**Legume Yellows Virus, a New Persistent Aphid-Transmitted Virus of Legumes in California**

James E. Duffus

Plant pathologist, U.S. Department of Agriculture, U.S. Agricultural Research Station, P. O. Box 5098, Salinas, CA 93915.

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**ABSTRACT**


Legume yellows virus, which has host range and vector characteristics similar to pea leaf roll virus (PeLRV) and other legume yellowing viruses, recently was found to be widely distributed in California legumes. It is transmitted in a persistent manner by *Acrlythosiphon pismum* and *A. solani* to a large number of leguminous species including *Pisum sativum*, *Trifolium incarnatum*, Vicia faba, *Cicer arietinum*, *Medicago sativa*, and *Glycine max*, but not to over 30 species in 13 nonleguminous plant families. Purified preparations containing monodisperse icosahedral particles ~ 25 nm in diameter proved to be infectious when fed to aphids through membranes. The virus is not identical but very similar to other legume-infesting yellowing viruses such as PeLRV or subterranean clover red leaf viruses (SCRLV). The virus differs markedly from beet western yellows virus (BWYV) in host range and vector specificity but was shown to be closely related to BWYV in reciprocal infectivity neutralization tests.

Recent studies indicated the susceptibility of a number of soybean cultivars (*Glycine max* L. Merr.) to beet western yellows virus (BWYV) (10). Serological studies have indicated that antisera prepared against two strains of soybean dwarf virus from Japan reacted positively with BWYV (7). Soybean dwarf virus was the first member of a group of legume yellowing viruses found in various parts of the world to be characterized (16). These legume yellowing viruses seem to be a distinct group, based on their host range and vector specificity. The serological relationship of BWYV to a member of this group of viruses, along with the known host range of BWYV in legumes (4,5,10,12), indicates that BWYV plays a role in the yellowing diseases of this group of plants and prompted a study of aphid transmitted yellowing viruses of legumes in California.

In random field indexing tests it became apparent that in addition to typical BWYV, a yellowing type pathogen differing from BWYV in vector and host range characteristics was common and widespread in California legumes.

The host, vector, and serological characteristics of this virus, herein termed legume yellows virus (LYV), were the subject of this study.

**MATERIALS AND METHODS**

Virus assays were conducted with detached leaves and stems from field or greenhouse infected plants. Field samples were washed, surface moisture was removed, and they were placed in petri dishes on moistened filter paper. Approximately 200 nonviruliferous aphids of the desired species were placed in the dishes with the plant tissue and the dishes were sealed with tape. In assays for LYV, aphids were allowed a 48-hr acquisition feeding on the material being tested. A 24-hr acquisition feeding was allowed for BWYV testing. Aphids then were transferred to test plants for a 48-hr infection feeding interval and were confined to the test plants by screened sleeve cages.

Nonviruliferous peach aphids (*Myzus persicae* [Sulzer]) were reared on radish (*Raphanus sativus* L.), *Acrlythosiphon pismum* (Harris) on broadbean (*Vicia faba* L.), and *Acrlythosiphon solani* (Kaltenbach) on *Geranium dissectum* L.

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ultracentrifugation. The pellets were resuspended to 0.05% of the original volume of sap.

Sera were prepared from the healthy pea antigen and LYV purified preparations by six intramuscular injections of rabbits at weekly intervals, using Freund’s complete adjuvant (Difco Bacto).

RESULTS

Virus occurrence. Early attempts to determine the occurrence of aphid-transmitted yellow type viruses in California legumes involved assays using M. persicae, A. pisum, and A. solani and the indicator species C. bursa-pastoris, T. subterraneum, M. hispida, and P. sativum. It soon became apparent that two groups or types of viruses were present in the legume species (Table 1). One type was typical BWYV. These isolates were shown later to be transmitted readily by M. persicae, poorly by A. pisum, and A. solani. The isolates produced good symptoms on C. bursa-pastoris. Isolates of the other virus group were not transmitted by M. persicae, but were transmitted by A. pisum and A. solani. These virus isolates produced good symptoms on T. subterraneum, Medicago hispida Gaertn. and P. sativum, but did not infect C. bursa-pastoris. There was some variation in the severity of the isolates on the clover species and pea but these severity differences were not investigated further. The isolates thus far analyzed are distinct on the basis of vector specificity and host range from previously recorded yellowing viruses of legumes and are termed herein legume yellows virus (LYV). The virus appears to have affinities to the PeLRY-SCRLV “group”, but closer alliance with these viruses will have to await further characterization.

Beet western yellows virus was recovered from P. sativum, M. hispida, V. faba, and C. arietinum from various parts of the state. The other virus, LYV, was recovered from M. sativa, V. faba and C. arietinum from widespread locations in the state.

Host range. Host range studies of one isolate (SV-31) of LYV were carried out by inoculation of 10–20 seedlings of a number of different species with A. pisum reared on diseased V. faba. A large number of insects were shaken from the virus source plants onto those being inoculated and were permitted to feed for at least 48 hr. Many of the same species also were inoculated with A. solani given a 48-hr acquisition feeding on infected T. subterraneum. Presence or absence of virus in each plant species tested for susceptibility was determined by aphid transfer to M. hispida or T. subterraneum seedlings about 30 days after inoculation.


Symptoms. Species infected by the LYV showed, in general, interveinal yellowing or reddening of the lower and intermediate leaves. These yellow or red leaves usually were thickened and brittle. Symptoms on a selected group of host plants follow:

Trifolium subterraneum. Infected subterranean clover varied somewhat in severity according to the cultivar tested but showed initial symptoms in 10-14 days. Leaflets of the older leaves folded inward and showed a reddening of the leaf margin. The younger leaves were reduced in size and the plants were stunted severely. Necrosis occurred at the leaflet margins at later stages of infection. Symptoms on subterranean clover are similar to, but not identical to, the symptoms induced on this species by subterranean clover red leaf virus (SCRLV) (19), pea leaf roll virus (PeLRY) (17) and the virus from Michigan alfalfa (18).

Pismum sativum. Pea cultivars differ markedly in reaction to LYV. The cultivars Dark Skin Perfection, Dart, Onyx, Laxton’s Superb, and W. R. Perfection showed severe stunting, shortened internodes and reduced leaf size. The upper leaves were light in color, whereas the intermediate and older leaves showed an intensive chlorotic mottle and were thickened and brittle. The cultivars Perfected Wales, Sprite, Rondo, and Alderman were only moderately stunted. The cultivar Mars was essentially symptomless until

<table>
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<th>Year and area of sampling</th>
<th>Plant species tested</th>
<th>Vector aphid species</th>
<th>Plants tested (no.)</th>
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<tr>
<td></td>
<td></td>
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<td>LYV</td>
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<tr>
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<tr>
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maturity, at which stage, maturation and seed set were severely delayed. Pea cultivars Mid Freezer, Wando, Ranger, Jade, Korozo, and Dwarf Telephone showed no signs of infection and LYV could not be recovered. The reactions of pea cultivars tested are similar to reactions of these same cultivars to PeLRV (2,3) and to the virus from Michigan alfalfa (18).

*Vicia faba.* Infected broad bean cultivars were virtually symptomless or showed marked interveinal yellowing symptoms on the lower and intermediate leaves of susceptible cultivars, such as Triple White. Affected leaves were usually thickened and brittle.

*Cicer arietinum.* Infected garbanzo beans were severely dwarfed, chlorotic and the leaves were curled downward. Symptoms resembled those induced by PeLRV in Iran (15) and by the yellowing isolate from Michigan alfalfa (18).

**Transmission. Mechanical.** Several attempts were made to transmit LYV mechanically by routine techniques that included the use of abrasives, phosphate buffer and sodium sulfite. The virus sources used in these tests were *T. subterraneum, M. hispida, V. faba,* and *P. sativum.* Plants inoculated included these and several other species found to be susceptible when inoculated by aphid vectors. All attempts to transmit the virus by mechanical means failed.

**Aphids.** The legume yellows virus was transmitted initially from field legumes by two aphid species, *Acrithosiphon pisum* and *A. solani.* Tests to determine whether some common aphid species are vectors of the virus were carried out with *T. subterraneum, M. hispida,* and *P. sativum,* as the virus source and test plants. Nonviraliferous aphids of the various species tested were placed on the source plants for 48 hr, and then 25 individuals were transferred to each of a number of test plants for an infection-feeding interval of about 48 hr. Under these conditions only *A. pisum* and *A. solani* transmitted the virus. *Acrithosiphon kondoi* Shinjii and K., *A. pelargonii zeronalphum* (Knowlton), *Aphis craccivora* Koch, *A. fabae* Scopoli, *Dactynotus sonchi* (L.), *Macrosiphum euphorbiae* (Thomas) and *Myzus persicae* failed to transmit the virus. In separate tests, BWYV was transmitted by both vectors of LYV, *A. pisum* and *A. solani,* from shepherd’s purse source plants to shepherd’s purse test plants.

**Virus-vector relationships.** Transmission of LYV by the two aphid vectors, *A. pisum* and *A. solani* is typically circulative. Aphids did not acquire the virus in brief probes. A minimum of 4 hr acquisition feeding was required before either species could acquire virus in these tests. The probability of acquiring virus increased with longer feeding periods up to 72 hr. Individual *A. solani* retained the virus for 24 days in daily serial transfers, and *A. pisum* for 17 days. Both vectors retained the virus after molting and both were more efficient during the first 10 transfers, with a definite loss in transmitting ability in later transfers. Individual *A. pisum* was a more consistent vector in these tests. Of 186 individual aphids transferred serially, 29.6% induced infection in one or more tests plants, compared to 18.6% for individual *A. solani.*

**Virus purification.** Early attempts to purify LYV involved variations of methods used successfully with BWYV. Tissue of *subterranean clover was ground in an equal volume of buffer in a food grinder, homogenized in a VirTis blender and squeezed through cheesecloth.* The expressed sap was heated to 45°. Concentration by ultracentrifugation and further purification by density gradient centrifugation resulted in an infectious zone 25 to 31 mm below the meniscus. The infectious zone was masked in most gradient scans by a large yellow band containing phytoferritin extending from 11 to 34 mm below the meniscus.

Separation of the virus band from the phytoferritin was accomplished by a modification of the methods used by Goodman et al (14). After heating the crude sap to 45° and low speed centrifugation, differential centrifugation was preceded by treatment with 6% polyethylene glycol. The infectious zone, 25 to 31 mm below the meniscus was removed from the gradients, diluted with buffer to fill a Beckman 30 centrifuge tube and frozen. The tubes were later thawed and subjected to differential centrifugation and density gradient centrifugation. These procedures resulted in a good separation of the infectious virus zone from the phytoferritin.

*Fig. 1.* Photometric scanning pattern of partially purified extract of legume yellows virus (LYV) from infected pea. The virus extracts were obtained from frozen plant material, clarified by heating to 45 C and low speed centrifugation (20 min at 12,100 g), and pelleted by ultracentrifugation (2 hr at 80,800 g). Virus samples were subjected to density-gradient centrifugation (4 hr at 59,600 g) and analyzed photometrically (upper curve). Separation of phytoferritin (18 mm) from the virus (28 mm) was accomplished (lower curve) by treatment of clarified sap with 6% polyethylene glycol and two cycles of density-gradient centrifugation.
Purified preparations contained mono-disperse icosahedral particles approximately 25 nm in diameter (Fig. 2). 

**Membrane feeding.** Transmission and symptom characteristics of LYV indicated the possible inclusion in the luteovirus group. Since several members of this group may be acquired through membranes, previously described techniques (8) were used in membrane feeding attempts. Early attempts with *A. pism*, *A. solani* and *M. persicae* on crude and concentrated preparations from subterranean clover were all negative. Later tests with *A. pism* from density gradient fractions from infected pea were successful. The infectious fraction in the density-gradient columns appeared to be in one zone 25 to 31 mm below the meniscus in SW 25 tubes and corresponded to a single peak in gradient scans.

**Serological tests.** Two antisera prepared against the SV-31 isolate of LYV were tested against two isolates of LYV (SV-31 and SP-71), and three strains of BWYV (ST-1, ST-7, and E-4). Antisera against healthy shepherd’s purse and healthy pea extracts were tested against the same isolates and served as controls (Table 2, 3).

The virus antiserum mixtures were subjected to density-gradient centrifugations, analyzed photometrically (1,10) and tested for virus neutralization (9,13).

A positive reaction was based on the reduction or elimination of virus antigen in the scanning patterns of sucrose density-gradient columns and the reduction or elimination of infectivity in the virus zone.

Antiserum prepared against LYV reacted positively with the two isolates of LYV tested and three isolates of BWYV (ST-1, ST-7, and E-4). Antisera prepared against the three isolates of BWYV (ST-1, ST-7, and E-4) reacted positively with the BWYV strains and the two isolates of LYV. Antiserum to healthy shepherd’s purse and healthy pea did not react with any of the virus isolates.

**DISCUSSION**

The legume yellows virus reported here has a high incidence and wide distribution in California. It is very significant economically, causing severe stunting diseases on pea, broad bean, garbanzo beans, lentil, and forage legumes. It occurs in high frequency in alfalfa but little is known of its affects on that crop. The virus has many similarities to pea leaf roll virus from Germany, The Netherlands, England, Iran, and New Zealand. It is also similar in many aspects to subterranean clover red leaf, subterranean clover stunt, soybean dwarf, and milk-vetch dwarf.
viruses from Australia, Japan, and New Zealand. Legume yellows virus is also very similar to the persistent, aphid-transmitted virus from alfalfa in Michigan (18).

The LYV differs from previously reported yellowing viruses of legumes in a number of ways. It has been transmitted by *Acyrthosiphon solani* and *A. pisum* but not by *Aphis craccivora*, *Macrostemum euphorbiaceae* or *Myzus persicae*. It differs from the other legume yellowing viruses in host range, especially in the Trifolium clovers and *Phaseolus vulgaris*.

The legume group of yellowing viruses appear to be very similar in host range (legumes only), aphid transmission (persistent), and symptomology, but differ in specific susceptibility of certain host plants and in vector specificity. At present, comparative host range studies with a number of these viruses are being conducted by workers in Europe, Asia, and North America and more specific information on host range similarities and differences certainly will result from these studies.

At present it would appear that a number of the viruses in the legume group are strains of the same virus, but how closely they are related to each other must await the results of chemical and serological studies of members of the group.

Beet western yellows virus differs significantly from these viruses in its much broader host range. The virus causes stunting and chlorosis on a wide range of dicotyledonous species including such economic hosts as sugar beet, table beet, spinach, lettuce, soybeans, broccoli, cauliflower, radish, turnip, pea, broad bean, chick pea, flax, sunflower, mustard, clover, cabbage, swede, rape, crambe, pepper, pumpkin, watermelons, cucumber, and tomato. Over 150 species in 23 dicotyledonous families have been shown to be susceptible and recent studies have shown that oats, a monocotyledon, is susceptible (11).

The close reciprocal serological relationships between LYV and BWYV as shown in virus neutralization tests in these studies, again indicate the close serological relationship of all members of the luteovirus group thus far studied. It would appear that the luteoviruses are a large family of serologically related viruses that differ in vector specificity, host range, and epidemiological characteristics.

It is important to note that the legume host range of BWYV is as wide or wider than the legume host range of LYV or any of the other legume yellowing viruses, thus special precautions should be exercised in determining the identity and serological characteristics of any aphid-transmitted yellowing component from legumes. Mixtures of BWYV and LYV frequently were found associated in the same plants in nature.

**LITERATURE CITED**


