An Application of Nuclear and Cytoplasmic Polyhedrosis Viruses Against *Lymantria fumida* BUTLER (Lepidoptera : Lymantridae)

By

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Résumés

A mixed suspension of nuclear and cytoplasmic polyhedrosis viruses was sprayed on the young larvae of *Lymantria fumida* in the second year of its occurrence. The nuclear polyhedrosis infected much more rapidly than the cytoplasmic polyhedrosis, and put a far greater proportion of the host larvae to death. A high percentage of mortality in pupae was recorded by the nuclear polyhedrosis virus, whereas the cytoplasmic polyhedrosis virus had little influence upon the host pupae. However, 31 % of the females and 14% of the males of the surviving adults were found infected with the cytoplasmic polyhedrosis virus. Thus, each virus seemed to have worked in such a way as to supply the deficiency to the other. Egg masses in the succeeding generation was then reduced approximately to one-tenth, and the number of eggs per mass was also diminished to one-helf. Meanwhile the rate of parasitized eggs increased some five-fold.

Introduction

Lymantria fumida is one of the major pests on the Japanese fir, *Abies firma* (HIJIKURO, 1927). It has generally been recognized that a nuclear polyhedrosis becomes epizootic in two to three years after the population of *L. fumida* has reached a certain level of abundance. The insect population is usually obliterated by this virus disease (HASEGAWA and KOYAMA, 1937 ; KOYAMA and KATAGIRI, 1959 ; KATAGIRI and KOYAMA, 1959 ; KOYAMA, 1963). This phenomenon is often called "Wipfelkrankheit".

Recently another virus disease, a cytoplasmic polyhedrosis, was found to be in *L. fumida* population. In 1965, infestation of *L. fumida* was noticed over 60 ha of a fir forest at Takao, Tokyo District, and was suspected of subsisting at least for 3 years, judging from the past records of outbreaks (KOYAMA and KATAGIRI, 1959). The dissemination of viruses against this insect was undertaken in 1966.

L. fumida completes only one generation in a year. The adult appears in the field in late June to early July and lays eggs on the trunk of a host tree in a lump. The egg overwinters and the new larva hatches in the next spring. The larva feeds on leaves up to early summer. The pupal period is about 10 days.

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This paper is concerned with the infection and mortality resulting from the field spray of a mixed suspension of nuclear and cytoplasmic polyhedrosis viruses at the early period in the second year of the insect occurrence.

Materials and methods

Preparation and application of virus suspension

Eggs of *L. fumida* were collected in February 1966 and kept at 25 °C in the laboratory. In about ten days the larvae hatched from these eggs. They were fed at first with new shoots of a fir, and when they reached the 5th instar the food was replaced by some leaves contaminated with viruses which were obtained in 1963 from *L. fumida* larvae collected in fir forests in Takao. After about ten days, death from viruses began to occur. Then the cadavers were removed and stored at 5 °C up to the end of April. Some cadavers were examined for the presence of polyhedra under microscope. During the process, a cytoplasmic polyhedrosis was found prevailing in the larvae of mass rearing. Nearly one-half of the total cadavers were infected with the cytoplasmic polyhedrosis virus.

All the dead larvae were ground up and macerated in water for some days and sieved through a cheese-cloth. The resulting suspension was diluted to the concentration of 5×10^5 polyhedra per ml, irrespective of nuclear or cytoplasmic polyhedra.

A neutralized sticker solution was added to the suspension at the rate of 1 : 2,000 just before field application.

The mixture was sprayed by a helicopter over 64 ha of the fir forest at the rate of 60 litres per ha.

The forest was a mixed stand of firs with some broad-leaf trees.

Observation and sampling plots

Six plots were established throughout the area. In each plot four trays, each 1 m×1 m in size, were placed under trees to collect every week frasses and cadavers falling from the crowns.

Mortality and morbidity were determined from samples of larvae collected at weekly intervals from these plots. At the 7 th day after the insects were collected in the field, the dead larvae were examined under microscope for a possible cause of death, and the living larvae were dissected a further 7 days later to examine the presence of any cytoplasmic polyhedra in mid-gut tissues and any parasitic insects. The incubation periods of two polyhedrosis are quite different. The nuclear polyhedrosis needs a little more than 7 days (KATAGIRI and KOYAMA, 1959) while cytoplasmic polyhedrosis takes many more days. This indicates that the larvae infected by the nuclear polyhedral virus were most likely dead, but the larvae containing cytoplasmic polyhedra were not always killed, at the time of examination in the laboratory.

A simple supposition was that all the larvae died within seven days and the larvae containing any cytoplasmic polyhedra were considered infected in the field. The percentage of mortality by different viruses was thus estimated.

Egg-mass collection

In February, March, July, November and December in 1966, egg-masses were collected repeatedly throughout the sprayed fir forest in the total of 58 man days. All egg-masses were kept at 25 °C to allow parasites to emerge. The rate of parasitism was estimated by counting the number of healthy and parasitized egg-masses and eggs. Density of egg-masses of the new generation was compared with that of the previous generation.

Results and discussions

Mortalities at larval stage

In about two weeks after the aerial spraying, a part of the larvae on the crowns, mostly of the 4th and 5th instar, dropped down to the ground. Thus the larval population was divided into two different groups, one group of larvae crawling on the ground and the other group of larvae that remained on the trees. Fig. 1 shows a curve indicating numerical decrease for the group of larvae remaining on the tree crowns. This is drawn on the basis of mortalities on the samples collected at weekly intervals after the dissemination of viruses. The average larval mortality finally reached 99.98 per cent of the initial population, ranging 93.40 (Plot 4) to 100 per cent (Plot 2). The mortality of 100 per cent was obtained in three weeks after spraying in Plot 2. Mortalities by different factors are shown in Fig. 2. In Plot 2, infection by nuclear polyhedrosis virus progressed rapidly, but no mortality by cytoplasmic polyhedrosis was observed in the early period. After reaching 100 per cent mortality in the sampled plot, there were actually many larvae in the crowns of trees in the neighbouring area. This indicates that many larvae which had dropped down from the host trees were again coming back to the trees. The

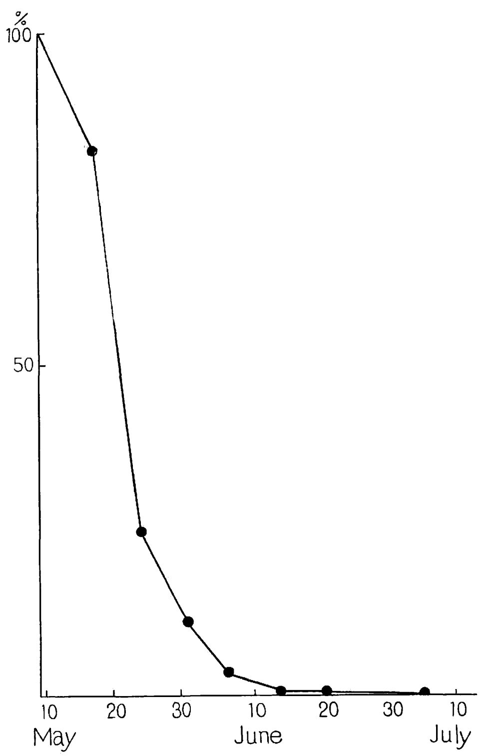

Fig. 1 Survivorship in the number of larvae of *L. fumida* that remained on the tree crowns after the aerial spray of viruses.

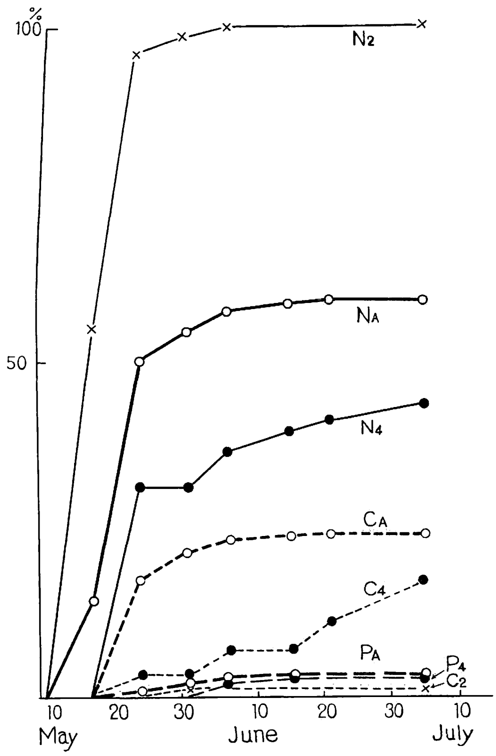

Fig. 2 Cumulative mortality curves by different factors.

N_A, N_2 and N_4 denote the mortalities by nuclear polyhedrosis in whole area, in Plot 2 and in Plot 4, respectively.

C_A, C_2 and C_4 denote the mortalities by cytoplasmic polyhedrosis in whole area, in Plot 2 and in Plot 4, respectively.

P_A and P_4 denote the mortalities by parasites in whole area and in Plot 4, respectively.

larvae on the ground were actively crawling about and tended to climb up any tree trunks regardless of the tree species. They were seemingly not in normal condition. All the larvae of this crawling group died of nuclear polyhedrosis in 2 or 3 days after being collected as samples. At the last period of larval stage, large lumps of cadavers were seen here and there at the tops of firs.

On the contrary, in Plot 4, a relatively high proportion (6.6 per cent) of the larvae survived to pupae. Mortality by the nuclear polyhedrosis virus was below 50 per cent. In addition to this low mortality caused by disseminated viruses, no predominent patterns of secondary infection were observed in this area, though such secondary infection was found in other areas after 4 or 5 weeks of aerial spraying. Incidences of mortality by the cytoplasmic polyhedrosis virus went gradually upward in the course of time and finally reached 17 per cent.

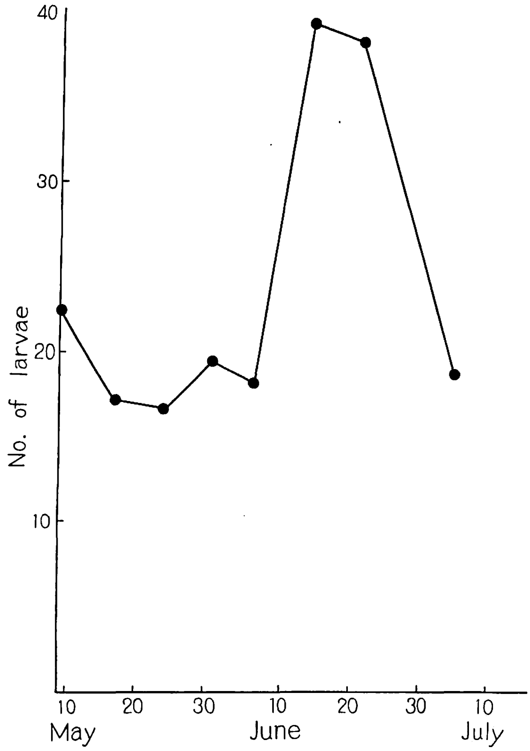

Fig. 3 The number of larvae per unit on the top of the tree, *Abies firma*.

After all, as SMIRNOFF et al. (1962) observed in the virus-sprayed population of *Neodiprion swainei* and STAIRS (1964) in the *Malacosoma* populations, two epizootical patterns of infection were seen; the initial infection with applied viruses and the secondary infection followed by the initial one. At about the beginning of the secondary infection, the larvae of the early 5th instar began to migrate to the top of the host tree, where they died, forming large cadaver lumps. The number of larvae per sample unit taken from an upper part of the host tree shows drastic migration of larvae into the upper part of trees during the late larval period (Fig. 3).

On the other hand mortality by the cytoplasmic polyhedrosis virus began to appear in about 2 weeks after spraying. The rates of mortality did not change widely with time. This indicated that the incidence of cytoplasmic disease was not so rapid as in the case of the nuclear one, and it became chronic in the insect population. No acute increase of mortality by the cytoplasmic polyhedrosis virus was observed. However, cytoplasmic disease prevailed well in the area where incidence of the nuclear polyhedrosis virus was low. In this area high parasitism by dipterous parasites was also recognized. Multiple infection of nuclear and cytoplasmic polyhedrosis was observed in all areas in certain rates.

The average mortality by the nuclear polyhedrosis virus was 59 per cent, that by the cytoplasmic one was 24 per cent, parasitization by dipterous insects 3 per cent, and mortality by other causes 13 per cent. As already stated, in about 2 weeks after the spraying the larvae began to drop to the ground and formed a large group. This group of larvae on the ground

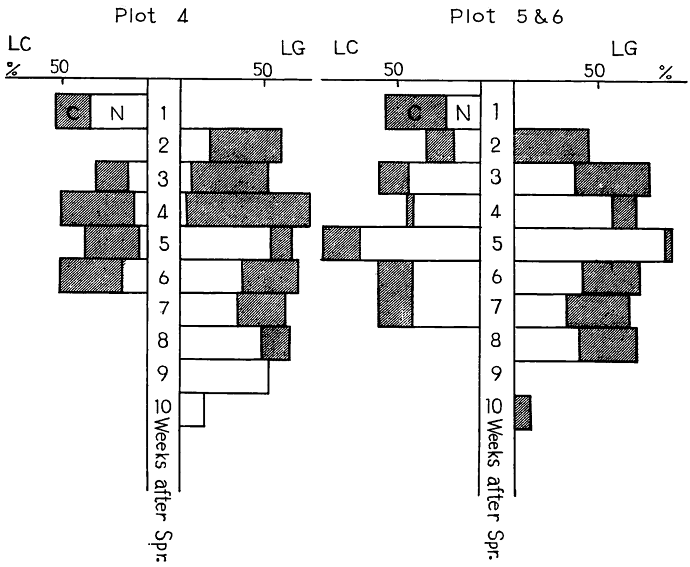

Fig. 4 Morbidities in percentage of the larvae on the crown and on the ground.
LC denotes larvae on the crown and LG larvae on the ground.

was so unstable that some larvae were always climbing the trees, while some were falling down. This irregular situation continued up to immediately before the pupal stage. Fig. 4 shows mortalities of these larvae by various factors. Mortality by virus diseases was revealed higher for the larvae on the ground, and the incidence of cytoplasmic polyhedrosis disease was more frequent in the larvae that had fallen to the ground in the earlier period.

In the area of Plot 3 where the density of *L. fumida* was low, no crawling larvae were found on the ground. Although the initiation of dropping coincided with the occurrence of highest mortality by the nuclear polyhedrosis, it was undeterminable whether this coincidence had any positive relation or not to the virus disease prevailing there. It is certain, however, that the dropping and crawling are very important means in dispersing and transmitting viruses and in making them epizootic.

Larvae that died and dropped from branches were collected in trays under tree crowns at one week intervals, and were examined for polyhedra and parasites. The results are shown in Fig. 5. Generally speaking, dead bodies from the nuclear polyhedrosis fell mostly during the initial period of infection and later they ceased to fall. This is probably because the infected larvae migrated to the top of the trunk and died there, forming cadaver lumps at the secondary infection period of late instar stages. As STAIRS (1965) said, the phenomenon of "Wipfelkrankheit" is important in regard to the transmission of viruses to the next generation or in preserving pathogens within the forest.

The larvae killed by the cytoplasmic polyhedrosis dropped mostly in around 4 to 5 weeks after spraying, but in such areas where comparably higher morbidity was recorded in crowns as well as on the ground (Plot 5, 6), dead larvae began to drop in one week after the spraying, showing the incidence of heavy infection of this polyhedrosis from the disseminated virus. Mortality by parasitization of dipterous insects began to appear in the 3rd week after spraying and attained the peak in about the 4th and 5th weeks. The larvae on the ground were

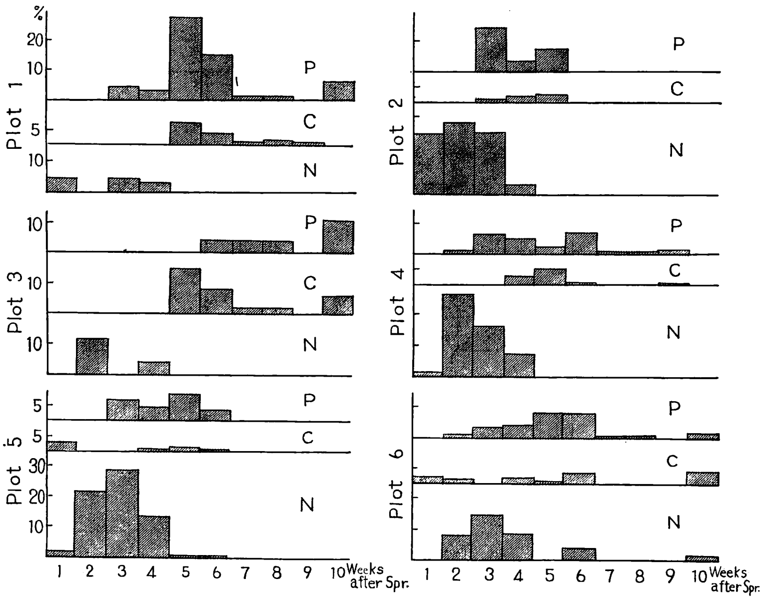

Fig. 5. Frequency distribution of death caliculated on the basis of the number collected in the frass trays at weekly intervals.
N : by nuclear polyhedrosis virus, C : by cytoplasmic polyhedrosis and P : by parasitic insects.

parasitised in greater proportion than the larvae in the crown. This difference in parasitism percentage may explain different preference on the part of parasitic insects.

Mortality by virus diseases in the present study was not equal throughout the sprayed area, and there was no clear correlation between the incidence of virus diseases and pest density (KATAGIRI and KOYAMA, 1959 ; STEINHAUS, 1954 ; MORRIS, 1963). The unevenness of mortality remains unexplained. It was due either to the unevennesses of spraying or to the diversity of host distribution. It is also suspected that an occult virus being inheritedly transmitted through the egg might have been activated differently by the physical and biotic environment of each particular plot (e.g. WELLINGTON 1962).

It can now be concluded that the nuclear and cytoplasmic polyhedrosis viruses, when disseminated to the young larvae of *L. fumida* in May, each virus having supplied the deficiency of the other, successfully brought about fairly high mortality among the larvae which resulted in the collapse of the pest population.

Mortalities at the pupal stage

About 0.02 per cent of the initial larval population survived to pupae in the average. No pupa was found in the areas where the incidence of virus diseases, especially of the nuclear polyhedrosis, showed 17.68 per cent by hymenopterous parasites, 1.66 per cent by dipterous parasites and only 0.55 per cent by the cytoplasmic polyhedrosis. All the pupae that had polyhedra in the mid-gut were also infected by the nuclear polyhedrosis. The rates of pupal mortality were clearly different depending on whether the pupae were collected on the host trees or on and near the ground. Hymenopterous parasites were obtained only from the pupae on the ground and on lower bushes (Fig. 6).

The percentage of mortality was found high in the nuclear polyhedrosis virus, while it was quite low in the cytoplasmic virus. This shows that the cytoplasmic virus in pupal tissues of

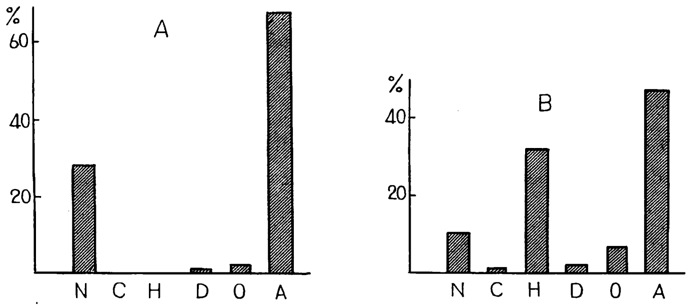

Fig. 6 Mortalities at pupal stage.
(A) Pupae collected on the host tree crown.
(B) Pupae collected on the lower bushes and near the ground.
N : Mortalities by nuclear polyhedrosis, C : by cytoplasmic polyhedrosis, D : by dipterous parasites, H : by hymenopterous parasites, O : by other causes and A : emerged adults in percentage.

L. fumida does not multiply at all or, the multiplication is at most kept below the level of abundance that is lethal to the host (c.f. NEILSON, 1965).

Mortalities at the adult stage

Although there is no definite method to identify the adult suffering from the nuclear polyhedrosis, the diseased moth of cytoplasmic polyhedrosis can easily be detected by the presence of polyhedra in the mid-gut cells (NEILSON, 1965 ; KATAGIRI, 1967). A little more than 57 per cent of the pupae collected in the field developed to adults and 31.25 per cent of the female moths were found infected by the cytoplasmic polyhedrosis. On the contrary 14.05 per cent of males were morbid. The average proportion of diseased adults was then 21.9 per cent. In the area where a relatively high survival rate was recorded, 70 per cent of the pupae grew to adults and morbidity of the adults was 37 per cent. Further information on the whereabouts of viruses contained in the adults was not available in the present study. It can only be suspected that the diseased adults would take some part in transmitting the viruses in the succeeding generations (BIRD, 1961 ; SMIRNOFF, 1962).

Surveys on the eggs of the next generation

Eggs and egg masses of the new generation were studied and compared with those of the previous generation (Table 1). The number of egg masses in February and March, decreased aproximately to one-tenth in the next generation. The number of eggs per mass was also diminished to one-half. Meanwhile, the rate of parasitism increased greatly ; that is, 3.05 per

Table 1. Collection of *Lymantria fumida* eggs and the parasitism percentage by egg parasites.

Date collected	No. collected		No. egg per mass	Parasitism percent	
	Total	Per man day		Masses	Eggs
	masses	masses		%	%
1966 Feb.-March	1210	40.5	157.4	60.0	3.05
July	27	2.8	61.7	77.8	9.54
Nov.-December	75	4.0	72.1	94.7	15.08

cent of parasitism in eggs in early spring went to 9.5 per cent in eggs of the next generation in July, and finally to 15 per cent in the overwintering period : more and more proportion of egg masses became parasitised in the course of time. Almost all egg masses in most areas were parasitized to some extent in November～December.

On the basis of these results it is concluded that the viruses, disseminated for the purpose of reducing the density of pests, have directly destroyed the pest population without causing any evil effects on the beneficial insect, which has consequently produced an increase in the percentage of parasitism by egg parasites.

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ハラアカマイマイ *Lymantria fumida* の多角体病ウィルスによる防除試験

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ハラアカマイマイは普通発生密度が高まってきて3世代目には自然に核型多角体病が流行し，大発生が終息する現象がくり返されてきていることが観察されている。この核型多角体病（N）についてはすでにその自然状態における流行状況や病理，病原ウィルスについて発表した。この核型多角体病ウィルスと，今回新しく発見された中腸細胞質型多角体病ウィルス（C）(スミシヤウィルスの1種）とを，大発生2世代目のハラアカマイマイ個体群を対象に散布導入し，ウィルス病がその host 個体群に与える影響について調べた。

対象林は高尾山国有林モミ林全域約 64 ha の占有面積のところで，1965年の世代から密度の高まりがみとめられた。ここに1966年春ハラアカマイマイ幼虫の若齢期に ha あたり 60*l* の割合でウィルス（多角体）懸濁液をヘリコプターにより散布した。散布液のウィルス濃度は多角体数で測って，5×10^5 個/m*l* で，これに 2,000 倍にした中性展着剤を加えた。

散布に用いた病原ウィルスは当研究室で冬期間にハラアカマイマイ卵のふ化促進を行なって飼育した幼虫を罹病させて量産したものである。

散布後のウィルス病流行状態をみると明らかに2つの流行の peak がみられた。すなわち，散布ウィルスによる病死がまず起こり，それにつづいて2次的な感染によると思われる病気の流行が観察された。

Nの発病はきわめて急性的で流行も急速に行なわれたが，Cには急性的な様相は観察されず，むしろハラアカマイマイ個体群に慢性病的になっていったことが推論された。

NとCの双方に感染している個体も，ある少率で観察された。

このようにこれらの多角体病ウィルスは，ハラアカマイマイの若齢幼虫期に散布すると幼虫の死亡率を高めて，よく幼虫個体群を破壊することができた。

さなぎ期ではNによる死亡が高率でみとめられたが，Cによる死亡はほとんどみとめられなかった。このことは，C型ウィルスはさなぎの中腸細胞中では全く増殖しないか，またはきわめて緩慢にしか増殖しないかのどちらかであると考えられる。

一方成虫についてみると，羽化した成虫のうち雌が31%，雄が14%Cに罹病していることがわかった。これはまた母蛾によるウィルスの次世代伝染を考える上に重要な現象である。

このようにして，これら2種のウィルスは互いに他の欠点を補うような働きをなした。

卵塊密度についてみると散布後の密度は前世代の約10%に減少し，また卵塊あたりの卵粒数も約半分になった。卵寄生バチによる寄生率は前世代の5倍に増大していた。この寄生率の上昇は散布ウィルスが天敵昆虫類に悪影響を与えずに対象害虫個体群を破壊することができたためであると考えられる。

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