

Title: Survey for Strawberry pallidosis disease and effect of these viruses in mixed infections (and some new viruses in strawberry in North America).

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During 2004 we sampled declining strawberry plants along the west coast from southern California north to the Fraser Valley in B.C., Canada. The most surprising result was the common occurrence of strawberry crinkle virus in northern Wash. and B.C., which had not been observed in strawberry fields in the Fraser Valley in the 1980's and early 90's when I was located in Vancouver. Also, it had not been observed in the 1960's or 1970's in that area (personnel communication with Dr. Frances Mellor, my predecessor in the Vancouver lab). In addition to strawberry crinkle, we did see more strawberry veinbanding than we had seen earlier. Strawberry mottle and strawberry mild yellow edge virus were both at high levels as we had seen earlier. We saw decline symptoms in 'Totem', 'Puget Reliance', 'Puget Summer' and 'Rainier' strawberries in B.C. Previously, these cultivars did not exhibit decline symptoms due to virus infection. The incidence of the above viruses was lower in Ore. and southern Wash. but the same viruses were associated with declining plants in this area.

The plants exhibiting the severe decline symptoms tested positive for three or four of the viruses, Strawberry mottle, strawberry crinkle, strawberry mild yellow edge and in most cases strawberry veinbanding. The reason for the high incidence of strawberry crinkle is not clear. In previous studies it was found that under ideal conditions it took aphids about 20 days after feeding on an infected plant before it could transmit the virus. Under cooler conditions this 'latent period' increased and it was thought that in northern areas the aphids died before they were able to transmit the virus. It is not clear at this point if we have a new strain of the virus or a new biotype of the aphid that result in transmission in less time such that aphids transmit strawberry crinkle before they die. The other possibility is that we are having warmer summers and the same virus and aphids that we had previously now able to spread and cause disease. We did not find strawberry pallidosis (SPaV) or Beet pseudo yellows virus (BPYV) in the material from Ore., Wash. or B.C. in 2004.

We have tested nursery material from California (fall of 2003 and summer of 2004) of the above cultivars and have not obtained any plants positive for strawberry crinkle virus, so it does not look like the virus is coming in on planting stock. We are doing more testing this fall (2004) on nursery material from California of the Northwest cultivars. The fall test results from the nurseries have not been completed yet.

In contrast the white fly transmitted viruses were the most common viruses in production fields in California, though the incidence in 2004 was much lower than in 2003. The situation in B.C. appears to be primarily caused by the aphid-borne virus

complex, though the occurrence of a new virus in the Pacific Northwest has not been ruled out and will be investigated further in 2005.

In California, the first year of the project we collected samples for virus testing from production fields in the Watsonville area and the south coast area of California and from low and high elevation nurseries. The nursery collections were done in early October trying to select a time that would be suitable for collecting at both locations. As it turned out the plants at the high elevation nurseries were going dormant and the low elevation nurseries were still very hot and the collection time was not optimal for either location. The preliminary test results from the nursery tests were not reliable and nursery testing was redone in 2004 as indicated above (testing still in progress).

In addition to testing for SPaV, BPYV and the aphid-borne viruses, we extracted dsRNA from declining plants and did random cloning and sequencing to look for viruses not known to infect strawberry in the USA. In the latter part of the project we identified two viruses not known to occur in the US, *Strawberry latent ringspot virus* (SLRSV), a European virus, and *Fragaria chiloensis latent virus* (FCILV), a virus only known in Chile. We then developed PCR tests for these two viruses and included them in the testing of declining plants and in subsequent survey work (Table 1). In addition, we identified another crinivirus similar to SPaV and BPYV in strawberry from Maryland and California.

We have developed RT-PCR based tests for each of the above viruses. In the testing that was done we found that SPaV is best detected in the late winter and spring before temperatures get too high when using RT-PCR. We are now investigating using nested PCR for the detection of SPaV throughout the year to see if we can detect the virus year round.

We did get a few samples for strawberry from Florida in 2004 and detected Beet pseudo yellows and strawberry mild yellow edge virus. The number of samples was limited so this probably does not represent a true picture of the virus situation in Florida. In samples previously collected in Maryland we did find, SPaV, BPYV, strawberry mild yellow edge virus and strawberry necrotic shock virus.

Table 1. Survey results from strawberry production fields (2003)*.

| Virus | Watsonville area | South Coast |
|--------------|-------------------------|--------------------|
| SPaV | 75% | 90% |
| BPYV | 60% | 20% |
| SMYEV | 20% | - |
| SCV | 20% | - |
| FCILV | 65% | - |
| SLRSV | 20% | - |

*The incidence of viruses in strawberry production fields in California was much lower in 2004 than in 2003. White fly numbers were also much lower, not sure if this is due to better control or due to environmental conditions.

Transmission and Host Range: Initial studies focused on identifying a vector associated with transmission of SPaV. It was already known that BPYV is transmitted by *T.*

vaporariorum. Several transmission experiments were conducted over the course of the year, with three resulting in sufficient transmission. Those three experiments are listed below in Table 2. All experiments were set up the same way. Whiteflies were fed on SPaV-infected strawberry plants for 48 hours to assure acquisition of virus by the vector. Whiteflies were then transferred to leaf cages and attached to SPaV-susceptible test plants (*Physalis wrightii* or *N. benthamiana*). After an additional 48 hours cages were removed, and insects killed with contact insecticide Resmethrin (PT-1200) (Whitmire). One week post-inoculation the inoculated leaf was removed to prevent any nymphs from maturing. Plants were transferred to the greenhouse and maintained for 6 weeks. At 5 weeks post-inoculation nucleic acid was extracted from a mature, pre-senescent, non-inoculated leaf. RT-PCR was conducted with SPaV-specific primers for the presence of SPaV. Results are presented in Table 2, below.

Table 2. SPaV is transmitted efficiently by the greenhouse whitefly (*T. vaporariorum*).

| Host Plant inoculated | GHWF ¹ | BWWF ² | SLWF ³ |
|--------------------------------|-------------------|-------------------|-------------------|
| <i>P. wrightii</i> (Test 1) | 3/3 | 0/3 | 0/3 |
| <i>N. benthamiana</i> | 3/3 | 0/3 | 0/3 |
| | | | |
| <i>P. wrightii</i> (Test 2) | 2/3 | 0/2 | 0/6 |
| <i>N. benthamiana</i> | 3/5 | 0/4 | 0/12 |
| | | | |
| <i>N. benthamiana</i> (Test 3) | 6/6 | 0/6 | 0/3 |
| | | | |
| TOTALS | 17/20 | 0/18 | 0/27 |

1. GHWF = Greenhouse Whitefly (*Trialeurodes vaporariorum*)
2. BWWF= Banded Wing Whitefly (*T. abutilonea*)
3. SLWF = Silver Leaf Whitefly (*Bemisia tabaci* biotype B or *B. argentifolii*).

These results confirmed that *T. vaporariorum* is the principal vector of SPaV. This vector is prevalent in and near strawberry fields in many areas along the US West Coast. Levels of this vector coincided with high levels of pallidosis associated disease in 2003 near Watsonville. Additional studies currently in progress are examining the amount of time the vector must feed on strawberry to acquire and transmit the virus from plant to plant. Separate experiments are examining the incidence of both SPaV and BPYV in weeds, and attempting to determine a more complete knowledge of the host range of SPaV. Studies conducted previously have determined the host range of BPYV (Duffus, 1965). To date, results indicate SPaV infects numerous *Fragaria* species, as well as nettle (*Urtica californica*), Malva (*Malva parviflora*), *Nicotiana benthamiana*, *Nicotiana clevelandii*. Additional local weed species are being tested.

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