Utilization of Granulosis Viruses for Controlling Leafrollers in Tea Fields

By TAKERU SATO*, NOBUHIKO OHO** AND SHIGEHIRO KODOMARI***

*,** Plant Protection Division, Fruit Tree Research Station
(Yatabe, Ibaraki, 305 Japan)
*** Shizuoka Tea Experiment Station
(Kikukawa, Shizuoka, 439 Japan)

Introduction

Many kinds of insect pests, such as leafrollers, tussockmoth, looper, thrips, leafhoppers and spider mites are commonly distributed in almost all tea fields of Japan.

For controlling these pests, chemical insecticides are frequently sprayed, and are making problems not only due to chemical residues but also through destruction of the natural enemies of these pests. This is especially true in the control of both leafrollers, the oriental tea tortrix *Homona magnanima* and the smaller tea tortrix *Adoxophyes* sp. (the scientific species name is pending). Nowadays the control of these insects depends on broad-spectrum insecticides, such as methomyl and salithion. If selective control agents would be developed against both the leafrollers, methods to control other insect pests attacking tea plants will also be improved, and the natural enemies fauna will be recovered. From these points of view, we intended to use Baculoviruses as a candidate of selective control agents. Fortunately, from both insects, 2 granulosis viruses were isolated by Oho et al.[3] and Sato et al.[4] However, on the use of mixture of viruses for mingled appearance of these insect pests, we have very little knowledge at present.

In this paper, the experimental results on the efficacy of the application of mixed viruses for both the leafrollers, and the influence of the virus application on natural enemies closely related to tea cultivation will be presented.

Present address:
** Department of Research & Experimental Farm, Japan Plant Protection Association (Kessoku, Ushiku, Ibaraki, 300-12 Japan)

Characteristics of the granulosis viruses of the leafrollers

The granulosis virus of the smaller tea tortrix is originally isolated from the summer fruit tortrix *Adoxophyes orana fasciata* which is a sibling species of the smaller tea tortrix. This virus was first reported by Aizawa and Nakazato[1] in 1963, and rediscovered by Drs. K. Honma and H. Sugawara in Iwate Prefecture, in 1967, while the granulosis virus of the oriental tea tortrix was detected from unhealthy larvae collected in the tea gardens of Fuji Branch of Shizuoka Tea Experiment Station (Fuji, Shizuoka Prefecture) by Sato et al..[4]

By electron microscopic observation, capsules of both viruses were shown as ellipsoidal, and virions as rod-shaped. Average size of virion of the virus of *Adoxophyes* is 290 nm in length and 70 nm in width, while the sizes of capsule and virion of virus of *Homona* are 385×220 nm, and 300×60 nm, respectively.

The larvae infected orally with the granulosis virus of *Adoxophyes* or *Homona* were grown up to the last instar, and they can be distinguished by their whitish body color. This symptom appears on the ventral side of the larva in the early stage of infection. Diseased larva gradually becomes sluggish and dies without pupating.

Both viruses act independently each other regarding their pathogenicity to larvae each of both the leafrollers in laboratory conditions, that is, the granulosis virus of *Adoxophyes* is highly infectious to the larvae of the smaller tea tortrix, but not infectious to the oriental tea tortrix larvae, whereas, the *Homona* granulosis virus is infectious only to the homologous insect. Then it would be necessary to mix both the viruses for simultaneous control of both *Adoxophyes* sp. and *Homona magnanima*.

In both the species, the earlier the developmental stage of larva the higher the susceptibility to the virus, and even when the infection takes place at the early stage of larval development, the diseased larva is able to survive even after the pupation of healthy larva.

The stability of the granulosis virus of the *Adoxophyes* was analyzed by Yamada and Oho[9] and Sato (unpublished). They revealed that the virus was inactivated by 10-min exposure to temperature of 60°C and 5-sec of ultraviolet-lamp irradiation (15 w, 30 cm apart). The virus was also inactivated with disinfectants such as formalin and sodium-hydrochlorate. The pathogenicity was lost when it was kept in a solution with pH 10.5 or higher alkalinity for 24 hr at room temperature. These characteristics of the stability almost coincided with those of *Homona* granulosis virus (Sato, unpublished data).

Safety tests of both the granulosis viruses using white mice did not show any negative results (Sato, unpublished).

Mass production of granulosis viruses

The granulosis virus of *Homona* was mass-produced by using its original host, while that of *Adoxophyes* was multiplied by using an alternative host, the smaller tea tortrix. The methods of mass-production for both granuloses were referred to Yamada and Oho,[8] Sato et al.,[4] and Sato.[5,6]

Evaluation of mixed virus application

1) *The design of treatments in an experimental field*

A field experiment was conducted in an experimental field of Fuji Branch of Shizuoka Tea Experiment Station. The field used for this experiment was surrounded by ordinary fields where control of these insects is practiced and it was divided into 4 plots, that is, 2 plots for virus-sprayed, one plot for chemical-spray and one plot without any spray. In the surrounding fields, chemicals were applied throughout the season of leafrollers infestation.

In the virus-sprayed plots, the mixed virus suspension was sprayed once with a power sprayer at the rate of 2,000 *l*/ha. The suspension was prepared by adding 1,000 mature diseased larvae equivalent (MDLE) virus-capsules of *Adoxophyes*, 500 MDLE of *Homona* and 50 m*l* of wetting agents into 100 *l* of water. The mean lethal concentration (LC_{50}) of this suspension against young larvae of the smaller tea tortrix and the oriental tea tortrix was about $1\times10^{-4.5}$ and $1\times10^{-4.0}$ MDLE per liter, respectively.

In the plots of virus-spray and the plot without any spray, chemicals which influence both leafrollers were not sprayed. In the chemical-sprayed plot, chemicals to control leafrollers were applied 6 times just like in surrounding ordinary fields.

Systematic sampling of insects was conducted once at the time of full-grown stage in each generation, and the number of larvae and pupae per 0.25 m^2 of filed was calculated. Larvae without clear symptoms of the disease were collected and reared individually on artificial diet in glass tubes for assessment of infection.

2) *Effects on mortality and population of leafrollers*

The change in mortality due to the granulosis virus diseases after the spray of the mixed viruses is shown in Fig. 1. In the virus-sprayed plots, high mortality (about 60–75%)

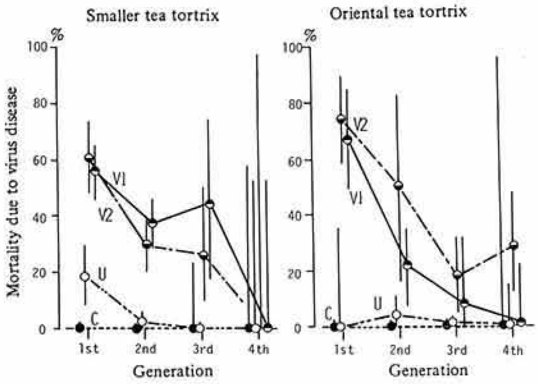

Fig. 1. Mortality increase in tea leafrollers caused by granulosis virus disease
V1, V2: Virus-sprayed plots, C: Chemical-sprayed plot, U: Unsprayed plot.
The bar indicates 90% confidence range.

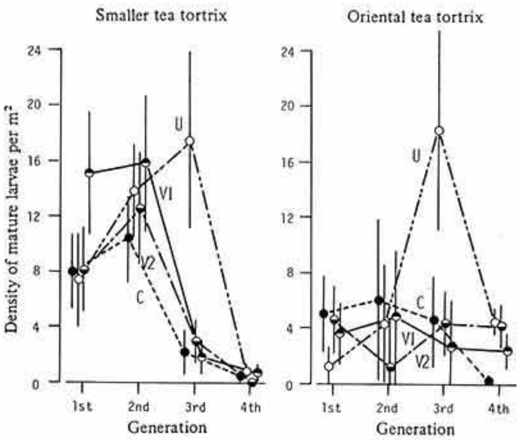

Fig. 2. Density of mature larvae of tea leafrollers as influenced by granulosis virus spray
Legend: See Fig. 1.

was attained in the treated generation of both pests. Even in the succeeding generations, mortality in the virus-sprayed plots was high as compared with that in the other plots. As shown in Fig. 2, in both species of the unsprayed plot, the larval density reached the highest level in the 3rd generation, whereas in the virus-sprayed plots, such a rise in density did not take place. The population trend was nearly the same as in the chemical-sprayed plot.

3) Influence on the natural enemies fauna and other miscellaneous causes

The change in the mortality caused by parasitoids after the virus application is shown in Fig. 3. In the treated generation, the rate of parasitized larvae of the smaller tea tortrix in the virus-sprayed plots and the unsprayed plot were significantly lower than in the chemical-sprayed plot. However in the

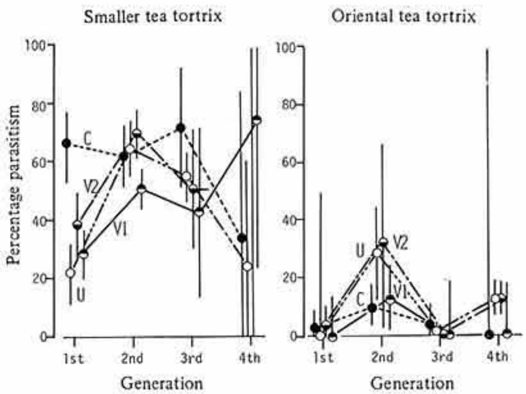

Fig. 3. Trend in percentage parasitism after the virus application
Legend: See Fig. 1.

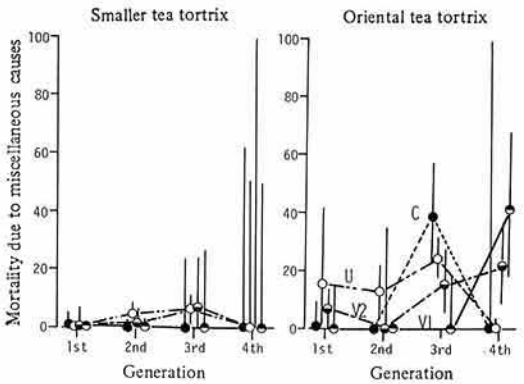

Fig. 4. Mortality due to miscellaneous causes such as fungal diseases and predation by spiders
Legend: See Fig. 1.

succeeding generations, no difference was observed.

The change in the mortality due to predation by spiders or other miscellaneous causes such as fungul diseases, is indicated inclusively in Fig. 4. In each generation of the smaller tea tortrix, the mortality rate was very low and similar for each plot. On the other hand in the oriental tea tortrix, the rate varied with plots and generations. It was mainly caused by fungal diseases.

4) Discussion

In general characteristic of susceptibility of lepidopterous insect to virus, the later developmental stage of larva is more resistant to the virus infection. In addition to this, in the field, tortrix larvae usually conceal themselves deeply in the layer of folded leaves at the middle to late stage of development. The virus suspension sprayed hardly reach inside the folded leaves. Consequently, they are seemingly much more resistant to virus infection than in laboratory condition. Thus in fields, spraying of virus suspension should be conducted during an early larval stage. According to the other spraying tests in which virus-spray was made at an early stage of each generation in a year, the mortality of 2 generations in the summer season was slightly lower than that of the spring and autumn generations.

From the characteristics of both viruses, that the infected larvae survived to the full-grown stage and they cause the similar damage to tea plants as that caused by healthy ones, and also from the view-point of the green-tea production in Japan, that the yield of the first picking is the most valuable, while that of the 2nd or 3rd picking is not so worthy in the current cultivation form, it can be said that the best timing of virus spraying must be at the early stage of the first generation.

The application of the mixed viruses caused a high mortality of the larvae directly exposed to the application, and it also induced significantly higher mortality in the succeeding 2 generations, but no effect was recognized in the next spring generation. Thus, the direct effectiveness of the virus application seems to last for only 3 or 4 generations. A persistant effectiveness of artificial dissemination of the viruses can not be expected even on an evergreen tree like tea plant.

Significantly high mortality due to parasitoids was observed in the treated generation of chemical-sprayed plot. The cause of this phenomenon has not been determined exactly, but we are considering that major parasitoids of leafrollers might acquire some adaptation to the chemical applied. We are also considering that the virus infection of their host larvae may exert some adverse effects to the development of dominant parasitoids, *Apanteles adoxophyes* and *Ascogaster reticulatus*. We noticed that the larvae of these parasitoids died mostly within the diseased larvae of their host.

As shown in Fig. 4, the rates of mortality caused by predation, fungul diseases etc., varied with generations and plots in both leafrollers. These rates might have been under-estimated, because predation could not be assessed exactly by only one survey in each generation. We obtained a distinct result with a survey on July 21, that is, no spider-webs could not observed in the chemical-sprayed plot, whereas large numbers of the webs were counted in the virus-sprayed plots.

Total mortality of leafrollers at the full-grown stage in the virus-sprayed plots was significantly higher than the other plots in each generation after the dissemination of viruses. In the smaller tea tortrix, it was as high as 85–97.5% in the sprayed generation, whereas 70% in the chemical-sprayed plot and 43% in the unsprayed plot. In the oriental tea tortrix also, total mortality in the sprayed generation attained to 67–87% in the virus-sprayed plot, but that was only 8% in the chemical-sprayed plot and 16% in the unsprayed plot.

It is noteworthy that spraying of mixed virus suspension practiced only once in an early stage of larval development was as effective as 6 times applications of chemicals in controlling leafrollers. If large scale field application is done, the more obvious effectiveness will be obtainable, because in the present small scale experiment, immigration of adult leafrollers might occur and disturb the result.

The economic injury levels with leafrollers were set up by Takagi,[7] that is, 8 larvae/m^2 for the smaller tea tortrix and 4 larvae/m^2 for the oriental tea tortrix. In our experiment, the larval density was suppressed to a level slightly higher than those levels.

The quality and quantity of tea picked in the virus-sprayed plots were slightly inferior to those of the chemical-sprayed plot, as crop damage by other pests may have increased due to no chemical application. We would like to emphasize that a new integrated control program including the use of these granulosis viruses as a key component should be established for other important pests of tea plants.

Conclusion

It depends on a characteristic of the granulosis viruses of the oriental tea tortrix and the smaller tea tortrix that the virus-infected larvae are able to survive until the full-grown stage of their development. As the result, the virus application did not show the acute efficacy in the generation exposed to the application. However the effect of the viruses appeared in the succeeding 2 generations by suppressing larval density. Moreover, the virus-application brought about no direct influence to the natural enemies of the leafrollers. The mixed-virus application must be emphasized as a promising candidate of control agent against both the leafroller pests.

At present, mass-production of the viruses depends only on the use of their host larvae, and virus-infected diseased larvae are collected individually. This procedure is the most time-consuming in the process of the virus mass-production. From the standpoint of the practical use of the virus application, the process of virus mass-production has to be improved and a system of mass-production on a large scale must be established.

References

1) Aizawa, K. & Nakazato, Y.: Diagnosis of diseases in insects: 1959–1962. *Mushi*, 37, 155–158 (1963).
2) Kodomari, S.: Use of granulosis viruses for leafrollers on tea plant. *Shokubutsu-boeki* (Plant Protection), 34, 462–466 (1880) [In Japanese].
3) Oho, N., Yamada, H. & Nakazawa, H.: A granulosis virus of the smaller tea tortrix, *Adoxophyes orana* Fischer von Roslerstamm (Lepidoptera: Tortricidae). *Mushi*, 48, 15–20 (1974).
4) Sato, T., Oho, N. & Kodomari, S.: A granulosis virus of the tea tortrix, *Homona magnanima* Diaknoff (Lepidoptera: Tortricidae); Its pathogenicity and mass-production method. *Appl. Entomol. Zool.*, 15, 409–415 (1980).
5) Sato, T.: Mass rearing method of a leafroller, *Adoxophyes* sp. for propagation of its granulosis virus. *Shokubutsu-boeki* (Plant Protection), 35, 233–237 (1981) [In Japanese].
6) Sato, T.: Mass production methods of Baculoviruses of *Adoxophyes* spp, *Shokubutsu-boeki* (Plant Protection), 38, 366–369 (1984) [In Japanese].
7) Takagi, K.: Integrated control of tea insect pests. *Nogyo-gijutsu* (Techniques of Agriculture), 31, 489–491 (1976) [In Japanese].
8) Yamada, H. & Oho, N.: A granulosis virus possible agent for control of *Adoxophyes orana* (Lepidoptera: Tortricidae) in apple orchards. I. Mass production method. *J. Invertebr. Pathol.*, 21, 144–148 (1973).
9) Yamada, H. & Oho, N.: Studies on the pathogenicity of granulosis virus of the summer-fruit tortrix, *Adoxophyes orana* Fischer von Roslerstamm, against the smaller tea tortrix, *Adoxophyes fasciata* Walsingham (Lepidoptera: Tortricidae). *Bull. Fruit Tree. Res. Sta.*, A3, 87–99 (1976) [In Japanese with English summary].

(Received for publication, July 31, 1985)