

# Characterization and Recent Advances in Detection of Strawberry Viruses

Commercial strawberry (*Fragaria* × *ananassa* Duchesne), which originated in Europe around 1750, is a hybrid between *F. virginiana* Duchesne from North America and *F. chiloensis* (L.) Duchesne from South America. Today *F.* × *ananassa* is grown worldwide for the red fruit that is consumed fresh or used in jam, yogurt, ice cream, and baked goods (12). White and red-fruited *F. chiloensis*, known for its intense fragrance and flavor, is cultivated in parts of South America; whereas another species, *F. vesca* L. ‘Alpine’ strawberry, is grown on small farms in parts of Europe.

Strawberries are propagated vegetatively and are subject to infection by viruses during plant propagation and fruit production stages. Reports of initial detections, symptoms, severities, and/or vectors for more than 30 viruses, virus-like diseases, and phytoplasmas affecting *Fragaria* spp. have been reviewed (8,81). Photographs of the symptoms caused by strawberry viruses have been published in those reports and will not be duplicated here. Only viruses will be considered in this article, because excellent work has been published on phytoplasmas in strawberry (1,29, 37,115). The names, acronyms, vectors, classification, and laboratory-based methods available for detection of the viruses discussed below are summarized in Table 1.

Initially, many of the virus diseases of strawberry were described based upon

symptoms in clones of *F. vesca* and *F. virginiana* plants induced when graft inoculated with various viruses (18). Since the last review on strawberry viruses (81), significant progress has been made in the molecular characterization of the aphid-borne viruses, the identification and characterization of several whitefly-borne viruses, and the molecular characterization of viruses associated with several of the “graft transmissible virus-like diseases” of strawberry. Prior to 1998, molecular data existed only for *Strawberry mild yellow edge virus* (SMYEV) (9,35). Currently, molecular-based reverse transcription–polymerase chain reaction (RT-PCR) detection methods are available for most of the viruses known to infect strawberry. The goals of this paper are to review symptoms induced by the viruses, describe advances in the detection of strawberry viruses, and demonstrate the application of these tests for characterizing the cause of recent outbreaks of a decline disorder in strawberry in the western United States and Canada. This disorder, in which plants develop a reddish coloration of the leaves, the roots appear to stop growing, the plants get progressively weaker and in some cases die (Fig. 1), has been observed in a number of cultivars in California. A similar decline also has been observed in Oregon, Washington, and British Columbia in cultivars such as ‘Totem’, ‘Rainier’, ‘Puget Reliance’, and ‘Puget Beauty’ (Fig. 2).

## Aphid-Transmitted Viruses

Seven aphid-transmitted viruses are reported in strawberry: *Strawberry crinkle virus* (SCV), SMYEV, *Strawberry mottle virus* (SMoV), *Strawberry vein banding virus* (SVBV), *Strawberry pseudo mild yellow edge virus* (SPMYEV), *Strawberry chlorotic fleck virus* (SCFV), and *Straw-*

*berry latent C virus* (SLCV). SCV, SMYEV, SMoV, and SVBV have been considered the four most economically important viruses of strawberry in the majority of production areas (8,81). These viruses are generally less important in annual cropping systems, because the plants are not grown in the field as long and there is less opportunity to get multiple infections, which are required for symptom expression in most cultivars of *F.* × *ananassa*. Nevertheless, it is important, even in annual production systems, to control the aphid vectors (primarily *Chaetosiphon fragaefolii*) in order to reduce virus infections. With the increase in whitefly-transmitted viruses in Mediterranean and subtropical climates, aphid control becomes more critical, as mixed infections of the aphid- and whitefly-transmitted viruses may lead to significant yield losses. It is also critical to keep plants virus-free during the propagation phase, where plants are grown for increase in the field for several years. Breeders have been effective at developing tolerance, and most strawberry cultivars grown today do not exhibit symptoms when infected with a single aphid- or whitefly-transmitted virus. However, under field conditions, these viruses often occur in complexes that may result in foliar symptoms and plant decline. These four major aphid-transmitted viruses have been studied in greatest detail and can be separated in the laboratory using serial transmissions by the common strawberry aphid (*C. fragaefolii*) onto indicator plants (81). Precautions should be taken when carrying out these experiments, since there may be considerable differences in transmission efficiency among virus isolates and a single colony of *C. fragaefolii* or between several colonies of *C. fragaefolii* and a single virus isolate. Laboratory tests, either

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RT-PCR or ELISA, are available for each of these viruses, with the exception of SLCV.

**Strawberry crinkle virus (SCV).** SCV was first reported in Oregon in 1932 (113) and in Great Britain in 1934 (62). In both cases, mild and severe forms of the virus were reported, suggesting that there were probably mixed infections. SCV occurs in strawberry in areas where the strawberry aphid is found, and is one of the most damaging viruses of strawberry. All species of *Fragaria* are susceptible to SCV. Great losses result from mixed infections of SCV with pallidosis virus and/or one or more of the other aphid-borne viruses. However, severe strains of SCV can cause disease symptoms on infected cultivars and reduce yield and fruit size. Sensitive *F. vesca* clones are reliable indicators for SCV infection, and symptoms include: deformed leaves and distorted petioles, leaflets with chlorotic spots that are often uneven in size, distorted, and crinkled, distorted petioles, and size reduction of leaves. In addition, necrotic lesions on runners, petioles, and petals often appear on indicators. SCV can be transmitted mechanically from strawberry to *Nicotiana occidentalis* P1 and then to *Physalis pubescens* (41). SCV caused local lesions and systemic symptoms on *N. occidentalis* P1 and systemic symptoms on *P. pubescens*, which was used for purification and characterization of the virus.

The virus is transmitted in a replicative persistent manner by aphids in the genus *Chaetosiphon*. In *C. fragaefolii*, the major aphid vector, SCV has a latent period of 10 to 19 days under optimal conditions; at cooler temperatures the latent period is

increased (42). Under cooler temperatures, transmission efficiency decreases (42). This longer latent period may explain the lack of SCV in many cooler strawberry production areas.

The entire nucleotide sequence of SCV has been determined, and it shows significant homology to the cytorhabdoviruses *Northern cereal mosaic virus* and *Lettuce necrotic yellows virus* (74). It has a single-stranded minus-sense RNA genome of 14,547 nucleotides that contains seven open reading frames in the complementary RNA. RT-PCR and real-time RT-PCR detection assays for SCV have been reported by several groups (57,65,89). Using RT-PCR assays, it was found that all 'Totem' plants with decline symptoms in British Columbia (B.C.), Canada, and Washington, USA, were infected with SCV as well as with SMoV and SMYEV. Many of the asymptomatic plants in the same fields were infected with SMoV and SMYEV. Previously, only the latter two viruses and SVBV had been detected in B.C. (81). The occurrence of SCV in B.C. may be due to the existence of new strains of SCV, new biotypes of the aphid vector, warmer temperatures that allow a shorter latent period and thus more efficient transmission of the virus, resistance to chemicals used for aphid control, or a combination of these factors.

**Strawberry mild yellow edge virus (SMYEV).** Strawberry mild yellow edge disease (SMYED) was first reported in California in 1922 (33) and in Europe in 1933 (27), and it is one of the most widespread virus diseases of cultivated strawberry. It also occurs in *F. chiloensis* in areas remote from cultivated strawberry in

Chile (31). Conflicting reports on the economic impact of SMYEV probably result from differences in virus strains, cultivars evaluated, the likelihood that mixed infections often occur in the field resulting in synergistic effects with SMYEV, and environmental conditions in the different studies. Reported yield losses have ranged from 0 to 30%. SMYEV is vectored efficiently in a persistent manner by aphids in the genus *Chaetosiphon*. Therefore, in areas where these aphids do not occur, the use of virus-free planting material will be an effective management strategy. There is no known resistance to SMYEV in the genus *Fragaria*, but most cultivars grown today are tolerant to infection with this virus by itself. Sensitive cultivars such as 'Hood', 'Puget Beauty', and 'Marshall' develop dwarfing, marginal chlorosis, leaf distortion, and small fruit, whereas tolerant cultivars such as 'Totem' and 'Sumas' do not show symptoms if infected doubly by SMYEV and SMoV or SVBV. However, infection by severe strains of three or more of these viruses leads to a decline of these cultivars.

Symptoms of SMYEV are similar on *F. vesca* 'UC-4', 'UC-5', and 'Alpine' seedlings, while 'UC-6' is usually symptomless. Symptoms first appear 8 to 10 days after inoculation with severe strains; whereas mild strains may induce delayed or no symptoms. The use of *F. vesca* 'EMC', which is infected with the latent A strain of SCV, may increase sensitivity to very mild strains of SMYEV. Initial symptoms on standard, healthy indicators include epinasty of the newly emerging leaves together with small chlorotic flecks. As the symptoms develop, the chlorosis

**Table 1.** Strawberry viruses, names, acronyms, natural modes of transmission, genera, and means of laboratory detection<sup>a</sup>

| Virus name                         | Acronym | Mode of transmission | Genus                    | Laboratory detection <sup>b</sup> |
|------------------------------------|---------|----------------------|--------------------------|-----------------------------------|
| Apple mosaic                       | ApMV    | Pollen, seed         | <i>Iarvirus</i>          | ELISA, RT-PCR                     |
| Arabis mosaic                      | ArMV    | Nematode, seed       | <i>Nepovirus</i>         | ELISA, RT-PCR                     |
| Beet pseudo-yellows                | BPYV    | Whitefly             | <i>Crinivirus</i>        | RT-PCR                            |
| <i>Fragaria chiloensis</i> cryptic | FCICV   | Unknown              | Unknown                  | RT-PCR                            |
| <i>Fragaria chiloensis</i> latent  | FCILV   | Pollen, seed         | <i>Iarvirus</i>          | ELISA, RT-PCR                     |
| Raspberry ringspot                 | RpRSV   | Nematode, seed       | <i>Nepovirus</i>         | ELISA, RT-PCR                     |
| Strawberry chlorotic fleck         | StCFV   | Aphid                | <i>Closterovirus</i>     | RT-PCR                            |
| Strawberry crinkle                 | SCV     | Aphid                | <i>Cytorhabdovirus</i>   | RT-PCR                            |
| Strawberry feather leaf            | NA      | Unknown              | Unknown                  | NA                                |
| Strawberry latent                  | StLV    | Unknown              | <i>Cripavirus</i>        | RT-PCR                            |
| Strawberry latent C                | SLCV    | Aphid                | <i>Nucleorhabdovirus</i> | NA                                |
| Strawberry latent ringspot         | SLRSV   | Nematode, seed       | <i>Sadwavirus</i>        | ELISA, RT-PCR                     |
| Strawberry mild yellow edge        | SMYEV   | Aphid                | <i>Potexvirus</i>        | ELISA, RT-PCR                     |
| Strawberry mottle                  | SMoV    | Aphid                | <i>Sadwavirus</i>        | RT-PCR                            |
| Strawberry necrotic shock          | SNSV    | Thrips, pollen, seed | <i>Iarvirus</i>          | ELISA, RT-PCR                     |
| Strawberry pallidosis associated   | SPaV    | Whitefly             | <i>Crinivirus</i>        | RT-PCR                            |
| Strawberry pseudo mild yellow edge | SPMYEV  | Aphid                | <i>Carlavirus</i>        | ELISA                             |
| Strawberry vein banding            | SVBV    | Aphid                | <i>Caulimovirus</i>      | PCR                               |
| Tobacco necrosis                   | TNV     | Oomycete             | <i>Necrovirus</i>        | ELISA, RT-PCR                     |
| Tomato black ring                  | TBRV    | Nematode, seed       | <i>Nepovirus</i>         | ELISA, RT-PCR                     |
| Tomato ringspot                    | ToRSV   | Nematode, seed       | <i>Nepovirus</i>         | ELISA, RT-PCR                     |

<sup>a</sup> NA = not available, indicates the virus disease has been described in the literature but that the authors are unaware of a known isolate of the virus currently maintained in a collection.

<sup>b</sup> Detection methods listed do not include sap inoculation, graft transmission, or vector transmission to indicator plants.



Fig. 1. Decline symptoms in strawberry plants near Watsonville, CA, U.S.A.: A, production field showing reddening, uneven growth, and decline associated with infection by multiple strawberry viruses in 'Ventana'; B, several plants of 'Ventana' at various stages of decline; C, close-up of 'Camarosa' strawberry plant that exhibits reddening, stunted and distorted leaves, and small fruit.



Fig. 2. Symptoms of decline in 'Totem' strawberry in British Columbia, Canada showing: A, vein reddening; B, leaf distortion, vein reddening, and lesions on petioles all associated with multiple virus infection.

increases and gradually becomes necrotic. Interveneal necrosis follows, and eventually the entire leaf collapses. New leaves continue to emerge and go through the same cycle of symptom development. Eight to 12 weeks after infection with severe strains of SMYEV, leaves that developed prior to infection appear healthy, a ring of dead leaves exists and the younger leaves exhibit the range of symptoms described above. The lack of symptoms on grafted 'UC-6' plants is helpful in identifying SMYEV.

SMYEV was the first strawberry virus cloned and sequenced (31). It is a member of the genus *Potexvirus* in the family *Flexiviridae*, and has a single-stranded positive-sense RNA containing 5,966 nucleotides excluding the 3' poly A-tail, that has five open reading frames. Using a full-length infectious clone of SMYEV, it was shown that this potexvirus was capable of causing SMYED in indicator plants (43) (Fig. 3). This was surprising, because it has long been known that field isolates of the causal agent of SMYED are transmitted in a persistent manner by the strawberry aphid, and it was anticipated that the causal agent would be a luteovirus. The potexvirus is not aphid-transmissible from symptomatic plants inoculated with the infectious clone, which suggests that there may be a helper virus that provides aphid transmission in the field. Some evidence of a luteovirus associated with SMYED exists, including the presence of isometric particles (47) and size and pattern of dsRNA (80). However, attempts to identify a luteovirus or other helper virus in plants infected with SMYEV have not been successful. SMYEV has been purified and used to produce monoclonal antibodies that can be used to detect a range of isolates from geographically diverse areas (69). SMYEV can be detected readily by RT-PCR or ELISA (69,89) and has been detected in all sources of SMYED characterized by symptoms on indicator plants.

**Strawberry mottle virus (SMoV).** SMoV was first recognized as a distinct virus in 1946 when it was separated from "mild yellow edge" based on the difference in aphid transmission properties (67). It is the most common virus of strawberry and occurs naturally in the genus *Fragaria* wherever strawberries are grown. Numerous strains of SMoV exist, and most are symptomless in strawberry cultivars, but severe strains may reduce vigor and yield by up to 30% (22). In nature, SMoV is vectored in a semi-persistent manner by the aphids *Chaetosiphon* sp. and *Aphis gossypii*. The virus can be acquired and transmitted with feeding times of a few minutes. In areas where *Chaetosiphon* spp. are the major vectors, the potential for simultaneous transmission of the other aphid-borne viruses of strawberry may result in a greater impact on yield. In areas with high populations of the aphid vectors

and the presence of other viruses, only tolerant cultivars can be grown without extensive use of insecticides for vector control. Because SMoV is transmitted by *A. gossypii*, this virus can be common in areas where the strawberry aphid does not occur, although it should not be a problem because most cultivars are tolerant to infection by SMoV alone.

Both *F. vesca* and *F. virginiana* clones are sensitive indicators for SMoV and produce a range of symptoms following inoculation by leaflet grafting or aphid transmission. *F. vesca* 'Alpine' and clone 'UC-5' are the most frequently used indicator plants for SMoV. Symptoms usually appear on *F. vesca* plants 7 to 10 days following inoculation, but may take longer for mild isolates. Symptoms on indicator clones range from barely discernible, to mild leaf mottle, to severe stunting and distortion, to plant death. The use of serial transmissions by aphid vectors has shown that individual plants from the field often carry a range of SMoV isolates, probably an indication of mixed infections with SMYEV or SVBV.

The complete nucleotide sequence of SMoV has been determined (88), and the sequence suggests SMoV is a member of the *Sadwavirus* genus in the family *Sequiviridae*. It has a bipartite genome with single-stranded positive-sense RNAs of 7,036 and 5,619 nucleotides, respectively, excluding the poly-A tail. Nucleotide sequence identity in a 327-base region of the large coat protein gene varied by as much as 25% among 16 geographically diverse isolates of SMoV (87). Based on conserved nucleotide sequence in the 3' non-coding region, primers have been developed that allowed detection of these same 16 isolates with a single primer pair, a good tool for general detection (87).

**Strawberry vein banding virus (SVBV).** SVBV is the least common of the four major aphid-transmitted viruses of strawberry and was first described in 1955 (16). SVBV usually occurs sporadically and at a

low incidence in strawberry fields, but under extreme aphid pressure, the incidence can approach 100% in third-year fields (92). SVBV is vectored primarily by *Chaetosiphon* spp. in a semipersistent manner. It also can be transmitted by aphids in five other genera, but their efficiency of transmission is low. It is likely that *Chaetosiphon* spp. are the most important vectors of SVBV in the field; however, in situations where other less efficient vectors reach high populations, they could have an important impact on the epidemiology of the disease. In areas where *Chaetosiphon* spp. are not found, control with virus-free planting stock usually provides excellent control of this virus. SVBV was reported to reduce runner production, yield, and fruit quality in the United States in commercial fields of 'Marshall', 'Tioga', and more recently in 'Carlsbad'. Symptoms also developed in SVBV-infected 'Gaviota', 'Cuesta', 'Pacifica', and 'Selva'. However, in most cultivars grown currently, SVBV is symptomless. Mixed infections of SVBV with SCV or *Strawberry latent C virus* led to more severe losses; whereas interactions with SMoV, SMYEV, or Strawberry pallidosis associated virus(es) result in a mild disease (46).

*F. vesca* and *F. virginiana* indicator clones all develop symptoms when infected with SVBV; 'UC-6' and 'UC-12' are the most sensitive of the two indicator species. The intensity of symptom development varies depending on the virus isolate and the indicator used. Three types of symptoms are known to be caused by different strains of SVBV: (i) vein banding, (ii) leaf curl, and (iii) necrosis. Vein banding symptoms, seen as chlorotic banding along the primary and secondary veins, are most intense in the first few leaves that develop after grafting. Leaves that develop later may show discontinuous streaks along the veins, mild chlorosis along the veins, or no symptoms. The vein banding symptoms tend to be more severe with



Fig. 3. *Fragaria vesca* inoculated with full-length clone of *Strawberry mild yellow edge virus* (SMYEV) (left) and uninoculated (right), demonstrating that the strawberry mild yellow edge potexvirus is capable of causing typical symptoms of the disease.

isolates from the western United States compared with those from the eastern United States. Symptoms of necrosis may develop on mature leaves. The net veins may become necrotic, followed by necrosis of the interveinal tissues. During chronic infections, premature discoloration often appears on older leaves. In the United States, the necrosis symptom is more common with eastern than with western strains. The vein banding symptom is diagnostic; whereas leaf curl and necrosis may be induced by other disease agents. Symptoms may be more severe in combination with SCV or be masked in combination with SMoV or by high levels of nitrogen in the potting medium.

Natural infections of SVBV are known only in species of *Fragaria*, but it has been transmitted to *Sanguisorba minor* experimentally. No symptoms have been detected in infected clones of *F. chiloensis* infected singly with SVBV. In mixed infections with SCV, SMYEV, and SMoV, some clones of *F. chiloensis* show decline symptoms (Fig. 4).

SVBV is a member of the *Caulimovirus* genus in the family *Caulimoviridae*. Particles 40 to 50 nm in diameter have been isolated, and cytoplasmic inclusion bodies typical of caulimoviruses have been observed in leaf tissues of infected plants. The genome of SVBV is a double-stranded circular molecule of DNA about 7,800 bp in size, and it has been cloned (86) and sequenced (63). SVBV has recently been verified as the causal agent of the typical North American vein banding disease (45). The coat protein of the virus is highly conserved, and SVBV can be detected readily by PCR using primers in the coat protein

open reading frame (46,56,89). A second virus may be associated with vein banding in Europe, because some isolates do not hybridize with a cDNA clone of SVBV of a North American isolate (56).

**Strawberry pseudo mild yellow edge virus (SPMYEV).** SPMYEV was first reported in 1966 from the eastern United States and also has been reported from Japan (17,112). It is not clear how widespread it is in Japan, where it was isolated from commercial strawberry plantings (111). There is only one report from the United States, in which it was isolated in Minnesota from the indicator clone 'M-1' of *F. virginiana*. SPMYEV is transmitted in a semi-persistent manner by *Chaetosiphon* sp. and *A. gossypii*. The disease is of no known economic significance in the United States, and its importance in Japan is unknown.

Infected strawberry cultivars are symptomless. Graft-inoculated *F. vesca* indicator clones as well as *F. virginiana* 'UC-12' develop symptoms of SPMYEV; whereas inoculated *F. virginiana* 'UC-10' and 'UC-11' remain symptomless. *F. virginiana* clone 'UC-12' develops yellow to reddish coloration with necrotic areas in older leaves. All *F. vesca* clones show mottled discoloration (yellow to red) followed by premature necrosis. The symptoms of SPMYEV can be confused with mild strains of SMYEV. In such cases, leaflet grafting onto *F. vesca* 'UC-6' can be used to differentiate the two viruses.

SPMYEV is a member of the *Carlavirus* genus in the family *Flexiviridae* and is serologically related to *Carnation latent virus*, the type member of the genus. The virus has been purified from strawberry

and an antiserum developed (110). SPMYEV can be detected by ELISA or immuno-dot blots directly from strawberry tissue (109). Sequence information is not available for this virus or for Strawberry latent C (see below); therefore, these viruses cannot be detected by RT-PCR or other nucleic acid based tests at this time.

**Strawberry chlorotic fleck virus (StCFV).** Strawberry chlorotic fleck disease was identified in the 1960s in Louisiana (32), and was named for the symptoms observed on *F. vesca* and *F. virginiana* indicators. The disease had a significant impact on plant growth and fruit yield in 'Headliner' in Louisiana (32). The causal agent of the disease was transmitted by *Aphis gossypii*, the cotton aphid (32). The National Clonal Germplasm Repository (NCGR) in Corvallis, OR, maintains the only known isolate of strawberry chlorotic fleck. Using standard procedures for dsRNA extraction, cloning, and sequencing, this plant was found to be infected with a new virus in the genus *Closterovirus* in the family *Closteroviridae* and the two criniviruses associated with strawberry pallidosis disease (described in more detail in the whitefly transmitted viruses section). This new closterovirus has similarities in genome organization and sequence to *Citrus tristeza virus* (I. E. Tzanetakis and R. R. Martin, unpublished results) and other aphid-transmitted members of the *Closterovirus* genus. Studies are underway in our laboratory to separate these three viruses and verify aphid transmission of this closterovirus with strawberry and cotton aphids, respectively, and determine if this new virus is associated with the symptoms observed in chlorotic fleck infected *F. vesca* plants. An RT-PCR has been developed in our laboratory for this new closterovirus, and the incidence of the virus in commercial fields and its role in strawberry decline are currently under investigation.

**Strawberry latent C virus (SLCV).** Strawberry latent C (SLC) is a poorly described disease, first reported in 1942 (28). Electron microscopy studies on infected material have identified a nucleorhabdovirus associated with the disease (111), which was designated as *Strawberry latent C virus* (SLCV). The disease has been reported from eastern North America, and studies have verified that the causal agent can be transmitted by grafting and several aphid species (8). A wide range of symptoms has been attributed to SLCV, although it is difficult to rule out the possibility that some isolates may be due to mixed infections with other viruses. The importance of the virus is mainly due to synergism with other strawberry viruses. Grafting and aphid transmission to indicator plants are the only available methods today to detect SLC disease. Sensitive *F. vesca* clones such as 'EMC' and 'UC-5' develop symptoms that include epinasty and dwarfing; however, verification of



Fig. 4. *Fragaria vesca* exhibiting peacock pattern symptoms and downward curling of leaves. The leaves shown are from a plant infected with *Apple mosaic*, *Beet pseudo yellows*, and *Strawberry pallidosis* associated viruses.

SLC disease also requires grafting on 'UC-4', which shows no symptoms when graft-inoculated with SLCV; whereas it develops symptoms when inoculated with nine other viruses that infect strawberry. To our knowledge, there is not a reference isolate of SLCV available in North America to use for further characterization.

### Whitefly-Transmitted Viruses

Whitefly-transmitted viruses are an emerging problem in world agriculture, due to migration and naturalization of the whitefly vectors and movement of plant germ plasm. Members of the *Crinivirus* genus of the *Closteroviridae* family comprise one of the predominant groups of whitefly-transmitted viruses that have emerged in the last decade (72,107,109). Closteroviruses have long filamentous particles of 700–2,000 × 11–12 nm and comprise the group of plant viruses with the largest positive-sense single-stranded RNA genomes. Criniviruses have bipartite or tripartite genomes and are transmitted in a semi-persistent manner by whiteflies belonging to the *Trialeurodes* or *Bemisia* genera. Until recently, there were no criniviruses identified in strawberry or any of the other small fruit crops. Modern molecular techniques have allowed identification of new criniviruses in crops not known to be infected by members of the group (50,76), because the inefficient sap transmission of all the known members of the genus did not allow their identification with the limited tools of the past. Two closely related criniviruses have now been associated with pallidosis disease of strawberry: the newly identified Strawberry pallidosis associated crinivirus (SPaV) (93) and *Beet pseudo-yellows virus* (BPYV) (104).

**Strawberry pallidosis disease.** Strawberry pallidosis was identified in the 1950s in both Australia and the United States (20). The disease was thought to be indigenous to North America because the plants in Australia originated in the United States (20). Pallidosis (pale or pallid appearance) is a disease caused by graft-transmissible agent(s), causing symptoms that include marginal leaf chlorosis and stunting on *F. virginiana* clones ('UC-10' and 'UC-11'); whereas *F. vesca* plants remain asymptomatic. Pallidosis may also cause root and runner reduction (10). Anecdotally, the major effect on strawberry production is due to the synergistic effects of the pallidosis agent(s) with other strawberry viruses. Symptoms are masked during the summer months unless plants are heavily shaded.

Pallidosis was considered a rarity until a survey in Maryland, USA, demonstrated that the disease was the most widespread of the graft-transmissible strawberry diseases in that state, with more than 70% of the plants tested being infected with the causal agent(s) (48).

**Strawberry pallidosis associated virus (SPaV).** SPaV is latent in most commercial cultivars grown today and in the *F. vesca* indicator clones. Infected *F. virginiana* 'UC-10' and 'UC-11' develop small chlorotic leaves; runners may be shortened; and it has been reported that severe strains can be lethal. In our studies with pallidosis, we never encountered strains that were lethal to indicator plants. It is possible that these strains reported in the past were actually mixed infections of pallidosis and one or more other strawberry viruses.

Sap transmission to 24 plant species was unsuccessful in our laboratory. Studies with the whitefly vectors of criniviruses indicated that *Trialeurodes vaporariorum*, the greenhouse whitefly, is a vector of the virus (94). The host range of SPaV, as in the case of most criniviruses, is narrow, including members of *Fragaria* and related genera and a few weed species (91). The virus has been found in the major strawberry production areas of the United States, and studies are currently in progress in our laboratory to determine if the virus occurs in other states in the United States and in other countries.

Anecdotal reports suggested that the pallidosis agent may be pollen-borne (10). However, more than 400 and 170 plants were tested for seed and pollen transmission, respectively, and all the plants tested negative for SPaV, suggesting that the virus either is not pollen- or seed-borne, or if it is, that transmission occurs at very low levels (91). Alternatively, the pallidosis isolates used in the previous transmission studies may have been infected with BPYV, the second virus associated with pallidosis disease, or the plants used for these studies may have been contaminated by the greenhouse whitefly, which was not known as a virus vector at the time.

SPaV is considered to be the major agent associated with pallidosis in United States because more than 97% of the plants identified as pallidosis positive by grafting were infected with the virus (93). The complete sequence of the virus has been determined (103). SPaV has a bipartite genome, similar to that of other criniviruses. Both molecular and immunological detection tests for SPaV have been developed, but the low titer of the virus, especially during summer, makes antibody-based detection unreliable. Laboratory detection by RT-PCR correlates well with symptom development in indicator plants.

**Beet pseudo-yellows virus (BPYV).** Tzanetakos et al. (93) tested 38 strawberry plants that were pallidosis positive by grafting for the identification of the agent(s). All but one of these plants tested positive for SPaV by RT-PCR. DsRNA cloned from this single plant negative for SPaV revealed that it was infected with BPYV. BPYV, a crinivirus like SPaV, has

the widest host range of all criniviruses (109), and new hosts continue to be identified (97,108). The virus is vectored by the greenhouse whitefly and has been an emerging problem in temperate regions worldwide because of the expanding range of the vector (107). The increased range and high fecundity of the greenhouse whitefly suggest that the incidence of BPYV and SPaV in strawberry production may increase in the future.

Two isolates of BPYV have been sequenced completely, one from cucumber (then referred to as Cucumber yellows virus) (30) and the other from strawberry (96). The genome organization of the BPYV isolate from cucumber is similar to that of SPaV, but there are significant differences between the two BPYV isolates. The differences include the presence of an additional open reading frame in the 3' end of RNA 1 of the strawberry isolate as well as an insertion in the middle of the replication-related polyprotein. The differences may be host- or geography-dependent, but additional data is needed to determine the origin of the diversity. Both molecular and immunological tests have been developed for BPYV, but as in the case of SPaV, the low titer of the virus makes immunological tests for BPYV less suitable for routine detection.

### Nematode-Transmitted Viruses

Five nematode-transmitted viruses are known to infect strawberry. Four belong to the genus *Nepovirus*, in the family *Comoviridae*; whereas one, *Strawberry latent ringspot virus* (SLRSV), is a possible member of the genus *Sadwavirus* in the family *Sequiviridae*. Nepoviruses and SLRSV have spherical (icosahedral) particles of ~28 to 30 nm in diameter and are efficiently transmitted by members of the nematode genera *Xiphinema* and *Longidorus* as well as via pollen and seed. The strawberry nematode-transmitted viruses have wide host ranges and can cause significant losses in the crop (6,21,44,54,83), especially when present in mixed infections with other viruses. Many of these viruses are quite diverse at the nucleotide level, possibly a result of their adaptation to a diversity of hosts (38). This genetic diversity makes detection based on RT-PCR a challenging task, as oligonucleotide primers may not detect all strains. Antisera are available for each of these viruses, and detection by ELISA is possible, but again strain diversity can be a problem with this assay.

The use of methyl bromide and other soil fumigants in most strawberry producing areas has reduced the importance of the nematode-borne viruses in strawberry. However, the restrictions on methyl bromide and pressure to reduce use of other chemical fumigants may result in a re-emergence of these diseases in the future. Because the vectors of these viruses have

been controlled, the diseases they cause have been very rare since 1975. As a result, the reaction of strawberry cultivars grown today to these viruses is largely unknown. This brief review will focus on the data obtained recently on nematode transmitted viruses. Extended reviews of the biological properties of the viruses in strawberry can be found elsewhere (8).

**Tomato ringspot virus (ToRSV).** ToRSV was first identified in *F. chiloensis* along the California coast in 1961 (21). The virus has a host range that includes plants in 35 families of both dicotyledonous and monocotyledonous plants (83). It can be a serious problem in raspberry production in the Pacific Northwest of the United States, but has not been a serious problem in strawberry, although it has been reported in strawberry (7). This may be due to several factors, such as the short period of time that strawberry plants are in the field combined with the use of soil fumigants (68). In some cultivars, such as 'Olympus' or 'Puget Beauty', ToRSV infection can have a dramatic effect on plant growth and yield, although this is not common. ToRSV causes severe necrosis in *F. virginiana* clones 'UC-10' or 'UC-11' that can result in death of the plant. In *F. vesca*, the symptoms are generally a mild mosaic and sometimes a reddish discoloration of the young petioles and leaves, although symptoms are not distinctive and fluctuate depending on environmental conditions.

The genome of ToRSV is typical of nepoviruses and picorna-like plant viruses. The genome is divided between two monocistronic genomic RNA molecules that encode polyproteins that are processed to mature proteins by a cysteine protease encoded by RNA 1 (70). As in the case of all nepoviruses, the 5' ends have a genome-linked virus protein (VPg), while ToRSV has a very long untranslated region at the 3' terminus of the genomic molecules ending with a poly adenosine tail. RNA 2 encodes the movement and coat proteins of the virus (71). The virus belongs to subgroup C of the *Nepovirus* genus and is transmitted by *Xiphinema* spp. ToRSV can be detected serologically with ELISA and by RT-PCR; however, there are significant strain differences, and one must take care to ensure that an appropriate test is used. The virus can also be easily detected by mechanical transmission to *Chenopodium quinoa* and *Nicotiana glauca*, which avoids the potential problem of strain variation in detection.

**Strawberry latent ringspot virus (SLRSV).** SLRSV often is found in association with *Arabis mosaic virus* (ArMV) in Europe, where both viruses are transmitted by the same nematode vector, *Xiphinema diversicaudatum* (5). The symptoms on strawberry plants are unknown because plants showing symptoms in field situations also were infected with ArMV. Until

recently, SLRSV was thought to be primarily a European virus. However, it was identified recently in strawberry in California in the United States and in British Columbia, Canada, and in mint samples from Nebraska, Ohio, and Maryland in the United States (49,66). Strawberry plants in the United States that were infected with SLRSV were either symptomless, or when symptomatic, were infected with at least one additional virus. SLRSV has been reported in North America previously, but there was no evidence that it had established or become widespread (2).

SLRSV has a host range that exceeds 125 plant species belonging to 27 families of both monocots and dicots, and it is transmitted by nematodes of the genus *Xiphinema* (60). SLRSV is listed as a member of the genus *Sadwavirus* (51); however, the taxonomic status of this virus is in flux and may change in the near future now that the complete sequence has been determined (102). Phylogenetic analysis of the RNA-dependent RNA polymerase and helicase motifs indicate that SLRSV is closely related to *Apple spherical latent virus* and *Cherry rasp leaf virus*, members of the *Cheravirus* genus; whereas analysis of the coat protein sequence shows it is also related to the *Sadwavirus* genus and the *Comoviridae* family. Unlike the nepoviruses, which encode a single coat protein, SLRSV has two coat proteins, a feature found in the *Sadwavirus* genus. It is likely that the status of the viruses in the *Sequiviridae* family will change as more sequence information becomes available.

Because of the previous quarantine status of SLRSV, detection of the virus was not included in virus surveys until recently. The wide host range of the virus and its wide geographical distribution in mint in the United States suggest it should be included in strawberry certification programs and possibly in other vegetatively propagated crop certification programs. On the other hand, the ornamental mint that was found infected with SLRSV is widely distributed and probably has been in the ornamental trade in the United States for many years. Alternate vectors for SLRSV should be further investigated, based on its unusual sequence compared with the nepoviruses. SLRSV also can be pollen and seed-borne, thus care should be taken by strawberry breeders that the virus is not introduced into their programs via germ plasm.

**Arabis mosaic virus (ArMV).** ArMV was first described in the 1940s (79). It infects almost 100 plant species belonging to more than 28 families, causing significant losses in many crops (58). The virus was reported to be widespread in strawberry prior to the introduction of chemical control of the nematode vector. It has also been reported to occur in strawberry in Germany, Hungary, Ireland, Poland, and

the former USSR (58). Most cultivars do not exhibit symptoms when infected only with ArMV; however, in some cultivars ArMV infection can cause a chlorotic leaf mottle and mild to severe stunting or death of plants in the field. In the United Kingdom, ArMV and SLRSV are commonly found in mixed infections where they occur due to their common vector, although ArMV has not been observed in strawberry plants infected with SLRSV in the United States (I. E. Tzanetakis and R. R. Martin, unpublished data). Most *F. vesca* indicator clones are symptomless when infected with ArMV.

ArMV is a member of subgroup A of the *Nepovirus* genus and is transmitted in nature mainly by *Xiphinema diversicaudatum*, although there are reports of other *Xiphinema* species that can transmit the virus (90). The efficiency of transmission by the vectors is highly strain-dependent (4). The complete nucleotide sequence of ArMV (105,106) confirmed the close relationship of ArMV with *Grapevine fanleaf virus* (GFLV), another member of subgroup A.

**Raspberry ringspot virus (RpRSV).** RpRSV was first identified in the 1950s as the putative causal agent of the raspberry leaf curl disease (6). The virus has been found throughout Europe but in strawberry has been found only in the United Kingdom and the former USSR (61). The *F. vesca* indicator clones initially show a yellow blotching, but the symptoms fade. In some strawberry cultivars, leaves develop yellow blotching, ring spots, crinkling, stunting, and eventually plants may die. The symptoms on susceptible cultivars include chlorosis, blotches, and crinkling, while in the second season, plant recovery may be observed.

RpRSV also belongs to subgroup A of the *Nepovirus* genus and is transmitted by members of the genus *Longidorus*, whereas there is a report of RpRSV transmission by members of the genera *Paratrichodorus* and *Xiphinema* (90). RpRSV infects dicotyledonous and monocotyledonous plants belonging to 14 families (61). The virus sequence (13) reveals shared characteristics with members of the subgroup GFLV and *Tobacco ringspot virus* as well as the comovirus *Cowpea severe mosaic virus*; this indicates the uniqueness of the virus and a possible insight into the evolution of the *Comoviridae*.

**Tomato black ring virus (TBRV).** TBRV was identified in 1946 (59) and is widespread in Europe. In Europe, the virus is often found together with RpRSV because they are both vectored by the nematode *Longidorus elongatus*, although TBRV tends to spread more slowly in the field than RpRSV, possibly reflecting differences in efficiency of transmission. Symptoms in *F. vesca* indicator clones may vary from being asymptomatic to leaf

blotching. Symptoms often diminish after the first season, as in the case of RpRSV. Symptoms in strawberry cultivars are similar to those caused by RpRSV.

TBRV belongs to subgroup B of the *Nepovirus* genus. The host range of the virus is as wide as those of the other nematode-transmitted viruses. The complete nucleotide sequence of the virus has been determined and confirms a close relationship of TBRV with *Grapevine chrome mosaic virus* and *Cycas necrotic stunt virus* (39).

### Oomycete-Transmitted Virus

**Tobacco necrosis virus (TNV).** The symptoms of TNV that are usually observed on strawberry indicator plants are similar to those caused by SMYE and SPMYE viruses. TNV has been associated with dwarfing and leaf and root necrosis in indicator clones and commercial cultivars (14). Fránová-Honetšlegrová et al. (14,15) have verified that TNV strain D is able to replicate in strawberry, while there is no information on the pathogenicity of TNV strain A in strawberry. Information on the incidence of the virus in commercial fields is limited (81), because detection is problematic due to low titer of the virus in the aboveground tissues (R. R. Martin, *personal observation*).

TNV belongs to the genus *Necrovirus* of the family *Tombusviridae*, and is transmitted by the oomycete *Oplidium brassicae* (34). In the past, TNV was considered a single species with diverse isolates, but sequence data suggest that there are at least two individual TNV species (53,55,114). TNV has icosahedral virions that are 26 nm in diameter and encapsidate the monopartite single-stranded positive-sense genomic RNA. A satellite RNA often is associated with the viruses and results in a dramatic change in symptoms. TNV is persistent in the soil and has a wide host range. The virus can be detected by ELISA and RT-PCR, but due to strain variation, it is important to carry out sap transmission tests to *Chenopodium quinoa* to avoid false negatives with the laboratory-based assays.

### Viruses with Unknown Vectors

**Iilarviruses.** Three ilarviruses are known to infect strawberry: Strawberry necrotic shock virus, (SNSV), *Fragaria chiloensis latent virus* (FCILV), and *Apple mosaic virus* (ApMV). Iilarviruses comprise the largest genus of the *Bromoviridae* family and are positive-sense RNA viruses with tripartite genomes (73). They can be transmitted via pollen to maternal tissue (horizontal transmission, spread within a field or within a generation) and through seed (vertical transmission, spread from one generation to the next). Thrips have been reported to transmit some of the ilarviruses (75). Since these viruses can be pollen-transmitted, there is not an effective way to prevent movement within a field

once the virus is established.

**Strawberry necrotic shock virus (SNSV).** SNSV was first identified in the 1950s (19) and was named after the symptoms observed on *F. vesca* clones when grafted with infected material. Grafted plants develop symptoms 6 to 14 days after grafting, and some isolates cause a severe necrotic reaction in newly formed leaves. After the initial severe reaction that develops on 1 to 3 young leaves, the subsequent leaves appear normal, and no further symptoms develop. SNSV is seed-transmitted up to 35%. The virus can have a significant impact on strawberry production, reducing yield by up to 15% and runner production by up to 75% (36). Early studies indicated that SNSV, which also infected *Rubus* species, was a strain of *Tobacco streak virus* (TSV) (40), the type member of the *Iilarvirus* genus (84). However, several lines of evidence indicated that strains from *Fragaria* and *Rubus* differed from the type strain of TSV. In immunodiffusion tests, SNSV formed spurs with antisera developed against other TSV strains (24), an indication of differences in the epitopes between the strains. In addition, SNSV isolates failed to cross-protect plants from other strains of TSV (26), and there was no cross-hybridization between strawberry and blackberry isolates and non-small-fruit strains of TSV in Northern blot analysis (85).

Several attempts to use RT-PCR for detection of TSV in small fruit crops were unsuccessful using primers developed against the type isolate of TSV. An isolate from strawberry was cloned and sequenced, and analysis of RNA 3 and part of RNA 2 revealed about 70% nucleotide sequence identity with TSV (95). This indicated that the strawberry ilarvirus belonged to a different species, distinct from TSV. The coat protein gene from 15 "TSV" isolates from *Fragaria* and *Rubus* were sequenced, and analysis indicated that all clustered with the strawberry isolate that was sequenced, which was then given its original historic name, SNSV. None of the strawberry and *Rubus* plants were found infected with TSV (95), an indication that TSV may not be a pathogen of *Fragaria* or *Rubus* species.

***Fragaria chiloensis latent virus* (FCILV).** FCILV was identified in *F. chiloensis* plants that originated in Chile (82). *F. chiloensis* is found along the west coast of the Americas except for the tropics. Graft indexing for detection of FCILV is problematic because it remains symptomless in *F. chiloensis* and strawberry cultivars, and only causes mild symptoms when grafted onto *F. vesca* 'UC-4'. It can be sap transmitted to *Chenopodium quinoa* and *Cucumis sativus*.

Molecular and immunological tests have been developed, and the presence of the virus was confirmed along the west coast of North America in 2003 (92) but was not

detected in hundreds of samples tested in 2004. This difference is unexplained at this time. The complete nucleotide sequence of FCILV has been determined (98), revealing unique features in the genome organization of the virus. Some ilarviruses encode a gene involved in suppression of RNA interference or gene silencing in RNA 2. FCILV lacks the suppressor of gene silencing but has an open reading frame in RNA 2 that shares homology to eukaryotic receptors, whose function in this virus is unknown. FCILV also has an open reading frame downstream from the coat protein gene in RNA 3 that is not present in any other ilarviruses that have been sequenced. Phylogenetic analysis places FCILV with *Prune dwarf virus* in subgroup 4 of the genus and also reveals a close relationship with *Prunus necrotic ringspot virus*, *Apple mosaic virus*, and *Alfalfa mosaic virus*.

**Apple mosaic virus (ApMV).** Strawberry leaf roll disease has been reported in northeastern North America and Kazakhstan (3). In *F. virginiana*, symptoms include a peacock pattern on the leaves, whereas a downward rolling is observed on some *F. vesca* indicators (Fig. 4). The only isolate of strawberry leaf roll currently known is maintained at the National Clonal Germplasm Repository in Corvallis, OR, and it was used in an attempt to characterize the strawberry leaf roll disease. This plant exhibited the peacock pattern on the leaves (Fig. 5). After cloning and sequencing dsRNA extracted from this source, it was found to be infected with three viruses, one of which was ApMV. This is the first report of this virus naturally infecting strawberry (99). The other two viruses identified in this plant were the criniviruses SPaV and BPYV. ApMV is a typical *Iilarvirus* (77,78) and belongs to subgroup 3 of the genus. The virus has a broad host range that exceeds 65 species in 19 families (25) and has many diverse strains (11,64). ApMV is one of several viruses that have been transmitted experimentally to strawberry from tree fruits (23).

The association of the virus with symptom development is under examination because the plant infected with leaf roll was also infected with the two criniviruses associated with pallidosis disease. The lack of testing for ApMV during virus surveys means the distribution and impact of this virus in strawberry production areas are unknown. Future testing for ApMV will be included in virus surveys of declining strawberry plants to determine its incidence and impact.

### Unclassified Viruses of Strawberry

***Fragaria chiloensis cryptic virus* (FCICV).** During the molecular characterization of FCILV, several cDNA clones corresponding to a novel virus with homology to cryptic viruses and also to plant dsRNA polymerases were identified (100).



**Fig. 5.** *Fragaria chiloensis* near Contulmo, Chile, naturally infected with **Strawberry crinkle virus**, **Strawberry mottle virus**, **Strawberry mild yellow edge virus**, and **Strawberry vein banding virus**.

A large portion of the RNA polymerase of this novel virus, designated *Fragaria chiloensis* cryptic virus (FCICV), has been sequenced, and an RT-PCR detection test has been developed. In order to minimize the possibility that the dsRNA molecule is encoded by the plant genome, multiple DNA extractions from plants that tested positive for FCICV were carried out and tested by PCR without a reverse transcription step. In all cases, no amplicons were obtained in PCR, while the cryptic virus was readily detectable after RT-PCR using either total RNA or dsRNA preparations (100). A band of ~1.8 kbp was extracted from the dsRNA gels and was both cloned and subjected to RT-PCR. Both tests confirmed that this band belonged to this cryptic virus. More than 20 FCILV-free *F. chiloensis* plants were tested for FCICV, and several tested positive for the virus, indicating that FCILV is not needed for replication of this cryptic virus (100).

**Strawberry latent virus (StLV).** Before the identification of SMEYV as the causal agent of the SMYE disease (43), a luteovirus was thought to be associated with the disease. Many attempts were made to isolate and characterize that virus. Martin and Converse (47) purified a spherical virus of 25 nm diameter from SMYE diseased plants, but they also identified this virus in SMYE-free plants, including several *F. vesca* seedlings. They named the agent 'cryptic' virus, because it caused no obvious symptoms on cultivars or indicators.

In the effort to develop detection protocols for all strawberry viruses and graft-transmittable diseases, a plant used in the previous study was obtained from National

Clonal Germplasm Repository in Corvallis, and dsRNA was extracted and cloned. The virus, now designated Strawberry latent virus (StLV), may be a link between plant and insect viruses (101). The genome organization of StLV appears similar to members of the *Cripavirus* genus, *Dicistroviridae* family. The members of the family are devastating insect pathogens with single-stranded, positive-sense RNA genomes that consist of two open reading frames encoding the viral replicase and coat proteins of the virus.

The virus can be purified from strawberry, which proves that it replicates in the plant and that it is a plant virus rather than an insect virus found in a plant. The complete sequence of the virus will determine if the virus encodes a movement protein or if it is translocated in the plant in a manner similar to that of other cryptic viruses. A detection protocol for the virus has been developed, while future plans include studies on the effect of the virus when found in complexes with other strawberry viruses.

**Strawberry feather-leaf disease.** Strawberry feather-leaf disease was first reported in Arkansas in 1970, and has since been detected in a few plants from Maryland, Michigan, and Missouri (52). No information on the natural transmission of the pathogen is available, but it was reported that 1 to 9 months were required for symptom development in indicator plants. In *F. vesca*, symptoms include feather-leaf symptoms that include dwarfing, narrowed strap-like and somewhat rugose leaf deformations with deeply serrated margins and leaflets that may be fused at the base. Symptoms may be obvious to obscure and often are exhibited on

only part of a crown or only on some crowns in a plant with multiple crowns. When grafted onto *F. virginiana*, symptoms typical of pallidosis were observed. As far as we are aware, no known reference isolates of this disease are available for development of laboratory tests or characterization of the pathogen. The possibility of a mixed virus infection of several of the viruses described above causing the disease seems remote, due to the long incubation time that may be required for symptom development in indicator plants and the unique combination of symptoms.

**Strawberry decline.** Since 2000, strawberry decline disease has been increasing in the Pacific Northwest (PNW), including British Columbia, Washington, and Oregon, and also in California. All cultivars can develop symptoms of the disease. Previously, cultivars in the PNW, such as 'Totem' and 'Puget Reliance', did not develop symptoms due to virus infections in the field. Analysis of declining plants in this area showed that SCV was present in the symptomatic plants in addition to SMYEV, SMOV, and SVBV. In the past, only the latter three viruses were observed in northern Washington and British Columbia. Aphid populations were generally very high in this area either due to lack of control efforts, or because aphid control had become ineffective due to development of resistance to the aphicides that had been used for many years. No whiteflies were observed in the strawberry fields in PNW, and the incidence of BPVY and SPaV was less than 5% (92).

In California, where strawberries are grown as an annual crop, symptoms of decline are generally rare. However, in 2002 and 2003, decline symptoms were common in strawberry fruit production fields in California. Declining strawberry plants from California were almost always infected with one of the whitefly-transmitted criniviruses plus one or more of the aphid-borne viruses. The preliminary testing done on strawberry plants suggests that the whitefly-transmitted viruses are an important component in the decline observed in California, in contrast to the PNW where the aphid-borne viruses are primarily responsible. The virus test results are in agreement with observations on vector abundance in strawberry fields in the two locations. In California, whiteflies were very common on strawberries in production fields in the south and central coast areas, with several whiteflies commonly observed on each leaflet (Fig. 6). Aphids also were present in these areas, but at much lower numbers than observed in the PNW.

In a visit to Chile in 2004, decline symptoms were observed on *F. chiloensis* grown for commercial fruit production near Contulmo, Chile (Fig. 5). Upon testing for viruses, it was found that these plants were infected with SCV, SMOV,

SMYEV, and SVBV, but not with SPaV or BPYV. The symptoms were similar to those observed in *F. × ananassa* cultivars in the PNW that were infected with these four viruses (Fig. 2).

## Conclusions

Over the past 50 years, breeders have been effective at selecting for virus tolerance in commercial strawberry cultivars, and as a result, very few cultivars grown today exhibit symptoms when infected with a single virus. Due to high aphid numbers and virus spread, cultivars that have become important in the PNW are tolerant to infection with multiple aphid-transmitted viruses. The only exception to this is the cultivar 'Hood', which commands a premium price for its use in the "high end" ice cream market, and is grown despite its sensitivity to virus infection and requirement for stringent aphid control. Selection for improved virus tolerance will become a priority for breeders as virus complexes become more common in the future due to reduced pesticide use, increased resistance to insecticides by vectors, and increased demand for organically grown fruit. Resistance to aphid- and whitefly-transmitted viruses in strawberry has not been reported in the *Fragaria* germ plasm used by breeders, which suggests that breeding for resistance in not an immediate option. However, the use of genetic engineering to produce virus resistant strawberry cultivars should be quite straightforward.

Strawberries are vegetatively propagated, and in most cases, the increase from nuclear stock to certified plants that are sold for fruit production takes place in the field, where there is potential for reintroduction of viruses. In the past, efforts during plant production have concentrated on controlling nematode and aphid vectors. The nematodes have been controlled efficiently with methyl bromide and other soil fumigants, and aphids through the use of insecticides, although development of resistance to the chemical insecticides has been a problem. With the discovery of whitefly-transmitted viruses that infect strawberry, attention must now be directed toward the control of whiteflies. The greenhouse whitefly has a very broad host range and recently has become naturalized in the strawberry growing areas of California and the southern United States (107). During the 2002 and 2003 growing seasons, incidences of plant infections with SPaV and BPYV infection reached as high as 90% in southern California strawberry fields, and over 70% in the Watsonville area along the central coast of California. In mixed infections with several of the aphid-borne viruses, whitefly-transmitted viruses caused a serious decline (92) (Fig. 1). In field visits in 2003, it was noted that whiteflies were present on most strawberry leaflets (Fig. 6), and this is consistent with



**Fig. 6. Strawberry near Watsonville, CA, with greenhouse whiteflies on the under surface of a leaf. Whiteflies have only recently become important pests of strawberry in California.**

the high infection rates with BPYV and SPaV.

With the recent advances in virus detection, it is now possible to quickly identify viruses in field plants, whether in single or multiple infections. These newly developed tests are being adopted in the certification programs in the United States. In addition, these technologies are being used to monitor nurseries at each stage of multiplication to determine if and when viruses might be introduced into the plant production scheme. Visual inspection is very inefficient in nurseries since most of the strawberry viruses do not cause any symptoms when they occur in single infections. Additionally, a coordinated effort is underway to validate the molecular tests that are now available for the detection of viruses in strawberries. The tests are being used in several laboratories to determine if they will detect a broad range of virus isolates. The development of specific RT-PCR tests for almost all of the known strawberry viruses allows for a re-evaluation of severe and mild strains reported in the literature for many of the strawberry viruses, to determine whether severe strains are actually mixed infections that were not separated by aphid transmission studies.

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