# Bean Yellow Mosaic Virus Isolate That Infects Peanut (*Arachis hypogaea*)

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**ABSTRACT**

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A virus isolate (T-1) recovered from arrowleaf clover (*Trifolium vesiculosum*) in the field was found to infect peanut (*Arachis hypogaea*) under greenhouse conditions. In peanut, initial symptoms were chlorotic rings and spots. After 2–3 wk, these symptoms faded and were no longer evident. Flexuous rod-shaped particles were observed under an electron microscope with leaf-dip preparations of infected peanut tissue. The virus was mechanically transmissible and was transmitted in a nonpersistent manner by the cowpea aphid (*Aphis craccivora*). Crystalline inclusions were observed in the cytoplasm and nuclei of infected peanut plants. No serological relationship was found to either of the potyviruses that commonly infect peanut (peanut mottle virus and peanut stripe virus). A strong serological reaction was obtained in enzyme-linked immunosorbent assays against clover yellow vein (CYVV Pratt) and bean yellow mosaic viruses (BYMV 204-1), two potyviruses not previously reported to infect peanut. Additional physical characteristics demonstrated that this virus was an isolate of BYMV. In a survey of commercial peanut fields in Georgia, BYMV was found to naturally infect peanuts in only one field in one of 12 counties surveyed.

Arrowleaf clover plants (*Trifolium vesiculosum*) collected from experimental plots in Texas showed systemic mosaic, crinkled leaf, and severe stunting symptoms (3). The virus infected peanut (*Arachis hypogaea*) under greenhouse conditions, and a similar isolate has since been recovered from naturally infected peanuts at one location in Georgia. The virus was shown by serological affinities, particle morphology, physical properties, and vector relationships to be a member of the potyvirus group. The virus differed in symptomatology and was not serologically related to the two major peanut-infecting potyviruses, peanut mottle virus (PMV) (11) and peanut stripe virus (PStV) (7). This paper details the specific characterization of the virus with host range, symptomatology, inclusion-body morphology. and serological tests. The virus was identified as an isolate of bean yellow mosaic virus (BYMV) (4).

## MATERIALS AND METHODS

**Culture and maintenance.** An arrowleaf clover plant showing severe mosaic symptoms was collected near a peanut field in Lavaco County, Texas, and was labeled isolate T-1. Symptomatic young clover leaves were triturated in 0.05 M phosphate buffer, pH 7.2, containing 0.01 M $Na_2SO_3$ and 1% Celite. Mechanical inoculations were made by rubbing the extract on leaves of test plants in the greenhouse. Eight to 10 plants of each test species were inoculated with the virus for studies. All plants were tested by enzyme-linked immunosorbent assay (ELISA) to determine if the plants were infected. Species inoculated were Argentine peanut, California Blackeye cowpea (*Vigna unguiculata* subsp. *unguiculata*), Crimson clover (*T. incarnatum*), Early Yellow Summer squash (*Cucurbita pepo* var. *melopepo*), Alaska pea (*Pisum sativum*), and Topcrop bean *Phaseolus vulgaris*). This inoculation procedure was followed in all subsequent greenhouse tests. Two single-lesion serial transfers were made from Topcrop bean to Topcrop bean before the isolate was returned to crimson clover. The virus was subsequently maintained in crimson clover.

**Properties in sap.** The physical properties of the virus were determined using sap from infected Alaska peas. The dilution end point was determined by diluting the sap in 0.025 M phosphate buffer, pH 7.2, from $10^{-1}$ to $10^{-6}$. A range of temperatures from 40 to 75 C (with increments of 5 C) was used with a dilution of $10^{-1}$ to determine thermal inactivation point. The sap (0.1 ml) was heated in a water bath for 10 min at the various temperatures. Longevity in vitro was determined using a $10^{-1}$ dilution of sap and incubation at 20 C for up to 10 days. Each day, an equal portion of the sap was inoculated to Alaska pea.

**Aphid transmission.** *Aphis craccivora* colonies were maintained on California Blackeye cowpea. The aphids were starved 12–16 hr and allowed an acquisition access period of 1 min on detached, infected Alaskan pea leaves. One insect was then transferred to each healthy Argentine peanut test plant, and after a 1-hr inoculation access period, aphids were removed and killed manually. All aphid-inoculated plants were assayed by ELISA.

**Yield loss assessment and seed transmission.** Eight replicates of three plants each of Argentine peanut were planted in pots containing steam-sterilized soil. The treatments included inoculation with isolate T-1, the mild strain of PMV, a mixture of both viruses, and inoculated controls. Each plant in the two- to four-leaf stage was inoculated twice with the virus and was tested 2 wk after the second inoculation by ELISA to verify that they were infected. The plants were grown to maturity and harvested. Seed weight was taken from each plant, and yield estimates were computed.

Seed from T-1-infected Argentine peanut plants were sown in steamed-sterilized soil in trays. Seedlings in the fifth-true-leaf stage were visually inspected and tested (five per well) by ELISA.

**Light and electron microscopy.** Viral inclusion bodies were examined in both peanut and pea tissue under a light microscope with tissue preparation and staining procedures outlined by Christie and Edwardson (5). For serum-specific electron microscopy (SSEM), carbon-coated Parlodion filmed 300-mesh grids were each floated on a 20-ml drop of 1:100 dilution of BYMV 204-1 antiserum in 0.1 M $Na_2HPO_4$-$NaH_2PO_4$ buffer at pH 7.0 and incubated for 30 min at room temperature. Serum-coated grids were floated on a 20-ml drop of antigen and incubated at room temperature for 1 hr. Grids were negatively stained with 2% ammonium molybdate and viewed under a Philips 201C electron microscope at ×20,000.

**Field survey.** Peanut fields in 12 counties in southern Georgia were surveyed for BYMV. The counties were Ben Hill, Berrien, Calhoun, Colquitt, Stewart, Sumter, Taylor, Terrell, Tift, Webster, Wilcox, and Worth. Two fields adjacent to major roads in each county were chosen for convenience, and random samples were taken throughout each field. Each sample was tested by ELISA to determine if the virus was present.

**Virus purification.** Infected crimson clover leaves were triturated in 0.1 M

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Tris-HCl buffer, pH 8.0 (1:4, w/v), containing 0.02 M $Na_2SO_3$ and 0.05 M EDTA and purified by the procedure described by Demski et al (7) for PStV.

**Antiserum.** One milliliter of purified virus was emulsified with an equal volume of Freund's incomplete adjuvant and injected intramuscularly into rabbits for 4 wk. A booster injection was given intravenously 1 wk after the last intramuscular injection. Serum was collected 2 wk after the last injection, and the titer was determined by the microprecipitin test (1) using purified virus.

**Serology.** The virus isolates and antisera used in this study were BYMV 204-1, from T. Pirone, University of Kentucky (9), and the Pratt isolate of CYVV, from O. W. Barnett, Clemson University (2).

The double-antibody sandwich ELISA procedure employed was similar to that described by Lister (12) and Rajeshwari et al (14), with the exception that antigen buffer contained 0.025 M phosphate and 0.1 M ethylenediaminetetraacetic acid. Isolate T-1, PMV, and PStV immunoglobulins, prepared by $(NH_4)_2SO_4$ precipitation, were used at 1 μg/ml, and all other antisera were used at 2 μg/ml. The enzyme conjugates were used at a 1/500 or 1/250 dilution.

Sodium dodecyl sulfate (SDS) immunodiffusion media contained 0.8% agar (Noble), 0.5% SDS, and 1% $NaN_3$ (w/v) in deionized water (8). Isolate T-1 was tested against both CYVV Pratt and BYMV 204-1 antigen and antiserum to determine any serological relationships.

**Table 1.** Comparative host range of isolate T-1, clover yellow vein virus (CYVV), and bean yellow mosaic virus (BYMV) 204-1

| Host species | Host reaction to virus | | |
|---|---|---|---|
| | T-1 | BYMV | CYVV |
| *Chenopodium amaranticolor* Coste & Reyn. | L[a] | L | L |
| *C. quinoa* Willd. | L | L | L |
| *Cucurbita pepo* var. *melopepo* | – | – | – |
| *Glycine max.* (L.) Merr. | | | |
| Bragg | – | M | M |
| Essex | – | – | – |
| Lee | – | – | – |
| Virginia | – | – | – |
| *Lupinus albus* L. | S,N | S,N | M |
| *Medicago sativa* L. | – | – | – |
| *Nicotiana tobacum* L. | | | |
| Burley 21 | – | – | – |
| *Phaseolus limensis* Maef. | M | – | – |
| *Phaseolus vulgaris* L. | | | |
| Bountiful | L | – | – |
| Topcrop | L,C | L,C | L,C |
| *Pisum sativum* L. | | | |
| Alaska | S | S | S |
| Little Marvel | M | M | M |
| *Trifolium incarnatum* L. | S | S | S |
| *T. vesiculosum* Savi. | S | NT | NT |
| *Vigna unguiculata* subsp. *unguiculata* | | | |
| California Blackeye | M | – | M |
| Clay | S | – | – |
| Coronet | – | – | M |

[a] L = chlorotic local lesions, M = mild systemic mosaic, S = severe systemic mosaic, C = systemic chlorosis, N = necrotic reaction, – = no infection detected, and NT = not tested.

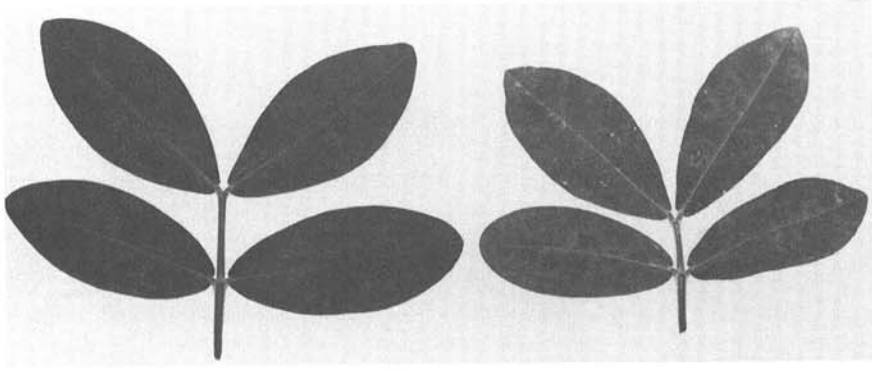

**Fig. 1.** Characteristic chlorotic rings and spots induced in Argentine peanut by an isolate (T-1) of bean yellow mosaic virus. (Left) Healthy plant and (right) diseased plant.

## RESULTS

**Symptomology and host range.** Extracts from a field sample of arrowleaf clover that showed severe mosaic symptoms were inoculated to a range of plants. Mild to severe mosaic symptoms were observed on inoculated leaves of Topcrop and lima bean (*Phaseolus limensis*), Clay and California Blackeye cowpea, Crimson clover; Little Marvel and Alaska pea, and white lupine (*Lupinus albus*) (Table 1). The peanut cultivars Argentine, Florigiant, Florunner, Sunbelt Runner, New Mexico Valencia A, New Mexico Valencia C, NC-7, Early Bunch, Starr, and Toalson also showed systemic symptoms (Table 2). All peanut cultivars developed the characteristic chlorotic spots and rings (Fig. 1), which faded as plants matured. Host plants that developed local infections only were *Chenopodium quinoa*, *C. amaranticolor*, and Bountiful bean.

**Aphid transmission.** Aphids transmitted the virus in a nonpersistent manner. Seventeen of 47 (36%) Argentine peanut plants inoculated using a single viruliferous aphid developed symptoms characteristic of T-1. All symptomatic plants were verified by ELISA as virus-infected.

**Physical properties.** Isolate T-1 remained infective for up to 4 days at 20 C. The dilution end point of the virus was $10^{-4}$. The thermal inactivation point was 50–55 C.

**Light and electron microscopy.** Long, flexuous rod-shaped particles were observed in SSEM with BYMV 204-1 antiserum of crude sap extracts from plants infected by isolate T-1. The particles had a diameter of 14 nm and a mean length of 757 nm. Light microscopic examinations of epidermal leaf strips showed crystalline inclusions in both the nucleus and cytoplasm.

**Seed transmission and yield loss assessment.** Six hundred ninety-one Argentine peanut plants were grown from seeds harvested from infected plants. No symptoms were observed and

**Table 2.** Comparative reactions of isolate T-1 and bean yellow mosaic virus (BYMV) 204-1 on selected peanut cultivars

| Cultivar | Reaction to virus | |
|---|---|---|
| | T-1 | BYMV 204-1 |
| Early Bunch | +[a] | + |
| Florigiant | + | + |
| Florunner | + | + |
| NC-7 | + | – |
| New Mexico Valencia A | + | + |
| New Mexico Valencia C | + | + |
| Pronto | – | + |
| Starr | + | – |
| Sunbelt Runner | + | + |
| Sunrunner | – | + |
| Tannet 74 | – | + |
| Toalson | + | + |

[a] + = Chlorotic spots and rings and – = no symptoms detected.

no positive reactions were detected by ELISA in any of these plants. Isolate T-1 caused no apparent yield loss to Argentine peanut in the greenhouse study. In combination with PMV-M, a 22% yield decrease was attained. This did not differ greatly from that obtained with PMV-M alone.

**Serological relationships.** Isolate T-1 reacted strongly with BYMV 204-1 and CYVV Pratt antisera in ELISA but did not react with antisera to the following potyviruses: PMV, blackeye cowpea mosaic virus, soybean mosaic virus, watermelon mosaic virus, potato virus Y, tobacco etch virus, and pepper veinal mottle virus. In reciprocal tests, BYMV 204-1 reacted with T-1 antiserum but CYVV Pratt did not. In SDS immunodiffusion tests, BYMV 204-1 reacted strongly with T-1 antiserum and produced no visible spurs. CYVV did not react to T-1 antiserum in this test.

**Field survey.** Peanut plants naturally infected with an isolate similar to T-1 were found only at one location in Georgia (Ben Hill County). Ten percent of the plants sampled in the Ben Hill County field were infected. Serologically, this isolate could not be distinguished from the T-1 isolate.

**Purification.** Crimson clover was an excellent purification host for T-1. After initial inoculation, the virus retained a high concentration for up to 60 days after inoculation. The procedure used was one developed for the purification of PStV and consistently yielded 25–30 mg (assuming an extinction coefficient of 3.0) of virus from 1 kg of tissue. The average uncorrected $A_{260/280}$ ratio was 1.25.

## DISCUSSION

A flexuous rod-shaped and nonpersistently aphid-vectored virus from clover was found to infect peanut. Its physical characteristics were similar to those of BYMV 204-1 and CYVV Pratt, and it reacted to both of their antisera in ELISA. The virus was determined to be a BYMV isolate because it did not produce symptoms on white clover as does CYVV Pratt. Also, BYMV 204-1 and T-1 produced a systemic necrosis on white lupine that CYVV Pratt did not produce. BYMV 204-1 reacted with T-1 antiserum but CYVV Pratt did not in both direct ELISA and SDS-agar double diffusion. Isolate T-1 also produced crystalline inclusion bodies in both the nucleus and cytoplasm of infected Alaska pea. BYMV 204-1 produced the same types of inclusion bodies, whereas CYVV Pratt produced crystalline inclusion bodies only in the cytoplasm (5).

BYMV isolate 204-1 is commonly found infecting clovers and other weed hosts that grow in and adjacent to peanut fields in Georgia (6,10,13). This BYMV isolate, along with T-1, has been shown to also infect several peanut cultivars (Table 2). Because of this abundance of potential inoculum sources, the virus tends to be widespread in these areas. Therefore, this first report of BYMV isolates infecting peanut is important because of the potential for a new virus disease becoming established in peanut.

Natural infection of peanuts with BYMV does not appear economically important at this time. The virus was not seed-transmitted in peanuts, and the incidence of BYMV naturally infecting peanuts was low; only 10% of the samples from one field in one county in Georgia were infected. A yield study in the greenhouse did not indicate significant yield reduction when Argentine peanut was infected with isolate T-1 alone or synergistic effects when infected in combination with PMV.

Care must be exercised when assaying for BYMV-infected peanuts. The easily observable symptoms that develop in peanuts 10–14 days after infection fade with time, and only a few faint chlorotic spots are observed on the later-developing leaves. The virus can be detected by ELISA in peanut plants infected with BYMV for 1 mo or more, and mechanical isolation is successful if young expanding leaves are used as inoculum sources. However, old leaves below the inoculated leaves always assayed negative by ELISA.

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