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Author(s)	Yuzaburo, Imai
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STUDIES ON THE TRANSMISSION OF BROAD BEAN MOSAIC

BY

YUZABURO IMAI

(今井 勇三郎)

It is a well known fact that insects play an important rôle in the dissemination of most virus diseases of plants. In our country the evidence that dwarf disease of rice plant is transmitted by the leaf hopper, *Nephotettix apicalis* MORSCH. var. *cincticeps* UHL. has been shown as early as in 1906 by the Imperial Agricultural Experiment Station and likewise by the Shiga Agricultural Experiment Station. This is the first virus disease of plants shown to be transmitted by an insect. KURIBAYASHI⁽¹⁾ (1926) has reported that bean mosaic is disseminated by the aphid, *Macrosiphum solani* KALT. as well as by the red spider. Recently he ⁽²⁾(1931) found that the leafhopper, *Delphacodes striatellus* FALL. transmits the stripe disease of rice plant. No other evidence has been shown to indicate the possible relationship between certain insects and virus diseases of plants in Japan. This paper has been prepared for the purpose of recording the experimental transmission of broad bean mosaic by three species of aphids, *Aphis laburni* KALT., *Acyrthosiphon pisi* KALT. and *Rhopalosiphum persicae* SULZ.

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Symptoms of broad bean mosaic

BÖNING⁽³⁾ (1927) described in detail the symptoms of broad bean mosaic, stating that there are two distinct types of disease, "Marmoriermosaik" and "Nervenmosaik". The symptoms of the disease under consideration closely resemble those of "Marmoriermosaik."

(1) KURIBAYASHI, K.—On the seed transmission of bean mosaic. (in Japanese) Journ. Pl. Protect. 13: 199-210. 1926

(2) ———, Studies on stripe disease of rice plant. (in Japanese) Nagano Agr. Exp. Sta. Bull. 2: 45-69. illust. 1931

(3) BÖNING, K.—Die Mosaikkrankheit der Ackerbohne (*Vicia faba* L.). Forsch. a. d. Gebiet Pflanzenkr. 4: 43-111. illust. 1927

The most remarkable features of the disease are mottling on the foliage and the dwarfness of the plant as in the other mosaic diseases of plants. The first symptom to be discerned is a slight clearing of the veins or numerous clear spottings on the leafblade. Subsequently light green areas appear along the veins gradually turning into yellowish green while dark green portions which are irregular in shape develop adjoining the light green areas and become bluish green. The dark green portions sometimes swell up producing blisters scattered on leaflets whereas the yellowish green areas on the newly developed leaves are markedly suppressed from growth and remain unthickened. Affected leaflets are smaller and narrower than the normal ones. Curling down, wrinkling and waving of the margin of the leaflets are generally observed on the leaves in advanced stages of the disease. Sometimes the point of the leaflet is divided into two portions and other malformed leaves frequently appear in mosaic plants. The young shoots and leaves which developed after infection invariably show the symptoms, while the older leaves which have almost completed their growth before infection appear entirely normal. The petioles of affected leaves are as long as the normal ones but occasionally shorter than normal. Mottling develops also on the stipules. The growth subsequent to infection is much arrested, the plant becomes remarkably stunted, the internodes shortened, and the succeeding shoots and leaves remain slender and small, while the axillary buds which should normally remain dormant grow into numerous diminutive shoots.

Transmission of broad bean mosaic by *Aphis laburni* KALT.

On June 30, 1932, the aphids, *Aphis laburni* KALT. were allowed to feed upon a broad bean plant affected with the mosaic disease. Two days later some of these aphids were transferred to a healthy broad bean plant enclosed in an insect proof cage. In two weeks the plant began to show the typical mottling of mosaic on the foliage. On July 3, certain aphids from the same culture as above were transferred to two healthy broad bean plants. In this case, too, infection was produced in one of these plants two weeks after the transfer of the aphids.

Accordingly a series of transmission experiments were carried out with this species of aphid. Five aphids reared on diseased broad bean plants were transferred to each young broad bean plant enclosed in an insect proof cage. Newly born offspring of the aphids were removed with a camel's hair brush every day in the first week and later every other day. In two weeks all the aphids on the plant were removed and killed. Seven out of 10 inoculated plants contracted the mosaic disease. In the other series of experiments the

aphids were allowed to feed upon healthy broad bean plants for 1 to 7 days. One hundred and twenty plants in all were infested with the aphids reared on diseased plants and 32 of them became diseased. The infective aphid was able to transmit the disease to a healthy plant after one day's feeding and the incubation period of the virus in the plant averaged 15 days under the writer's conditions.

The attempts to transmit the disease to beans, garden peas and sweet peas by this aphid were successful, 10, 6 and 12, respectively out of 30 inoculated plants having contracted the disease. The details of the experimental results and the symptoms developed on these plants will be reported in another publication. All the attempts to transmit the disease to lucerne, Adzuki beans and soy beans failed. The parthenogenetically produced progeny of viruliferous aphids could not transmit the disease showing that the infective principle of the broad bean mosaic is not "hereditary."

Transmission of broad bean mosaic by *Acyrtosiphon pisi* KALT.

On May 7, 1932, Mr. K. KAWAI brought the writer some broad bean plants affected with mosaic disease which had been collected in the Experimental Fields of the Imperial Agricultural Experiment Station at Nishigahara, Tokyo. The aphids, *Acyrtosiphon pisi* KALT. (= *Macrosiphum pisi* KALT.) began to appear on these plants in a few days. These aphids were transferred to healthy broad bean plants in order to test their ability to transmit the disease. In two weeks one of these plants began to show faint mosaic symptoms which subsequently became more and more pronounced. A series of transmission experiments were, therefore, carried out with this aphid in the same way as in the case of *Aphis laburni*. Thirty-eight plants in all were infested with the aphids bred on diseased broad bean plants and infection was secured in only 4 of them. It seems that this aphid transmits broad bean mosaic less readily than *Aphis laburni* KALT. Thirty plants of sweet peas were also inoculated by means of the aphid and 4 of them contracted the disease.

Transmission of broad bean mosaic by *Rhopalosiphum persicae* SULZ.

In the course of these experiments it happened that some individuals of the peach aphid, *Rhopalosiphum persicae* SULZ. which were abundant on tobacco plants in the green house migrated to the broad bean plants affected with mosaic disease. Some of these aphids were found feeding upon healthy garden pea plants which had been placed by the mosaic broad bean plants. In about two weeks the garden pea plants began to show the symptom of mosaic. Con-

sequently it was supposed that some aphids which had picked up the virus migrated to garden pea plants producing infections in these plants. A series of experiments were carried out in order to obtain more definite evidence to indicate it. Throughout three experiments 30 broad bean plants were subjected to the infestation of the aphids reared on the mosaic broad bean plants and 13 of them became infected, the percentage of infection amounting to 43 %.

Artificial inoculation with the juice of mosaic broad bean plant

Thirty grams of the leaves and stems of mosaic broad bean plants were thoroughly ground in a sterilized mortar adding 20 cc. of sterilized water and filtered through two thicknesses of thin cotton cloth. A large drop of the inoculum thus prepared was placed with a sterile scalpel on a leaf axil and with a sterile needle about 30 pricks were made through the fluid. Two inoculations were made on each plant on the axils at the basal part of the stem. Sixty young broad bean plants were thus inoculated by needle pricks. In 6 to 15 days after inoculation 20 plants showed the symptom of mosaic. The other plants remained healthy until the experiments had been finished. Fourteen check plants which had been pricked with a sterile needle were also free from the disease.

The attempts to infect young broad bean plants with the juice of crushed viruliferous aphids gave negative results.

Discussion and conclusion

As a result of these experiments it was ascertained that broad bean mosaic was transmitted by three species of aphid, i. e. *Aphis laburni* KALT. (= *A. rumicis*), *Acyrtosiphon pisi* KALT. (= *Macrosiphum pisi*) and *Rhopalosiphum persicae* SULZ. (= *Myzus persicae*). BÖNING⁽¹⁾ (1927) was the first to prove experimentally that certain aphids are responsible for the dissemination of broad bean mosaic. According to him *Aphis fabae* (= *A. rumicis*), *Macrosiphum pisi* and *Rhopalosiphum viciae* transmit it. It was subsequently confirmed by MEULEN⁽²⁾ (1928) and MERKEL⁽³⁾ (1929). The present writer found that the peach aphid, *Rhopalosiphum persicae* also acts as an insect vector of the mosaic disease of the broad bean. It appears that these species of aphids are unequally endowed with the

(1) loc. cit.

(2) MEULEN, J. G. J. VAN—Voorloopige onderzoek naar de specialisatie en de infectiebronnen der mosaikziekten van landbouwgewassen. Tijdschr. Pflanzenziekt. 34 : 155-176. 1928 (original not seen)

(3) MERKEL, L.—Beiträge zur Kenntnis der Mosaikkrankheit der Familie der Papilionaceen. Zeitschr. Pflanzenkr. 39 : 289-347. illust. 1929

ability to transmit the disease, *Aphis laburni* being capable of transmitting the disease most easily, *Rhopalosiphum persicae* the next, and *Acyrtosiphon pisi* least readily. In this connection it is noteworthy that *Aphis laburni* multiplies itself most readily on the broad bean plant, *Acyrtosiphon pisi* the next and *Rhopalosiphum persicae* is less abundant on this plant. As a matter of fact the former two species only are found feeding upon the broad bean plant in the fields.

It was rather striking that some plants infested with viruliferous aphids did not contract the disease whereas the same individuals of aphids produced infection in the other plants under similar conditions. This might be explained on the assumption of any one of the following cases: either certain viruliferous aphids sometimes fail to produce infection in healthy plants for some unknown reason or certain individual plants resist the virus of mosaic disease. When numerous aphids were allowed to feed upon one plant, infection was produced more easily as compared with the case when a plant was subjected to the feeding of a few aphids. This may be due to the fact that there exist non-viruliferous aphids which were bred on mosaic plants.

The broad bean mosaic was transmitted to the garden pea, sweet pea and bean by *Aphis laburni* and to the sweet pea by the agency of *Acyrtosiphon pisi*. The former aphid failed to transmit the disease to the lucerne, Adzuki bean and soy bean. The infective aphid was able to transmit the virus to a healthy broad bean plant after one day's feeding. The incubation period of the virus in the plant was generally 6 to 10 days but rarely as long as 3 to 4 weeks while that in the insect carrier seemed to be very short. The parthenogenetically produced progeny of viruliferous aphids could not transmit the disease showing that the infective principle is not "hereditary."

It is worthy of note that BÖNING was unable to transmit the broad bean mosaic to healthy plants by artificial inoculation with the mosaic plant juice, whereas the writer successfully transmitted the disease through the plant juice. However, it is not definitely known whether both the disease under consideration and BÖNING's broad bean mosaic are caused by the same virus.