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# Immigrating Aphid Species and their Importance as Vectors of *Passionfruit woodiness virus* in the Fields of Amami Oshima Island, Japan

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## Summary

At least ten or more species of aphids were trapped in orchards of hybrid passionfruit (*Passiflora edulis* x *P. edulis f. flavicarpa*) on Amami Oshima Island in Kagoshima Prefecture, Japan. The sowthistle aphid (*Hyperomyzus lactucae*), an inhabitant of milk thistle (*Sonchus oleraceus*) grass around orchards, was newly found to have a particularly high efficiency of transmission of PWV-AO (Amami Oshima isolate). The green peach aphid (*Myzus persicae*) and the cotton aphid (*Aphis gossypii*), already known as PWV-vectors abroad, also transmitted the virus though less than the sowthistle aphid.

**Key words:** *passionfruit woodiness virus*, sowthistle aphid, aphid transmission, potyvirus, passionfruit

## Introduction

In 1986, on Amami Oshima Island in Japan, some plants of a hybrid cultivar of passionfruit (*P. edulis* Sims. x *P. edulis* Sims. f. *flavicarpa* Deg., lately registered as "Summer queen") showed systemic mosaic and rugose foliar symptoms and produced severely malformed and woody fruits. Iwai et al. [2] showed that the disease was caused by an isolate of the *Passionfruit woodiness virus* (PWV), which was named PWV-AO for convenience sake. PWV-AO had been transmitted by the green peach aphid (*Myzus persicae* Sulzer) and the grafting process. In the 1980s, a species, the purple passionfruit (*P. edulis* Sims.) was cultivated mainly for processing of the fruits. The damage caused by PWV was not so serious because the purple passionfruit was propagated by seed, and PWV is not seed-transmissible. However, after 1992, hybrid passionfruits for the fresh market replaced this species, and by 1997 the virus had spread to various regions of the island [3]. The distribution of infected shoots is responsible for the geographic spread of the disease, while virus transmission between orchards or trees may be attributable to aphid vectors. However, on Amami Oshima Island,

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the common aphid species, including green peach aphid and cotton aphid (*Aphis gossypii* Glover), have not been found to form colonies on passionfruit trees. Thus, we supposed that winged-type aphids arbitrarily arriving from outside the orchards were responsible for the transmission of PWV. In this report we carried out a field survey to determine which species of aphids is responsible for PWV transmission in the field.

## Materials and Methods

### A survey of aphid species in orchards

Two orchards on Amami Oshima Island, one in Naze-City and the other in Tatsugo-Town, were selected as the survey spots. From May to December 2000, we set out yellow pan traps (plates having a size of 600 mm-diameter and 75mm-depth, painted in bright yellow equivalent to the JIS standardized 5Y, 8.5, and filled with soapy water) at the entrance of the orchards. The aphids were collected every one to two weeks and observed under the stereomicroscope. Some specimens were preserved for the detailed identification of species.

### The double antibody sandwich-enzyme-linked immuno-sorbent assay (DAS-ELISA) for detecting the PWV-AO

The PWV-AO particles were purified from infected "Summer queen" (SQ) leaves (200g) as previously described [2]. Antiserum to PWV-AO was prepared in New Zealand white rabbits by injecting a total of 2.5mg purified virus. The animals received a series of three intramuscular injections of 0.5mg virus in adjuvant followed by two intravenous injections of 0.5mg virus, at intervals of 7 days. Seven days after the final injection the animals were exsanguinated to obtain antiserum. The titer of the antiserum was 1:512 in the micro precipitin test against the purified virus (100$\mu$g/ml). The ELISA procedures followed those described by Clark and Adams [1]. The optimal concentration of $\gamma$-globulin for coating plates was 2.5$\mu$g/ml; the optimal dilution of conjugate was 400. The minimum concentration of purified PWV for detection by ELISA was 16ng/ml.

### The potential of PWV transmission of the prime aphids found in orchards

The capacities for PWV-transmission of the prime aphids found in orchards were investigated. As the PWV-source, a PWV-AO infected SQ was employed. Twigs of SQ having one node and leaf were planted in the seeding mold "YOSAKU N-100" (horticultural bed ingredients of vermiculite and peat moss and enriched with fertilizer) (Chisso-Asahi Fertilizer Co., Ltd.). One month after planting, rooted twigs were transplanted to a soil mix of clay loam and compost at a ratio of 9:1. Six months after being transplanted, various aphid species were allowed access to the leaves of young plants showing leaf mosaic symptoms.

The following winged and non-winged wild aphid species were collected from their respective host plants: corn leaf aphid (*Rhopalosiphum maidis* Fitch) from maize (*Zea mays* L.), cotton aphid from dasheen (*Clocasia esculenta* (L.) Schott), sowthistle aphid (*Hyperomyzus lactucae* L.) from milk thistle (*Sonchus oleraceus* L.), green peach aphid from cabbage (*Brassica oleracea* L. var. *capitata* L.), mealy plum aphid (*Hyalopterus pruni* Geoffroy) from Japanese plum (*Prunus salicina* Lindley), spiraea aphid (*Aphis spiraecola* Patch) from Tankan (*Citrus reticulata* Blanco var. *tankan* (Hayata) H H Hu), turnip aphid (*Lipaphis erysimi* Kaltenbatch) from Japanese radish (*Raphanus sativus* L. var. *macropodus* Makino), and udofutao aphid (*Cavariella araliae* Takahashi) from Japanese angelica tree (*Aralia elata* (Mig.) Seem.). Among these eight species, only non-winged aphids were used for mealy

plum and spiraea aphids.

For PWV inoculation by non-winged aphids, aphids collected from the field were starved for at least 2 hours, fed on the infected leaves for 30 to 60 sec, then transferred to healthy SQ plants confirmed not to have PWV by DAS-ELISA. For each of five treatments, five non-winged aphids were fed on one healthy plant at the 4th to 5th leaf stage. The leaves were covered by a disposable plastic petri dish, 90 mm in diameter, with a hole cut at the edge for the petiole to pass through, and the plants and aphids were incubated in the growth-chamber at 25℃. For PWV inoculation by winged aphids, twenty aphids per plant were fed on two plants; for each of two treatments, infected and healthy plants were incubated together with aphids in boxes with mesh-covered windows under greenhouse conditions. After incubation for 5 days, the plants were removed from the dishes and boxes, released from the insects, and incubated another 25 to 35 days in a glass-cage house (nursery house having glass ceiling and wire netting-screened wall). Newly grown leaves were tested by DAS-ELISA.

## Results and Discussion

As shown in Table 1, over 10 species of aphids were present on the island in both spring and autumn, with larger populations in the spring. They included corn leaf aphid, cotton aphid, sowthistle aphid, green peach aphid, mealy plum aphid, spiraea aphid, turnip aphid, and udofutao aphid. Setokuchi [8] reported that the main aphid species arriving in the fields of sugar cane - the most important crop in the Amami islands - are cotton aphids, sowthistle aphids, green peach aphids, and turnip aphids, all of which show high populations in spring and autumn. The result of our survey showed the same tendencies, but the populations in the passionfruit orchards were much smaller than those in the sugar cane fields, and the most frequent species were mealy plum aphids. This distinctive tendency might reflect aspects of the plant habitat around orchards surrounded by hills.

Table 1. The number of aphids trapped by the yellow pan traps in the passionfruit orchards

| | Number of aphids trapped[a] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Aphid species | May | Jun | July | Aug | Sep | Oct | Nov | Dec | Total |
| *Aphis gossyppi* | 26[b] | 2 | 0 | 0 | 2 | 4 | 0 | 2 | 36 |
| *Aphis spiraecola* | 9 | 6 | 0 | 0 | 5 | 1 | 0 | 0 | 21 |
| *Cavariella araliae* | 22 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 22 |
| *Hyalopterus pruni* | 99 | 39 | 0 | 0 | 1 | 0 | 0 | 0 | 139 |
| *Hyperomyzus lactucae* | 18 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 22 |
| *Lipaphis erysimi* | 12 | 1 | 0 | 0 | 0 | 0 | 3 | 0 | 16 |
| *Myzus persicae* | 16 | 14 | 0 | 0 | 0 | 1 | 0 | 0 | 31 |
| *Rhopalosiphum maidis* | 1 | 1 | 0 | 0 | 34 | 4 | 0 | 0 | 40 |
| Others[c] | 51 | 30 | 2 | 0 | 8 | 13 | 4 | 2 | 110 |
| Total | 254 | 93 | 2 | 0 | 50 | 27 | 7 | 4 | 437 |

a) Trapped aphids were sampled at intervals of 1 to 2 weeks.
b) Figures indicate the sum of the total numbers of aphids trapped at two orchards, one in Ashikebu, Naze-City, and the other in Tatsugo, Tatsugo-Town.
c) These species were excluded from consideration because the eight-month totals were negligible, ranging from 1 to 6 in number.

As shown in Table 2, aphid species that transmitted PWV included the cotton aphid (1/5 by

non-winged and 2/2 by winged aphids), sowthistle aphid (3/5 by non-winged and 2/2 by winged aphids), and green peach aphid (0/5 by non-winged and 2/2 by winged aphids). The green peach aphid, previously reported to be a vector for PWV [2], was not as consistently efficient as the other two aphid species; in all five cases, application of non-winged green peach aphids failed to transmit the PWV-AO. On the other hand, the corn leaf aphid, mealy plum aphid, spiraea aphid, turnip aphid and udofutao aphid did not transmit the virus. Cotton, green peach and potato aphids have been reported to be vectors for PWV [4, 6]. As a result of our experiment, the sowthistle aphid was added to the list of PWV vector aphids. Cotton, green peach and potato aphids are colonized in many plant species including the families of Cruciferae, Solanaceae, Cucurbitaceae and Compositae [5]. In contrast, the sowthistle aphid has a narrow host range in compositae, predominantly inhabiting the grass of milk thistle [5]. Milk thistle is quite common all over the world, and is found abundantly in and around the passionfruit orchards on Amami Oshima Island. As we expected, it was revealed that the sowthistle aphids actively colonized the milk thistle surrounding the passionfruit orchards and were PWV carriers.

Table 2. Transmission of PWV by aphids in passionfruits

| Aphid species | No. of infected plants | |
|---|---|---|
| | non-winged adult insects[a] | winged adult insects[b] |
| *A. gossyppi* | 1/5 | 2/2 |
| *A. spiraecola* | 0/5 | -[c] |
| *C. araliae* | 0/5 | 0/2 |
| *H. pruni* | 0/5 | - |
| *H. lactucae* | 3/5 | 2/2 |
| *L. erysimi* | 0/5 | 0/2 |
| *M. persicae* | 0/5 | 2/2 |
| *R. maidis* | 0/5 | 0/2 |

a) For each of five treatments, five aphids were fed on the PWV-AO infected plant for 30 to 60 sec, then transferred to a healthy plant for five-day incubation. Twenty-five to 35 days after the treatment, PWV infection in the inoculated plant was detected by DAS-ELISA and symptomatology.
b) For each of two treatments, twenty aphids were applied to boxes including a diseased and a healthy plant for five-day incubation. Infection was detected by methods described above.
c) Not tested.

In the preliminary survey, we could not observe any aphid colonies on the passionfruit plants in the orchards. However, recently we recognized that the spreading of PWV in the orchards was related to the direction of the wind, blowing from the sea to inland [7]. The details of this phenomenon are still being investigated. In any case, based on the high efficiency of PWV transmission by sowthistle aphids shown in Table 2, we can conclude that the winged-type sowthistle aphids, which appear to be transported to the passionfruit orchards from the surrounding grassy areas of milk thistle by the wind, play an important role in the spreading of PWV originating from the introduction of a few infected scions.

We reported previously that healthy passionfruits planted in November began to show viral symptoms in April of the next year and that the virus had spread throughout the orchard by July [7]. There are no reports for the period of latent infection with aphid-transmitted PWV, but based on the results of this experiment, we predict that the spring and fall, and especially the former, are the influential infection times. The use of shade nets or insect screen nets over the trees and along the sides of the orchards in early spring, or a plastic greenhouse, might be effective measures to

protect against aphids and thereby to prevent the woodiness disease.

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