunted, and the foliage was characterized by very narrow (filiform) deformed leaflets (Fig. 1). Infected plants appeared bushy because of proliferation of the axillary buds. Fewer pods usually formed on CFV-infected plants, and seeds frequently were small and misshapen. Chickpeas in the field infected with CFV have been observed only at Central Ferry. The incidence of naturally infected chickpeas was very sporadic and usually <1%.

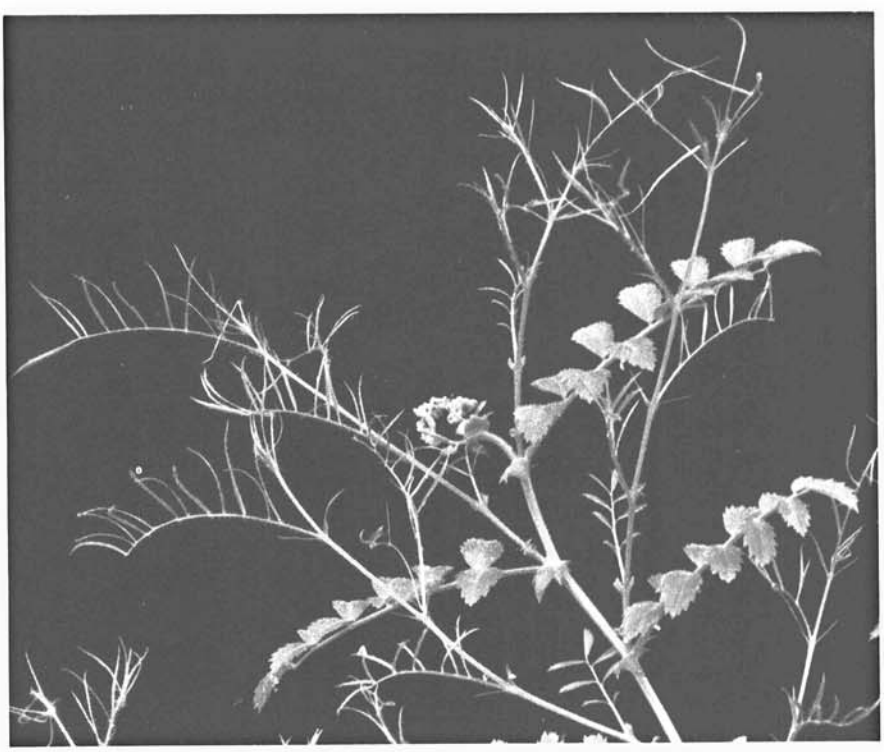

Fig. 1. Filiform leaves on the foliage of chickpea PI 458870 inoculated with chickpea filiform virus.

Host-range studies. Of 25 plant species tested, CFV infected seven species in four families. Systemic symptoms occurred in seven species of *Cicer*, fenugreek, and lentil (*Lens culinaris* Medik.) (Fabaceae). All cultivated chickpea accessions (>25) inoculated with CFV were susceptible. The initial symptoms in chickpea were wilting and necrosis of apical tissues followed by development of filiform leaflets (Fig. 1). Infected plants were stunted, and pod formation was reduced by >50%. Additionally, filiform symptoms were incited in the following inoculated wild *Cicer* spp.: *C. bijugum* K. H. Rech. (PI 458550), *C. cuneatum* Hochst. ex Rich. (PI 458554), *C. echinospermum* P. H. Davis (PI 489776), *C. judaicum* Boiss (PI 458558), *C. pinnatifidum* Jaub. et Spach (PI 458556), and *C. reticulatum* Ladiz. (PI 489777). All plants of *C. bijugum* wilted and died within 27 days of inoculation. Mosaic and leaf curling were observed in lentil cultivars Chilean 78 and Red Chief. Virus-infected fenugreek showed mosaic and stunting symptoms. *Nicotiana clevelandii* Gray (Solanaceae) was a symptomless carrier of the virus. CFV produced local lesions without systemic spread in *Chenopodium amaranticolor* Coste & Reyn., *C. quinoa* (Chenopodiaceae), and *Gomphrena globosa* L. (Amaranthaceae).

No symptoms developed on, and no virus was recovered from, *Cucumis sativus* L. 'Ohio MR-17' (Cucurbitaceae); *N. glutinosa* L., *N. tabacum* 'Havana 423' and 'Samsun NN' (Solanaceae); *Tetragonia tetragonioides* (Pall.) Ktze. (Aizoaceae); *Cassia occidentalis* L., *Cyamposis tetragonoloba* (L.) Taub., *Glycine max* (L.) Merr. 'Bragg,' *Medicago sativa* L. 'Hairy Peruvian,' *M. lupulina* L., *Melilotus alba* Medik., *Phaseolus lunatus* L. 'Jackson Wonder,' *P. vulgaris* L. 'Apollo,' 'Black Turtle Soup,' 'Bountiful,' 'Gold Crop,' 'Long Tom,' 'Red Kidney,' 'Sanilac,' 'Stringless Green Refugee,' 'Tendercrop,' and 'Topcrop,' *Pisum sativum* L. 'Dark Skin Perfection' and 'Perfected Wales,' *Trifolium repens* L. 'New Zealand,' *Vicia faba* 'Herz Freya' and 'Diana,' *V. villosa* Roth, *Vigna radiata* (L.) Wilczek var. *radiata* 'Berken,' and *V. unguiculata* (L.) Walp. subsp. *unguiculata* 'California Blackeye' (Fabaceae).

Vector transmission. Both *Myzus persicae* and *Acyrthosiphon pisum* transmitted CFV from virus-infected chickpea to healthy chickpea and fenugreek seedlings in a styletborne (nonpersistent) manner. Transmission by single aphids was <20%, whereas groups of five aphids transmitted the virus to >50% of the test plants. Disease symptoms in chickpea resulting from aphid transmission were identical to those that developed on mechanically inoculated plants.

Seed transmission. No transmission of CFV was detected in 297 and 173 germinating seeds from chickpea PI 458870 and fenugreek, respectively, which were inoculated before flower initiations.

Yield loss assessment. Field inoculations of chickpea PI 458870 at prebloom or full bloom with CFV reduced seed yields by 80 and 46%, respectively (Table 1). Virus infection resulted in a reduction in seed size. Inoculation at prebloom resulted in the largest reduction in seed size for the 9.6- and 6.4-mm size ranges, whereas the full-bloom treatment caused intermediate reductions in seed size between the prebloom and healthy control treatments (Table 1). Plant survival was not reduced by inoculation with CFV, although prebloom-inoculated plants were markedly stunted at maturity.

Virus purification. CFV infectivity appeared high in chickpea as indicated by assay on the local lesion host *C. quinoa*. Because of the filiform symptoms, however, only small amounts of tissue were available for virus purification. Small amounts of virus were purified from systemically infected chickpea and the local lesion host *C. quinoa* by centrifuging PEG precipitate on PEG-

Table 1. Effects of infection by the chickpea filiform virus on seed yield and seed size of chickpea PI 458870 inoculated at two stages of growth at Central Ferry, WA

Treatment[w]	Mean seed yield[x] (g)	Seed size (mm)[y] Percentage of yield retained by metal sieves 9.6	8.0	6.4	4.8
Healthy control	1,573 a[z]	28.7 a	62.9 a	7.6 c	0.9 a
Inoculated prebloom	316 c	14.4 c	62.8 a	19.5 a	3.3 a
Inoculated full bloom	852 b	22.6 b	62.2 a	13.1 b	2.1 a

[w] Prebloom and full bloom plots were inoculated 45 and 79 days after planting, respectively.
[x] Mean yield is based on seed harvested from 25 plants in four single-row plots, each 5.3 m long.
[y] Seeds were sized through metal sieves with four different-sized round holes.
[z] Numbers in the same column followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's new multiple range test.

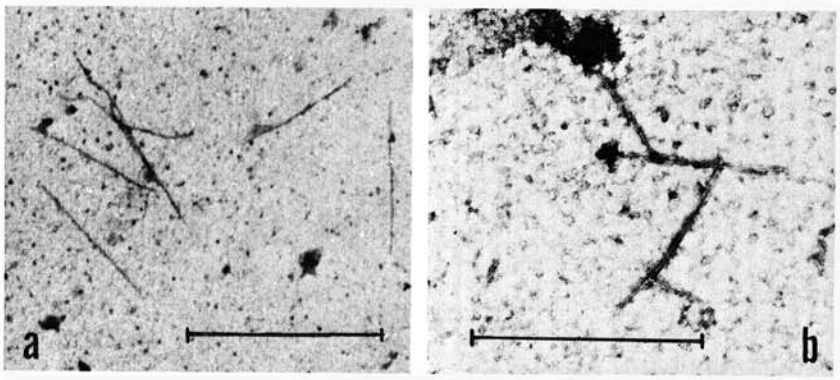

Fig. 2. Electron micrographs of the chickpea filiform virus (CFV) from chickpea. **(A)** Negatively stained particles of CFV from chickpea sap and **(B)** particles of CFV decorated with antiserum of bean yellow mosaic virus diluted 1:10 and negatively stained. Scale bar = 1 μm.

Table 2. Serological relationship of the chickpea filiform virus (CFV) to some potyviruses of legumes by indirect ELISA[a]

Antiserum[b]	A_{405nm}[c] CFV	Homologous antigen
BCMV (NL-3)	−0.10	1.21
BCMV (NY-15)	−0.22	0.66
BlCMV	0.54	1.45
BYMV	0.68	0.83
CAMV	0.21	0.76
CYVV	−0.15	1.03
PMV	0.02	0.15
PSbMV	0.00	0.67

[a] Chickpea tissues were triturated in pH 7.4 phosphate-buffered saline-Tween 20 (0.05%) containing ovalbumin (0.2%) and polyvinylpyrrolidone (2%), 1 g/10 ml, and absorbed to microtiter plate wells during incubation for 1 hr at room temperature. Antisera were diluted 1:1,000 with the above buffer and incubated 1 hr. Antirabbit alkaline phosphatase conjugate was diluted 1:3,200 in the above buffer and incubated 3 hr at room temperature.
[b] BCMV = bean common mosaic virus, BlCMV = blackeye cowpea mosaic virus, BYMV = bean yellow mosaic virus, CAMV = cowpea aphidborne mosaic virus, CYVV = clover yellow vein virus, PMV = peanut mottle virus, and PSbMV = pea seedborne mosaic virus.
[c] Absorbance readings of the healthy controls were subtracted from the absorbance readings of infected test samples.

solubility gradients as described by Clark and Lister (5). A virus band was taken from reverse PEG-solubility gradients for production of antibodies. The virus preparation was not pure, as evidenced by much light-scattering material. Attempts to further purify the virus by sucrose density gradient centrifugation resulted in complete loss of the virus, probably because of aggregation.

Virus identification. Long flexuous particles typical of the potyvirus group were seen when sap from chickpea showing filiform leaflets (Fig. 2A) was examined with the electron microscope. The modal length of 27 particles was 770 nm. A serological relationship with BYMV, BlCMV, and CAMV was suggested by indirect ELISA using antisera specific for BCMV, BlCMV, BYMV, CAMV, CYVV, PMV, and PSbMV and homologous antigens as controls (Table 2). At pH 7.4 BYMV, BlCMV, and CAMV antisera detected CFV, but at pH 9.6, CFV was detected by antisera to BCMV, BlCMV, BYMV, CAMV, and CYVV. PMV and PSbMV antisera did not detect CFV at the latter pH. A CFV-BYMV relationship was further confirmed using serum specific decoration of particles seen in the electron microscope (Fig. 2B). Decoration of particles was achieved only with BYMV antibodies. Ascitic fluid produced against purified CFV gave positive ELISA reactions for CFV but not for BCMV, BlCMV, BYMV, CAMV, CYVV, PMV, and PSbMV (Table 3).

DISCUSSION

Although indirect ELISA suggests a relationship of CFV to BYMV, BlCMV, and CAMV, host range studies indicate that the three viruses are different. BlCMV and CAMV are not known to infect chickpea and are known to be associated with tropical legumes (1,15–17,20). We failed to infect chickpeas when plants were inoculated with two isolates of BlCMV and one isolate of CAMV. Additionally, BlCMV and CAMV have not been reported from the Pacific Northwest (1,17; *unpublished*). CFV, in our opinion, appears to be more closely related to BYMV than BlCMV or CAMV. A number of strains of BYMV have been identified worldwide that vary in symptomatology and host range (2,9). Natural infection of chickpea by BYMV has been reported in India (3), Iran (10,11), and the United States (7,14,18). In Iran, Kaiser and Danesh (11) isolated several strains of BYMV, one of which induced filiform symptoms in chickpea similar to CFV; however, the filiform-inducing isolate of Kaiser and Danesh (11) had a much wider host range in the Fabaceae. In the Palouse region of eastern Washington and northern Idaho, the authors have tested the host ranges of more than 50 isolates of BYMV from different naturally infected legumes. Most isolates infectious to chickpea induced wilting, yellowing, and/or stunting symptoms but not filiform symptoms. Additionally, all of these BYMV isolates infected Bountiful bean and/or Herz Freya broadbean; however, CFV did not infect either of these hosts. CFV caused systemic symptoms in only three species of the Fabaceae. Chickpea-infecting isolates of BYMV have a widespread distribution in the Palouse region, but the filiform virus has been found only in the Central Ferry area. One of the primary reservoir and overwintering hosts of BYMV in the region is white sweet clover (*M. alba*), but those of CFV are, as yet, unknown. In some years, diseased plants were not observed in the seed increase plots at Central Ferry.

Serological evidence indicates that CFV is possibly related to BYMV, BlCMV, and CAMV. This relationship is based on positive ELISA reactions with several BYMV, BlCMV, and CAMV antisera and decoration of CFV particles with BYMV antibodies in serologically specific electron microscopy tests. It should be noted, however, that ascitic fluid produced in mice against CFV did not react in ELISA with BYMV, BlCMV, and CAMV. Because CFV has a very restricted host range compared with other BYMV strains (2,3,9,11,19) and many potyviruses show some serological relatedness, further research will be needed to clarify the relationship of the virus to BYMV, BlCMV, and CAMV.

As the designated repository for the *Cicer* germ plasm in the United States, the Pullman station is continually receiving seeds of *Cicer* species from different countries and distributing seeds to researchers worldwide. Seed transmission is an important means of spread of some chickpea pathogens, such as *Ascochyta rabiei* (13). However, to date we do not have evidence that any of the five viruses infecting chickpeas in Washington and Idaho is seedborne in this host. At Central Ferry, virus diseases are potentially very important to the project's seed increase program, particularly in years with mild winters when there is a large buildup of aphids on virus-infected reservoir hosts. For example, in 1983 aphid populations increased rapidly early in the growing season and by full bloom, more than 50% of some chickpea accessions at Central Ferry were virus-infected (W. J. Kaiser, *unpublished*). Virus infection decreases chickpea yields (e.g., CFV decreased yields by 46–80%) and quality (higher percentage of small seeds). Virus infection may also adversely affect germination and vigor of chickpea seedlings.

Table 3. Results of indirect ELISA of chickpea filiform virus and other legume viruses using mouse polyvalent ascitic fluid produced against the chickpea filiform virus[a]

Virus isolate[b]	A_{405nm}[c]
BCMV	0.03
BlCMV	−0.06
BYMV	0.06
CAMV	0.00
CFV	1.33
CYVV	−0.10
PSbMV	0.01

[a] Indirect ELISA employed polyvalent ascitic fluid at 3 μg/ml. Details described in Materials and Methods.

[b] Virus samples were prepared by grinding tissue in phosphate-buffered saline containing Tween-20 (0.05%), ovalbumin (0.2%), and polyvinylpyrrolidone (2%), 1 g tissue per 10 ml. BCMV = bean common mosaic virus, BlCMV = blackeye cowpea mosaic virus, BYMV = bean yellow mosaic virus, CAMV = cowpea aphidborne mosaic virus, CFV = chickpea filiform virus, CYVV = clover yellow vein virus, and PSbMV = pea seedborne mosaic virus.

[c] Absorbance readings of healthy samples were subtracted from virus sample readings.

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