Influence of Baculovirus AdorGV on the Mortality of Larvae and Pupae of Summer Fruit Tortrix *Adoxophyes orana* in Laboratory Conditions

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Abstract

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The mortality of larvae and pupae of *Adoxophyes orana* was examined by keeping larvae of each larval instar (L1–L5) on an artificial diet in laboratory conditions. Larvae were infected by using an artificial diet containing AdorGV-based CAPEX® 2. Samples of uninfected larvae from each instar served as controls. The mortality of larvae infected in the 1st instar was 100%, compared to a mortality of 68% in the control. In both, the larvae died before the 5th larval instar. With larvae infected in subsequent instars the mortality rate declined gradually (96%–72%–40%–12%) and death occurred predominantly in the 5th larval instar. The mortality of larvae in the controls was low (12%–12%–0%–0%). The mortality of pupae from larvae infected in the 2nd, 3rd and 4th instar was high (100%–86%–93%), and the mortality of larvae and pupae combined was close to 100%. Mortality of pupae developed from larvae infected in the 5th instar was 27% and that of larvae and pupae combined was 36%. The mortality of pupae developed from uninfected larvae in all controls was low (max. 8%). These results demonstrated the high efficacy of AdorGV to cause high mortality of larvae and pupae of *Adoxophyes orana* in laboratory conditions.

Keywords: summer fruit tortrix*; Adoxophyes orana*; AdorGV; mortality; larvae; pupae

The summer fruit tortrix *Adoxophyes orana* (Fischer von Röslerstamm, 1834) is a serious pest in apple orchards in the Czech Republic and in many other European countries. It has two generations per year. Females of the 1st generation lay up to 150 eggs from which larvae emerge within a few days. The larvae feed close to the main vein of leaves in a protective silk mesh. Later they spin leaves together and feed from here mostly on the tips of shoots. Older larvae also feed on ripening fruits, hereby producing the superficial damage typical for this species. The 2nd generation of moths that appears in August deposits its eggs on leaves and on fruits. The larvae feed mainly on fruits before hibernating in silk cocoons in crevices in the bark. They reappear in March or early April to feed 1st on the developing buds and later on the flowers. In the middle of May, the caterpillars pupate to emerge as moths in May/June (Andermatt 2005). Although damage

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to fruit is not great, it enables fungal pathogens to attack, causing a decrease in the quality of the fruit. Consequently, the economic threshold of this pest is low and its population must be monitored and regulated exactly (Morgan 1991). Geest and Evenhuis (1991) state that summer fruit tortrix became the most important pest in apple orchards in many regions of Holland, Austria, southern Switzerland, Germany, the Balkan countries and Eastern Europe. Also, according to Sekita (1996), this insect is one of the most important pests in many countries due to damage done to leaves and fruit on apples trees and to its acquired resistance to chemical insecticides. Kocourek *et al*. (2007) state that significant damage caused by *A. orana* has been observed in apple orchards in Eastern and Central Bohemia since 1999.

Baculoviruses are the most numerous as well as the most studied group of entomopathogenic viruses. They are divided into two groups: Nucleopolyhedroviruses and Granuloviruses. They attack only invertebrates (most commonly Lepidoptera). All the Granuloviruses have been isolated only from lepidopterous insects worldwide thus far (Asano 2005). Baculoviruses do not show any negative effect on the health of plants and vertebrates (Blissard & Rohrmann 1990). Biopesticides based on baculoviruses are safe and have a very selective mechanism. They mostly attack only one host species and there are no known occurrences of resistance being developed (Moscardi 1999). The insect (in most cases a Lepidoptera larva) is infected during feeding on plants or by parasitoids. The insect's body disintegrates when it dies and the virus contaminates the surrounding vegetation from which it is spread by wind, birds and insects (Boucias *et al*. 1987; Fuxa 1987; Rohrmann 1992). The post-larval effect of baculovirus infection can cause a smaller size of pupa and imago and consequently reduce their ability to reproduce and their lifespan (Moscardi 1999). Various species of baculoviruses have different efficacy. Kundu *et al*. (2003) discovered that the persistence of *Cydia pomonella* granulovirus (CpGV) among the surviving population of *C. pomonella* is low, whereas the mortality of larvae of *C. pomonella* caused by this virus is high. Consequently, this virus is effective in reducing the insect population density in fruit orchards. On the other hand, *Lymantria dispar* nucleopolyhedrovirus (LdMNPV) causes low mortality of infected *L. dispar* larvae, whereas the frequency of the virus persistence in the surviving insect population is high (Cunningham *et al*. 1991).

AdorGV was 1st detected in 1960 by Aizawa and Nakazato (1963) from diseased larvae of *Adoxophyes orana* and was described as a pathogen of the summer fruit tortrix. It was reisolated from its larvae in 1967 (Asano 2005) and its influence on the larvae mortality and effectiveness as a possible biological agent against *Adoxophyes orana* was demonstrated in laboratory conditions and in apple orchards in 1970 and 1971 (Shiga *et al*. 1973; Yamada & Oho 1973).

AdorGV is a granulovirus with a slow effect so that infected larvae die only in the last instar regardless of the time when they were infected by the virus (Winstanley & O'Reilly 1999). Also, according to Oho (1975), the virus has very slow-acting pathogenity and newly hatched larvae infected by AdorGV die only in the 5th instar. Geest and Evenhuis (1991) state that infected larvae dying at the end of the last instar change colour from dark green to milk yellow. Yamada and Oho (1973) reported that summer fruit tortrix larvae are highly susceptible to infection with AdorGV during early larval instars, but that susceptibility decreases remarkably after the 4th and 5th instars.

Stará *et al*. (2007) state that AdorGV is characterised by strong persistence in surviving larvae after direct treatment with AdorGV in apple orchards causing high mortality of larvae in subsequent generations. According to Ito *et al*. (1977), spraying of the virus against the first generation larvae in a large area can reduce the number of individuals of *A. orana* in subsequent generations as well as their damage to the fruits to a very low level. Spraying of chemical insecticides is not so effective in reducing the population density of subsequent generations. Similarly, Shiga *et al*. (1973) reported that artificial dissemination of a granulosis virus of *A. orana* (apple race) had a marked effect in raising larval mortality not only in the generations sprayed (74% mortality), but also in the subsequent two generations (37.3% mortality in the 2nd and 50.7% mortality in the 3rd generation). Among generations of *A. orana* the virus AdorGV can persist in shady places in the orchard, from where it can infect larvae of the subsequent generations and multiply in them (Charmilot 1992).

In 1989, a preparation called CAPEX® based on AdorGV was registered in Switzerland (An-

DERMATT 1991). Of primary importance in the use of such products is correct timing. CAPEX® must be sprayed while the larvae are still small (L1–L3). The advantage of a virus application over conventional chemical pesticides is that this virus preparation is very specific and only acts against the summer fruit tortrix. It is non-toxic to other tortricids. Even naturally occurring parasites can develop on virus-infected larvae. There is no danger whatsoever for warm-blooded animals. Infected larvae will not die before the last instar. Therefore, only applications of CAPEX® against the young overwintering larvae reduce fruit damage. Applications against the young larvae of the summer- and autumn-generation do not reduce the damage by the treated generation, but the population density of the following generation (ANDERMATT 2005). To make CAPEX®, AdorGV is produced *in vivo* from infected summer fruit tortrix larvae in a laboratory colony. But despite its high efficacy and high specific action the use of CAPEX® is limited by its high production costs (CHARMILOT 1992). LUISIER and BENZ (1994) state three main problems in using AdorGV for protection against summer fruit tortrix. The 1st are the high treatment costs, the 2nd is the short persistence of the virus caused by its susceptibility to UV radiation, and the 3rd is the fact that infected larvae do not die before the last instar. At present, CAPEX® is registered in Austria, Belgium, Germany, Greece, Italy, Spain, Slovenia and Switzerland (ANDERMATT 2005; KABALUK & GAZDIK 2005) and CAPEX® 2 is registered in Germany (KABALUK & GAZDIK 2005). In the Czech Republic, the registration of CAPEX® 2 is planned.

The aim of this paper is to ascertain the influence of the baculovirus AdorGV on the mortality of summer fruit tortrix larvae and pupae compared to an uninfected control.

MATERIAL AND METHODS

Insect. *Adoxophyes orana* larvae from a laboratory colony were used for the experiment. The colony had been established in spring 2004 from hibernating *A. orana* larvae collected in the orchard of the Research and Breeding Institute of Pomology at Holovousy. The larvae were kept on a natural diet (leaves) until October 2004 (approximately five generations) in laboratory conditions. From October until the start of the experiment in January 2005, they were kept on an artificial diet as described by ANKERSMIT *et al.* (1977) in laboratory conditions (three generations). The egg masses were obtained by caging males and females in a plastic container composed from two connected 250 ml plastic cups. Egg masses were cut from the wall of these cups and sterilised in 10% formaldehyde.

Virus. For the experiment, AdorGV as a component of CAPEX® 2 (0.01 ml/40 ml H_2O containing 5×10^{10} granules/ml) (Andermatt Biocontrol AG, Grossdietwil, Switzerland) was used. The virus was applied by immersing the 2 × 1 × 0.5 cm shreds of artificial diet into a solution of CAPEX® 2 for a short time. The shreds of the diet were then put on filter paper to let excess water evaporate. Larvae were fed on the artificial diet containing AdorGV and thus infected.

Bioassay. The experiment was carried out in laboratory conditions (a constant temperature of 20°C and photoperiods 14 h of light and 10 h of darkness) at the Research Institute of Crop Production in 2004. Twenty-five larvae from the 1st (0 day old), 2nd (13 days old), 3rd (20 days old), 4th (27 days old) and 5th (33 days old) instar were infected with the AdorGV virus, and the same number of uninfected larvae from each instar served as control. That a larva had reached the next instar was determined by using a microscope and on the basis of changes in size, colour and morphology compared to larvae of the previous instar. The 25 larvae from each infected and control set were subdivided into groups of five individuals and these placed in 50 ml plastic cups with artificial diet. The number of surviving and dead larvae as well as the number of pupae and adults in each treatment and control was counted in 2 days intervals. From the data of each treatment and control the mortality rate of larvae and pupae was evaluated. In the case of larvae, mortality in the 5th instar compared to total larvae mortality was also evaluated. Statistical analyses were performed using the paired *t*-test.

RESULTS AND DISCUSSION

Mortality of larvae

The mortality rate of larvae infected by AdorGV in the 1st and 2nd instar was 100% and 96 %, respectively. The mortality of larvae infected in subsequent instars decreased considerably; with those infected in the 3rd instar it was 72%, in the

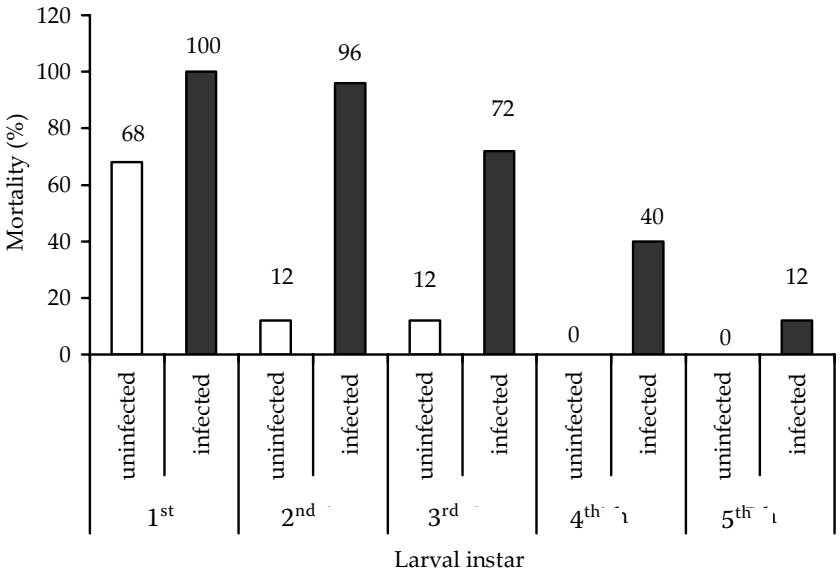

Figure 1. Mortality (%) of *Adoxophyes orana* larvae infected with AdorGV in each instar and mortality (%) of uninfected larvae in each control

4th instar 40% and in the 5th instar 12% (Figure 1). In the controls, the mortality rate of uninfected larvae of the 1st instar was high (68%), whereas that of uninfected larvae from subsequent instars was low (max. 12%). Statistical analysis showed highly significant differences between the numbers of pupae developed from larvae infected in the 1st, 2nd, 3rd and 4th instar and the numbers of pupae developed from uninfected larvae in the controls. When we look at the numbers of pupae developed from larvae that were infected in the 5th instar and from uninfected larvae in the control the differences are insignificant (Table 2). A possible explanation for the high mortality rate of uninfected larvae of the 1st instar could be that the newly hatched larvae in the 1st instar had not yet adapted to the artificial diet (short time to adapt to artificial diet for all populations – only three generations).

All the larvae infected in the 1st instar and half of the larvae infected in the 2nd instar died even before reaching the 5th larval instar (they all died during the instar in which they were infected). Statistical analysis shows highly significant differences between numbers of larvae which died in the same instar in which they were infected in case of the 1st and 2nd instar and numbers of larvae which died in the controls. No significant differences were found between these numbers in subsequent instars (Table 4).

This does not agree with reports from several authors, e.g. Winstanley and O'Reilly (1999),

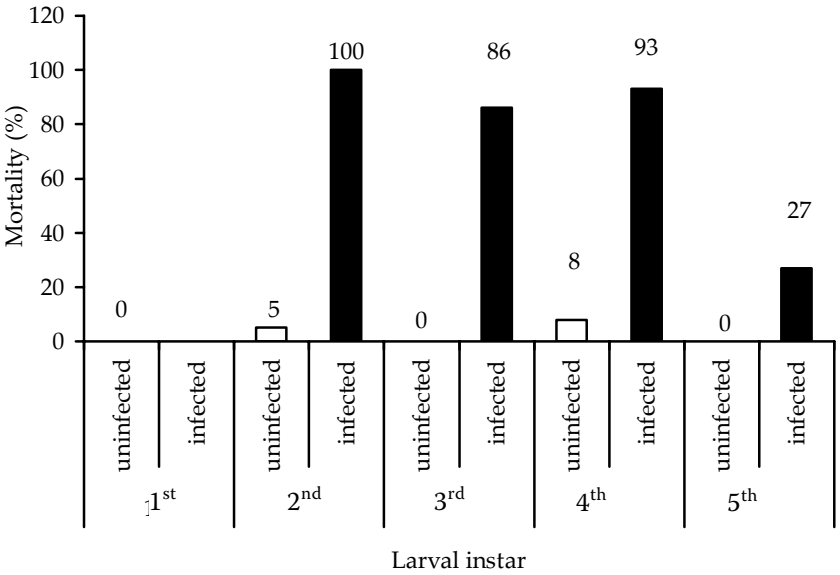

Figure 2. Mortality (%) of pupae of *Adoxophyes orana* developed from surviving larvae infected in each instar with AdorGV or from uninfected larvae of each control

Table 1. Proportion of the mortality of *Adoxophyes orana* larvae in the 5th instar from total mortality of larvae infected with AdorGV in each instar and from the uninfected larvae in each control

Instar	Variant	Larvae mortality (%)	
		in total	5th instar from total larvae mortality
1st	infected larvae	100	**0**
	uninfected larvae	68	0
2nd	infected larvae	96	**50**
	uninfected larvae	12	0
3th	infected larvae	72	**89**
	uninfected larvae	12	67
4th	infected larvae	40	**90**
	uninfected larvae	0	0
5th	infected larvae	12	**100**
	uninfected larvae	0	0

Oho (1975), Geest and Evenhuis (1991) and Andermatt (2005) who stated that larvae infected with AdorGV died only in the last (5th) instar regardless when they had been infected. Andermatt (2005) stated that infected larvae will not die before the last instar and, therefore, only applications of CAPEX® against the young over-wintering larvae might reduce fruit damage. In the present study, a possible explanation for the high mortality rate of larvae in early instars shortly after infection could be that the virus had weakened the young larvae, thereby increasing their sensitivity to less suitable conditions (in this case the unnatural feed). Therefore, the application of virus against larvae in early instars could reduce the damage of the treated generation. Larvae infected in subsequent instars died predominantly in the 5th instar (Table 1). Dead larvae of the 5th instar infected with AdorGV did not change dark green to milk yellow as described by Geest and Evenhuis (1991), but became even darker (Figure 4).

Table 2. Comparison of the number of pupae of *Adoxophyes orana* developed from surviving larvae infected with AdorGV in each instar and from uninfected larvae in each control, and a comparison of the number of adults of *Adoxophyes orana* hatched from these pupae

Instar	Number of pupae developed from surviving larvae – in each cup (total)		Difference	Number of adults hatched from surviving pupae – in each cup (total)		Difference
	infected	uninfected control		infected	uninfected control	
1st	0, 0, 0, 0, 0 (0)	2, 1, 1, 2, 2 (8)	++	0, 0, 0, 0, 0 (0)	2, 1, 1, 2, 2 (8)	++
2nd	0, 0, 0, 0, 1 (1)	5, 4, 4, 5, 4 (22)	++	0, 0, 0, 0, 0 (0)	5, 3, 4, 5, 4 (21)	++
3th	1, 3, 1, 1, 1 (7)	5, 4, 4, 5, 4 (22)	++	0, 1, 0, 0, 0 (1)	5, 4, 4, 5, 4 (22)	++
4th	2, 3, 4, 3, 3 (15)	5, 5, 5, 5, 5 (25)	++	0, 0, 0, 0, 1 (1)	5, 5, 4, 5, 4 (23)	++
5th	5, 4, 5, 5, 3 (22)	5, 5, 5, 5, 5 (25)	–	2, 2, 4, 5, 3 (16)	5, 5, 5, 5, 5 (25)	+

– insignificant difference (α = 0.05); + moderately significant difference (α = 0.05); ++ highly significant difference (α = 0.01)

Table 3. Comparison between the relative number of adults of *Adoxophyes orana* hatched from survived pupae which developed from larvae infected in each instar and the number of adults from uninfected larvae of each control

Instar	RN of adults hatched from pupae (5 = 100%) in each cup (total)		Difference
	infected	uninfected control	
1st	/	1, 1, 1, 1, 1 (5)	NT
2nd	/	1, 0.75, 1, 1, 1 (4.75)	NT
3th	0, 0.33, 0, 0, 0 (0.33)	1, 1, 1, 1, 1 (5)	++
4th	0, 0, 0, 0, 0.33 (0.33)	1, 1, 0.8, 1, 0.8 (4.6)	++
5th	0.4, 0.5, 0.8, 1, 1 (3.7)	1, 1, 1, 1, 1 (5)	–

RN – relative number; NT – not tested; / low number of adults; – insignificant difference (α = 0.05); + moderately significant difference (α = 0.05); ++ highly significant difference (α = 0.01)

Mortality of pupae

The mortality of pupae developed from infected larvae was high. With pupae developed from larvae infected in the 2nd instar it was 100%, in the 3rd instar 86%, in the 4th instar 93% and in the 5th instar 27%. The mortality of pupae developed from uninfected larvae was low (max. 8%) in each control (Figure 2). Statistical analysis shows highly significant differences between the relative number of adults developed from pupated larvae infected in the 3rd and 4th instar and adults hatched from pupated larvae in the controls. If we compare the relative numbers of adults hatched from pupated larvae infected in the 5th instar and that of adults hatched from pupated larvae in the control, the differences are insignificant (Table 3).

The high mortality of pupae developed from infected larvae in this study indicate a much higher potential of AdorGV for decreasing the population density of *A. orana* than the authors cited earlier had reasoned from investigating only the effect of AdorGV on the mortality of the larval stadium.

Total mortality

The combined mortality rates of larvae and pupae after infection of larvae with AdorGV in the 1st and 2nd instar were 100%, in the 3rd and 4th instar it was 96% and in the 5th instar 36% (Figure 3). Statistical analyses found highly significant differences between the numbers of adults developed from pupated larvae infected in the 1st, 2nd, 3rd and

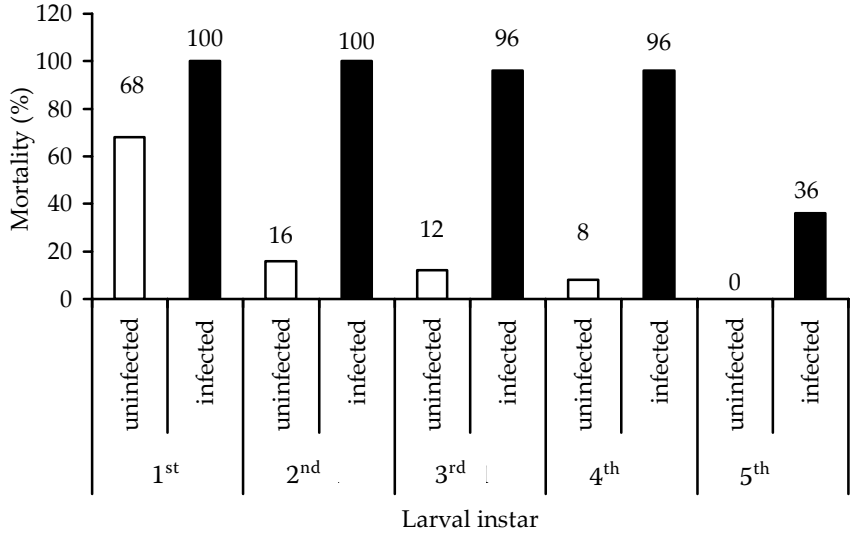

Figure 3. Combined mortality (%) of larvae and pupae of *Adoxophyes orana* after infection of larvae with AdorGV in each instar and of uninfected larvae and pupae from each instar

Figure 4. Dead *Adoxophyes orana* larva of the 5th instar infected by AdorGV

4th instar and the numbers of adults developed from pupated uninfected larvae in the controls. Significant differences were recorded between the numbers of adults developed from pupated larvae infected in the 5th instar and those of adults hatched from pupated uninfected larvae in the control (Table 2).

This implies that the susceptibility of larvae to the virus did not decrease from the 1st to the 4th instar (only mortality rates in the larvae decreased) because if individuals did not die as larvae, they subsequently died as pupae. The susceptibility decreased markedly only in the 5th instar. As was reviewed by Yamada and Oho (1973), summer fruit tortrix larvae are highly susceptible to infection with AdorGV in early instars and that susceptibility decreased remarkably after the 4th and 5th instars.

As well as showing the high efficacy of CpGV-based preparation in reducing the *Cydia pomonella* population density (the persistence of CpGV among the *C. pomonella* individuals surviving CpGV treatment is low) in fruit orchards (Kundu *et al*. 2003), the high mortality of *Adoxophyes orana* larvae and pupae in laboratory conditions demonstrated the high efficacy of the virus used. That corresponds with results obtained by Kocourek *et al*. (2007) who investigated the efficacy of AdorGV in apple orchards in the Czech Republic in comparison with chemical insecticides; they found that AdorGV had a high efficacy similar to the most effective chemical insecticide Cascade 5 EC. On the other hand, e.g. in the case of *Lymantria dispar* infected with *Lymantria dispar* nucleopolyhedrovirus (LdMNPV), the mortality of larvae was low, whereas the frequency of virus persistence in the surviving insect population was high (Cunningham *et al*. 1991). Whether AdorGV causes mortality in infected larvae or continues into subsequent generations probably depends on the quantity of the virus administered and of course on the larval instar which became infected. Stará *et al*. (2007) discovered a strong persistence of AdorGV in surviving larvae after direct treatment with

Table 4. Comparison between the number of larvae that died in the same instar in which they were infected by virus AdorGV and the number of larvae that died in that instar of each control

Instar	Infected		Uninfected control		Difference
	number of dead larvae in each cup (total)	proportion from total larvae mortality (%) of larvae that died in the same instar in which they were infected	number of dead larvae in each cup (total)	proportion from total larvae mortality (%) of larvae that died in the same instar in the control variant	
1st	5, 5, 5, 5, 5 (25)	100	3, 3, 4, 4, 3 (17)	100	++
2nd	2, 3, 3, 2, 2 (12)	50	0, 1, 1, 0, 1 (3)	100	++
3th	0, 1, 0, 0, 1 (2)	11	0, 0, 1, 0, 0 (0)	33	–
4th	0, 1, 0, 0, 0 (1)	10	0, 0, 0, 0, 0 (0)	0	–
5th	1, 1, 0, 1, 0 (3)	100	0, 0, 0, 0, 0 (0)	0	–

– insignificant difference (α = 0.05); + moderately significant difference (α = 0.05); ++ highly significant difference (α = 0.01)

AdorGV in apple orchards, causing high mortality of larvae in subsequent generations (after the 2nd or 3rd year of AdorGV treatment, the population density of *A. orana* was reduced to zero in each of three treated orchards).

The results of this study imply that the effect of AdorGV on reducing the population of *Adoxophyes orana* among subsequent generations is greater than published up to now. In addition to the virus causing high mortality rates in larvae infected in the 1st, 2nd, 3rd and 4th instar, it also causes high mortality rates in pupae developed from surviving larvae. Furthermore, results of this study show that the virus increases the sensitivity of newly hatched larvae and larvae of the 1st and 2nd instar (with decreasing effectiveness) to unfavourable conditions and thus reduces their vitality.

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