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BARD

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PROJECT NO. US-659-83

Strawberry Virus Detection

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ABSTRACT

Two aphid-transmitted viruses of major economic importance to commercial strawberry production in the United States and Israel, strawberry mild yellow-edge virus (SMYEV) and strawberry mottle virus (SMV), were investigated.

SMYEV was found to have three double-stranded ribonucleic acid components that are similar in size to those of several well-studied luteoviruses. Serological evidence also relates SMYEV to the well-known luteovirus, beet western yellows virus, strengthening the hypothesis that SMYEV is a luteovirus. The experimental host range of SMYEV was found to include Rubus rosafolius, a plant from which SMYEV-related nucleic acid is much more easily processed than from strawberry. Strains of SMYEV exist, each usually more efficiently transmitted by the strain of vector aphid (Chaetosiphon fragaefolii) with which is naturally associated than by random C. fragaefolii strains.

SMV is an isometric virus of unknown virus group relationship that is transmitted in a semi-persistent manner by C. fragaefolii, Aphis gossypii, and other aphid species. It is mechanically transmissible to herbaceous plants like Chenopodium quinoa and was successfully returned by C. fragaefolii to wild strawberry (Fragaria vesca) where it caused typical symptoms of strawberry mottle disease, thereby demonstrating that the virus(es) mechanically transmitted to C. quinoa is the cause of strawberry mottle disease rather than being a chance associated virus. Partial purification of SMV from C. quinoa showed sucrose density gradient peaks of viruslike particles 28-30 nm in diameter.

D. Research Objectives

The aim of this project was to develop rapid and reliable diagnostic methods for detection of the strawberry viruses known or suspected of being of major importance in both countries: strawberry mild yellow-edge virus, strawberry mottle virus, and pallidosis agent. The purpose of developing such diagnostic methods is to make them available to pathologists and horticulturists for use in evaluating plant health of breeding and nursery stocks and to use these diagnostic tests in improving control of virus infection in the field and to lessen crop damage from these viruses through improved breeding and management practices.

E. Technical research report

(Note: citations in the text without a journal paging reference are listed in Section H of this report.)

1. Studies of strawberry mild yellow-edge virus (SMYEV)

a.) Cytopathology. The cytopathology of isolate MY-18 of SMYEV was examined at intervals after aphid inoculation of the susceptible host Fragaria vesca cv UC-4. ③ (Florance et al 1986). From electron microscope studies of negatively stained (PTA) ultrathin sections, viruslike particles (ca 30 nm in diameter), not seen in uninoculated UC-4 plants, were first located in phloem parenchyma cells of the youngest leaves of plants inoculated 4 weeks previously. Aggregates of viruslike particles were found in some cells, an unusual occurrence for luteoviruses, of which SMYEV has been shown to me a member (see later). These viruslike particles were spherical to hexagonal in outline, had a densely stained core, and appeared quite different from the 22-25 nm diameter viruslike particles reported for SMYEV-infected strawberries from Japan by Yoshikawa (Ann. Phytop. Soc. Japan 50:658), or the 23 nm diameter viruslike particles reported from purified SMYEV isolate MY-18 by Martin and Converse (Phytop. Zeitschr. 114:21-30). The significance of morphological variation in SMYEV among these three studies has not yet been determined.

b) Host range. The current standard detection procedure for SMYEV is to graft leaflets of suspect plants into healthy susceptible plants of Fragaria vesca L. cvs like Alpine and UC-4 and watch for diagnostic symptom formation. In hot climates like Israel, F. vesca indicator plants are difficult to grow in the greenhouse, and symptom development in graft-inoculated indicators is often obscured by physiological heat spot on new leaves. In Israel, the Indian strawberry, Duchesnea indica (Andr.) Focke, was found to grow well in the greenhouse in the summer without developing heat spot symptoms. D. indica can be successfully graft-inoculated with SMYEV without producing

symptoms but expresses strong symptoms when inoculated with strawberry mottle virus (SMV), making it possible to detect SMV in Israel at will throughout the year (Spiegel 1986).

The reported experimental host range of SMYEV has been limited to the genera Fragaria, Duchesnea and Sanguisorba (Converse, ed., 1987, USDA Ag Handbook 631, and Spiegel, 1986). Purification of SMYEV from infected Fragaria plants has been hampered by the high levels of polyphenols and mucilaginous substances present in leaves of species in this genus (Martin and Converse op. cit., 1985). Accordingly, the rosaceous plants Duchesnea indica, Potentilla anserina L., Rubus niveus Thunb., R. rosafolius Smith, Sanguisorba minor Scop., and Waldsteinia fragarioides (Michx.) Tratt. were tested for their susceptibility to infection by SMYEV by leaflet graft inoculation. Only R. rosafolius and D. indica were found to be infected by SMYEV by this method (Tian, Kowalczyk and Converse, 1988). Because the genus Rubus has been found in previous studies to be a host superior to Fragaria for virus purification, R. rosafolius was subsequently used for further study of SMYEV purification, as will be outlined subsequently.

c.) SMYEV strain specificity. Martin and Converse op. cit. (1985) observed that an SMYEV isolate was more efficiently transmitted by the isolate of vector aphid [Chaetosiphon fragaefolii (Ckll.)] from the exact same strawberry plant from which that isolate was collected in the field than it was by a stock insectary culture of C. fragaefolii. Expanding on this lead, D. L. Clement, a postdoctoral research associate on this BARD project, and subsequently Y-N. Tian, a PhD student on this BARD project, collected several more SMYEV isolates and their field-associated C. fragaefolii aphid strains and used them for comparative transmission tests, each virus isolate with its own "matched aphid" and with other C. fragaefolii aphid cultures. Among 9 virus-aphid pairs examined, several SMYEV isolates were transmitted only, or almost exclusively, by the matched aphid. Some virus isolates were transmitted by unmatched aphids, sometimes in reciprocal fashion, sometimes not. Quantitative differences occurred in virus transmission efficiency among heterologous pairs, as well as considerable differences among isolates as to symptomatology and chronology of symptom expression. Mr. Tian has encountered too much variation in repeated experiments to prepare this work for publication at this time, but the data clearly point to the generalization that a given SMYEV isolate from the field is likely to be most efficiently transmitted by the C. fragaefolii isolate with which it is naturally associated in the field.

In 1982, Martin and Converse (Phytopathology 72:989) reported on the occurrence of a virus that was not detectable by leaf graft analysis on indicator plants that were sensitive to known strains of SMYEV but that co-sedimented with an isolate of SMYEV. Detection of this unknown latent virus is currently accomplished only by electron microscopy of sucrose density gradient peaks to check for 23 nm isometric viruslike particles. A large number of plants were regenerated after prolonged heat

therapy and shoot apex culture of 100 micron shoot tips from latent-virus-infected Benton strawberry plants. Purification of viruslike particles from a few such heat-treated, shoot-apex-cultured plants indicated that these viruslike particles were still present. The relationship of these viruslike particles to SMYEV and their successful elimination by therapy must await the final development of anti-SMYEV probes.

d.) SMYEV purification. The work of Martin and Converse op. cit. (1985) in partially purifying SMYEV from F. vesca leaves was confirmed and expanded by Mr. Tian and Drs Spiegel and Clement. Using published procedures, they found appreciable quantities of viruslike particles ca 23 nm in diameter to be routinely associated with sucrose density gradient peaks from SMYEV-infected F. vesca leaves but not from uninoculated leaves. However, the heterogeneity of particles in fractions containing viruslike particles indicates that plant material is still present and therefore that the purification procedures need to be modified. Purifications from SMYEV-infected R. rosafolius using the same methods gave rise to minute quantities of viruslike particles, as judged by density gradient ultraviolet absorbance traces and by electron microscopy (Tian et al 1988).

e.) dsRNA studies. Spiegel ⁵ (1987), working with SMYEV-infected cultivated strawberry plants, was able to detect three distinct bands of double-stranded ribonucleic acid (dsRNA) of relative molecular masses of 3.8, 2.8, and 1.3 million daltons, that were absent from plants of the same cultivars that indexed negative for SMYEV on F. vesca. She also found several dsRNA bands with relative molecular masses of 0.9 and 0.5 million daltons that were common both to SMYEV-inoculated and to uninoculated strawberry cultivars. The identity of these bands is at present unknown. Tian et al. (1988) confirmed the work of Spiegel (1987) in F. vesca and in R. rosafolius, but failed to find the same dsRNA bands in uninoculated F. vesca cv UC-4 or in uninoculated R. rosafolius. Although we feel that SMYEV does have a set of characteristic dsRNA bands that are similar to those reported for several other luteoviruses, we believe that they are present in too low a concentration in infected tissue to be useful for routine diagnosis of this virus. Using infected R. rosafolius, Martin et al (1988) have successfully developed complementary DNA probes against SMYEV. This cDNA work was facilitated by the present BARD work on R. rosafolius as an experimental host of SMYEV, but is itself outside the scope of the present BARD project.

f.) SMYEV serology. Spiegel, Cohen and Converse ⁶ (1986) found that a partially purified Israeli isolate of SMYEV was trapped by antiserum against the luteovirus beet western yellows virus (BWYV) in serologically specific electron microscopy (SSEM) tests. This work constitutes the first serological evidence that SMYEV is a luteovirus. Because the trapped SMYEV viruslike particles were not subsequently decorated by BWYV antiserum, we conclude that the serological relationship between the two

viruses is not a close one.

Using standard procedures with mice and partially purified preparations of SMYEV from F. vesca, Converse was not able to develop monoclonal antibodies specific against SMYEV. Instead, a large number of hybridomas were formed that recognized healthy F. vesca antigens. In order to reduce the number of mouse B-cells programmed against healthy plant antigens from highly purified plant virus preparations, George and Converse (1987 and 1988) developed a model system for selective elimination of undesired B cells, using cauliflower mosaic virus (CaMV) in Chinese cabbage as a system. An additional, brief, simple step was added to the hybridoma formation procedure, in which B cells programmed against healthy plant antigens were adsorbed by partially purified sterile plant antigens anchored to a solid phase. This additional step resulted in a 72% increase in anti-CaMV B cells. This technique is now ready to be used in future preparation of hybridomas against SMYEV.

In anticipation of detecting serological differences among SMYEV isolates when specific poly- or monoclonal antibodies were developed in this laboratory, Clement and Converse (1986) successfully applied the quantitative ELISA method developed by Clark and Barbara (J. Gen. Virol. 58:315) to several serologically distinct strains of tobacco streak virus in order to calculate serological differentiation indices (SDIs) among them. This technique is also now ready in this laboratory for the quantitative evaluation of serological differences among SMYEV isolates.

g.) Control of SMYEV. Although the original terms of this BARD proposal did not include control studies, subsequent reports in the literature on the value of certain synthetic pyrethroids on the control of several other plant viruses led Converse and Aliniaze (1987 and 1988) jointly to investigate their effects on aphids and on SMYEV incidence in field studies. Dr. M.T., Aliniaze is a non-BARD member of the Oregon State University Department of Entomology, who cooperated on this study because of his interest in fruit insects. In a two-year study with a strawberry cultivar that is popular commercially in this area (Totem), we found that all of the insecticides tested (organophosphates and pyrethroids) significantly reduced populations of C. fragaefolii but that these reductions were not directly related to incidence of SMYEV in these test plants. For instance, the best aphid control was achieved by use of the pyrethroid bifenthrin, which did not significantly reduce incidence of SMYEV below that of unsprayed plants. On the other hand, applications of the pyrethroid cypermethrin resulted in appreciable populations of vector aphids but resulted in a significant reduction of SMYEV incidence- to 44% of the incidence in unsprayed plants. Clearly, in evaluating the value of insecticides in controlling SMYEV, factors other than the survival of vector aphid populations must be considered.

II. Studies of strawberry mottle virus (SMV).

a.) Completion of Koch's Postulates. In 1968, Frazier (Pl. Dis. Repr. 52:64) reported on the mechanical transmission of a viruslike agent from SMV-infected F. vesca to Chenopodium quinoa, with the production of faint mottle in the latter, but Fraizer was unable to return this viruslike agent to strawberry. Kitajima et al in electron microscope ultrathin section studies conducted in Brazil in 1971 (Cienca e Cultura 23:649) associated 25-30 nm diameter isometric viruslike particles with SMV infection.

Working in Converse's laboratory partly on non-BARD grant funds, Dr. Ruperto F. Hepp, Associate Professor of Plant Pathology, Universidad de Concepcion, Chile, was able to transmit a virus mechanically from F. vesca plants infected with a severe strain of SMV to C. quinoa. This virus was then successfully returned in about 5% of cases to F. vesca by the aphid Chaetosiphon fragaefolii and caused symptoms typical of SMV in F. vesca. From those infected F. vesca plants, SMV was successfully transmitted by C. fragaefolii to other F. vesca plants. This work clearly demonstrates that the virus (or viruses) transmitted mechanically to C. quinoa is competent to cause the complete typical symptomatology associated with SMV (Hepp and Converse, 1987 and 1988).

b.) Electron microscopy of SMV. Electron microscopy of ultrathin sections of C. quinoa infected with SMV revealed numerous isometric viruslike particles 28 nm in diameter occurring singly and occasionally in groups in the cytoplasm of cells in phloem tissues, and absent from uninoculated C. quinoa plants (Hepp and Converse 1987 and 1988).

c.) Partial purification of SMV. A severe strain of SMV was partially purified from infected C. quinoa by a modification of the method of Van Vloten-Doting (Virology 65:215). After the homogenized leaf sap in buffer was passed through cheesecloth and clarified by extraction with butanol-chloroform, it was precipitated with polyethylene glycol and pelleted by low-speed centrifugation. The resuspended pellet was then given a high-speed centrifugation through a sucrose pad, and given either a sucrose rate-zonal density gradient centrifugation, or an equilibrium ultracentrifugation in CsCl. An absorbance (A_{256nm}) peak in the former and a band at a density of 1.35 in the latter both contained ca 30 nm isometric particles lacking in parallel preparations made from uninoculated C. quinoa plants. Along with these isometric particles we observed a quantity of fibrillar material that was also present in parallel preparations from uninoculated C. quinoa. Immunoabsorption of these virus preparations with anti-healthy C. quinoa antiserum did not reduce the height of the resulting sucrose density gradient peak

associated with viruslike particles, nor did it eliminate the fibrillar material in the preparation. The removal of these lingering contaminants will now be a necessary prelude to the successful use of these preparations for preparing antisera specific for SMV. (Hepp and Converse 1987, and 1988).

III. Studies on pallidosis agent. Pallidosis disease was first described by Frazier and Stubbs in 1969 (Pl. Dis. Reprtr. 53:524). Current detection methods require the use of F. virginiana clones like UC-10,11, or 12 for leaf graft bioassay (Frazier, Pl. Dis. Reprtr. 58:28). In our laboratory, we have found UC-10 to be the preferred indicator host for pallidosis agent (PA), but we are unable to detect it in the presence of other strawberry viruses because the symptoms of PA are either obscured or duplicated by the other viruses found in strawberry. Like other investigators who have worked with PA, we have detected it by this bioassay when PA occurred alone in several strawberry cultivars from eastern and western U.S. and from Canada. It has not been detected in Israel to date. Preliminary greenhouse studies on the effects of PA on growth of UC-10 indicate that a severe strain of PA from N.W. Frazier (Rip 174) causes a statistically significant 15% reduction in petiole length, accompanied by severe distortion and chlorosis of leaves.

F. Description of cooperation.

Formal evidence of cooperation is available by scanning the List of Publications in Section H of this report. In addition to Drs. R.H. Converse (Principal Investigator), and Cooperating Investigators Drs. T. C. Allen (U.S.) and S. Spiegel (Israel), the following persons cooperated and were involved in publication of work arising from this BARD project:

In the United States: M.T. Aliniaze, D.L. Clement, E. Florance., R.A. George, R.F. Hepp, K.L. Kowalczyk, and Y.-N. Tian.

In Canada: W. Jelkmann, and R.R. Martin,

In Israel: J. Cohen

In addition, visits to Converse's laboratory were made by Dr. Spiegel in November 1985, and again in September 1987. Converse scheduled a visit to Dr Spiegel's lab in May 1988, not involving BARD funds, but one purpose of the visit was to complete this final BARD report and plan future cooperative non-BARD research work between our two laboratories.

Extensive interchange of messages has gone on throughout the course of this BARD project between Converse and Spiegel by letter and by electronic mail (bitnet), resulting in continual awareness by each participant of the progress and problems in the other laboratory.

9. Main research achievements. These can be placed into two virus-related groups, for strawberry mild yellow-edge virus, and for strawberry mottle virus.

For strawberry mild yellow-edge virus (SMYEV):

1. Determination that SMYEV has three double-stranded RNA moieties that are comparable with those found in several other luteoviruses, to which SMYEV was heretofore tentatively assigned on other, less critical, grounds

2. Discovery of Rubus rosafolius as an experimental host for SMYEV, making possible its use as a biochemically superior host for purification of SMYEV nucleic acids when compared to strawberry. This led to the confirmation of the existence of the three dsRNA SMYEV bands reported by Spiegel from infected strawberry cultivars (item 1. above), and was the key step needed to make possible the development of complementary DNA probes to SMYEV in Dr. Martin's laboratory in Canada, in conjunction with Jelkmann, Spiegel and Converse.

3. Determination that SMYEV is composed of more than one aphid-specific virus strain with respect to efficient transmission. SMYEV is reported in some of the previous literature as being very inefficiently transmitted. It now appears that when an isolate of SMYEV is acquired by a strain of the aphid Chaetosiphon fragaefolii with which that isolate was naturally associated in nature, transmission is likely to be very efficient.

4. Development of a simple B-cell enrichment procedure, to selectively remove unwanted B-cell populations from harvested splenocytes before production of hybridomas based on fusion with the remaining B cells. This work provides a needed tool for improving chances to develop monoclonal antibodies against SMYEV isolates.

For strawberry mottle virus (SMV):

1. Koch's Postulates as applied to plant viruses have been fulfilled, showing that the virus(es) that were successfully mechanically transmitted to Chenopodium quinoa were competent to cause the complete symptomatology of SMV when returned to Fragaria vesca by vector aphid. This work opens the door to the use of purifying SMV from mechanically inoculated C. quinoa by removing earlier doubts that this mechanically transmitted virus was actually competent to cause typical strawberry mottle disease symptoms.

2. The success in partial purification of SMV from C. quinoa now places us in the position to make rapid progress toward the development of mono- and polyclonal antisera against this virus.

H. List of Publications

- ① Clement, D.L. and R.H. Converse. 1986. Serological relationships among four tobacco streak virus isolates. (Abstract) *Phytopathology* 76:842.

Converse, R.H. and M.T. Aliniaze. 1987. The reduction in incidence of aphid-borne strawberry viruses in the field by use of pyrethroid insecticides. (Abstract) *Phytopathology* 77:1762.

- ② Converse, R.H. and M.T. Aliniaze. 1988. Effects of insecticides applied in the field on the incidence of aphid-borne viruses in cultivated strawberry. *Plant Disease* 72:127-129.

Converse, R.H., S. Spiegel and others. 1988. Laboratory detection of viruses and mycoplasma-like organisms in strawberry. accepted for *Plant Disease*.

- ③ Florance, E. T.C. Allen, and R.H. Converse. 1986. The ultrastructure of strawberry mild yellow-edge virus, a luteovirus, in cells of Fragaria vesca, cultivar UC-4. (Abstract) *Phytopathology* 76:843.

George, R.A. and R.H. Converse. 1987. Reduction of unwanted B-cell populations by adsorption or complement-mediated cytotoxicity prior to anti-cauliflower mosaic virus hybridoma formation. (Abstract) *Phytopathology* 77:1238.

George, R.A. and R.H. Converse. 1988. Methods for the enrichment of desired B-cell populations prior to anti-cauliflower mosaic virus hybridoma formation. accepted for *Phytopathology*.

Hepp, R.F. and R.H. Converse. 1987. Transmission of strawberry mottle virus between Chenopodium quinoa and Fragaria vesca by aphid and sap inoculation. (Abstract) *Phytopathology* 77:1239.

Hepp, R.F. and R.H. Converse. 1988. Mechanical transmission of strawberry mottle virus from Chenopodium quinoa to Fragaria vesca. submitted to *Phytopathology*

Martin, R.R., S. Spiegel, W. Jelkmann, and R.H. Converse. 1988. Molecular cloning of the dsRNAs associated with strawberry mild yellow-edge virus. (Abstract) *Acta Horticulturae* (in press).

- ④ Spiegel, S. 1986. An improved indexing procedure for some strawberry viruses in a hot climate area. *Acta Horticulturae* 186:77-82.

- ⑤ Spiegel, S., J. Cohen, and R.H. Converse. 1986. Detection of

strawberry mild yellow-edge virus by serologically specific electron microscopy. (Abstract) Acta Horticulturae 186:95.

- ⑤ Spiegel, S. 1987. Double-stranded RNA in strawberry plants infected with strawberry mild yellow-edge virus. Phytopathology 77:1492-1494.

Tian, Y-N., K. L. Kowalczyk, and R.H. Converse. 1988. Rubus rosafolius, an experimental host for strawberry mild yellow-edge virus. (submitted to Phytopathology).