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Production and field evaluation of a Granulosis Virus for control of *Sesamia cretica* Led. (Lep., Noctuidae) in maize fields in Egypt

G. Fédière,[1] M.A.K. El-Sheikh,[1] A. M. Semeada,[2] A. El-Hefny,[1] M. Masri[1] and S. El-Sherif[2]
[1]Entomovirology Laboratory, ORSTOM, and [2]Corn Borers Research Laboratory, Department of Economical Entomology and Pesticides, Faculty of Agriculture, Cairo University, Giza, Egypt

Abstract: Pilot field trials were conducted against *Sesamia cretica* Led. using a Granulosis Virus recently isolated from the corn borer infesting maize at Kafr El-Sheikh Governorate. The efficacy of spray application of *Sc*GV was compared with the currently recommended chemical insecticides (Methomyl and Monocrotophos) as two different control strategies against *S. cretica* larvae on maize. The viral insecticide is a highly purified suspension of granules in water plus 10% molasses as a sticker as well as a feeding stimulant additive. The spray containing 0.1 g of granules/l (*i.e.* 1.48×10^{10} I.B./ml) was applied twice at the rate of 1.25 and 2.5 l/0.01 feddan (1 feddan = 4200 m^2) at 2 week intervals, starting 3 weeks after sowing. For the chemical insecticides, the methomyl was applied at the same time as the *Sc*GV suspension against *S. cretica* larvae, and the monocrotophos was applice twice, 2 months after sowing, and 2 weeks later, against *Ostrinia nubilalis* and *Chilo agamemnon* larvae. All treatments were made using a portable knapsack sprayer. Additional plots were left as control. Results revealed that both tested treatments (microbial and chemical insecticides) noticeably reduced the numbers of *S. cretica* larvae. Regarding yield, the mean weight of seeds was higher for the plots which were treated with either virus or insecticide than untreated ones. However, the chemical insecticide treatment was more effective than the formulated virus treatment. The corrected yield in ardab (1 ardab = 145 kg)/feddan were 20.11, 16.04 and 12.79, for chemical insecticide, viral insecticide and control treatments, respectively. It is concluded that *Sc*GV seems to be pathogenic to the field population of *S. cretica* and may be considered a promising biocontrol agent to support the Integrated Pest Management Programme of such an important corn borer.

1 Introduction

The Pink Borer, *Sesamia cretica* Led., is one of the most important corn borer in Egypt. Until now, no viral agent has been tested against this pest. Some Granulosis Viruses (GV) from family Baculoviridae have already been tested as biological insecticides to control pests and appear to be promising candidates. Large-scale applications of GV in pest management programmes have been made against certain destructive insects, such as the potato tuber moth *Phthorimaea operculella* (Matthiessen et al., 1978), the codling moth *Cydia pomonella*, (Huber and Dickler, 1977), the Indian meal moth *Plodia interpunctella* (McGaughey, 1975), the cabbage worm *Pieris rapae* (Tatchell and Payne, 1984) and *Agrotis segetum* (Caballero et al., 1991).

The present work tests the field efficacy of spray application of a recently isolated GV (Fédière et al., 1993), infecting *S. cretica* (*Sc*GV) as a part of the program concerning the interrelationship between corn pests and their complex of viral diseases in Egypt. The objective is to select pathogenic viruses which could be proposed as biological control candidates.

2 Materials and methods

2.1 Production and purification of the Granulosis Virus

A stock culture of *S. cretica* larvae was maintained under laboratory conditions and intentionally infected with the wild-type Granulosis Virus isolated from Egypt and described by Fédière et al., (1993). The virus suspension used in the field experiments was a highly purified preparation of *Sc*GVat the appropriate concentration of 1.0 OD420/ml (1 OD420 = 1.48×10^{10} capsule/ml and 1 ml at 1 OD420 = 0. 125 mg capsule/ml, Chang and Tanada, 1978) mixed in distilled water.

2.2 Formulation of chemical insecticides

The recommended Methomyl (Lannate 90% W.P.) against *S. cretica* larvae and Monocrotophos (Nuvacron 40% S.L.) against *Ostrinia nubilalis* and *Chilo agamemnon* larvae were used.

2.3 Experimental design and spray applications

2.3.1 Locations

The experimental trials were carried out at two different localities of Egypt during the summer season of 1995: the north of the Nile Delta, at Sakha Agricultural Research Station, Kafr El-Sheikh Governorate, and the Nile Valley of middle Egypt, at Sids Agricultural Research Station, Beni-Suef Governorate.

Unfortunately, the results obtained from both untreated and treated plots of Sids Experimental Research Station were incomparable, since the population density of the corn borers was very low. Accordingly, this study concentrated on the results from Sakha Agricultural Research Station, only.

2.3.2 Field plots and sowing method

For every station, three blocks of 15 plots each, were specified for untreated (control), chemical insecticide treated (Lannate

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and Nuvacron) and biological insecticide treated (*Sc*GV) trials. The 15 plots of each block included five plots for regular sampling, five plots for yield assessment and five plots for collection of corn borers material (mainly larvae). The experimental unit plot was equivalent to 1/100 feddan (i.e. $42 m^2 = 6 m \times 7 m$). The total area of one block, including 15 plots, plus the borders and irrigation canals was about $1260 m^2$, i.e. a total area of $3780 m^2$. Every plot consisted of 10 rows with 20 hills for seeds separated by 30 cm. Maize variety 'Giza 2' was used for cultivating all plots. Sowing of seeds was conducted on April 23.

2.3.3 *Spray applications*

For Lannate 90% WP, the spray contained 3 g/l/0.01 feddan for the first application, 3 weeks after sowing, and 3 g/2 l/0.01 feddan, 2 weeks later.

For Nuvacron 40% SL, the rate was 15 ml/4 l of water/0.01 feddan, applied twice, at 2 week intervals, starting 2 months after sowing.

The viral suspension of *Sc*GV was used at the final concentration of 0.1 g of granules/l (i.e. 1.0 OD420/ml), plus 10% of molasse of sugar-cane, as a sticker, a feeding stimulant and a sunlight protector. The rate of the spray was 1.25 l/0.01 feddan for the first application, 3 weeks after sowing, and 2.5 l/0.01 feddan for the second, 2 weeks later.

2.4 Sampling procedure

Regular samples of maize plants were taken every 2 weeks, beginning the day of the first insecticide application and continued during the 12 forthcoming weeks. The first sampling is considered to have measured the larval population before treatments.

At each sampling date, 20 plants of each plot were pulled, following the zig-zag method. Plants were inspected for *S. cretica* larvae and other corn borer larvae.

2.5 Evaluation of the efficacy of the insecticides and viral diagnostic

The insects were classified in the field and kept individually in small plastic tubes, then transferred to the laboratory and kept in the freezer until required.

At harvest time, ears of a random row from each plot were weighed and shilled then their moisture content was measured using a universal moisture tester. Data representing yield in ardabs (1 ardab = 145 kg)/feddan for both treated and untreated plots was corrected at 15.5% moisture content by applying the following formula:

$$\text{Corrected yield} = \frac{\text{Shilled yield} \times (100 - \text{Seeds moisture Content})}{(100 - \text{allowed moisture content})}$$

Percent yield reduction for untreated plots was also worked out by applying the following formula:

$$\%\ \text{yield reduction} = [\text{Corrected yield} - \text{Shilled yield}] \times [100 - \text{moisture content}]$$

A digoxigenin-labelled *Sc*GV DNA probe was used according to the protocol recommended by the suppliers (Boerhinger). The dot blot method of hybridization (FÉDIÈRE et al., 1993) was applied for detecting a possible presence of viral DNA in the larvae.

3 Results and discussion

3.1 *Production of Granulosis Virus*

The average yield of purified *Sc*GV granules was 14.25 mg of granule/1 g of larvae, based on 10 separate extractions of virus from different batches of infected larvae. For a mean larval weight of 0.4 g for the last instar (fifth) dead larva, the production square with an average weight of 5.7 mg of granule/larva, i.e. 6.83×10^{11} granules/larva.

3.2 Epidemiological studies

Before the evaluation of the efficacy of the entomopathogenic viruses as biological control agents, investigation on the presence of the virus in the natural populations was required. This investigation was aimed towards an epidemiological study of the viral disease during 1995 season. Table 1 demonstrates the natural incidence of the virus at Kafr El Sheikh region. The percentage of estimated rates of virus occurrence showed that most of the larval population of *S. cretica* in the first generation harboured the virus, without developing any signs of disease under natural field conditions. Those larvae which appeared healthy usually became diseased when they were transferred to the laboratory for monitoring and rearing under different (stress) factors, and the majority of them die showing viral infection symptoms. Such stress factors often take place when the insects are reared at a high population density. Similarly, an outbreak of the pest in the field leads to a natural epizootic of the disease.

As shown in table 1, the number of recorded larvae decreased gradually from the first to the last larval instars (210/plants on May 28–21/plants on June 25). This result is mainly due to the various natural factors affecting larval population throughout the first generation.

In conclusion, the massive introduction of the specified virus could be applied, as a stress factor, to reduce the pest's population by causing an induced epizootic.

3.3 Evaluation of the efficacy of the biological insecticide

The efficacy of the *Sc*GV in comparaison with methomyl and monocotrophos, as control agents against *S. cretica* larvae on corn are shown in tables 2 and 3.

It is worth mentioning that biological control using *Sc*GV in this work, has its effect only on *S. cretica* larvae and that this effect does not extend to the other corn borer species (*Ostrinia nubilalis* and *Chilo agamemnon*)

Table 1. *Natural occurrence level of Granulosis Virus estimated in the 1st generation of* Sesamia cretica *larval population, Kefr El-Sheikh region, 1995. The virus was detected by using nucleic probe*

Date	Number of larvae	% Occurrence
May 28	210	96.92%
June 11	121	97.14%
June 25	21	100%
July 6	0	—

Table 2. Number of Sesamia cretica *larvae/100 plants from five plots, Kafr Sheikh region,* 1995

Sampling date	Chemical insecticide treated plots	Biological insecticide treated plots	Untreated plots
May 14*	Egg masses	Egg masses	Egg masses
May 28*	75	84	210
June 11	7	60	121
June 25	7	12	21
July 9	0	0	0

* Dates of insecticides applications.

or even to any other pests. On the contrary, the use of the 2 chemical insecticides had resulted in killing all present insects.

The evaluation of the efficacy of the biological insecticide was studied by two parameters, the population density and the crop yield assessment.

3.3.1 Population density

Data presented in table 2 clearly demonstrate that introduction of any of the tested control strategies (the chemical or biological insecticide) had resulted in a remarkable reduction in the population of larvae, compared to the control. A sharp reduction in the larval population (from 75 to 7) was recorded in the case of using chemical insecticide. However, with the biological insecticide treatment, population was declined slowely (from 84 to 60) during 2 weeks. This may be due to the one week incubation period required by the Granulosis Virus to be capable to induce its effect. Two weeks later, a relatively higher reduction (from 60 to 12) was recorded in the number of larvae, showing the persistent effect of the virus treatment.

3.3.2 Yield assessment

Data presented in table 3 demonstrate that the infestation with *S. cretica* has greatly influenced the total number of plants. The mean number of plants did not exceed 17 690/feddan in the untreated plots, while it reached 22 140 in the chemical insecticide treated plots. However, this mean number was in the middle (19 800 plants) in the case of the *Sc*GV treated plots. This result is expected since the infestation with *S. cretica* is characterized by causing the death of young plants (dead-heart symptoms).

Table 3 confirms that the same trend of results as infestation greatly affected the number of produced ears. The mean number of ears/feddan was the highest (21 970) in the chemical insecticide-treated plots, followed by the *Sc*GV treated plots (18 020) and the control (14 460). Comparison between the number of produced ears and the number of plants shows that the ratio of such relationship was high (0.99) for the chemical insecticide treatment, followed by 0.91 in the case of *Sc*GV treatment and dropped to 0.81 in the untreated plots. An explanation of the reduction in the number of ears is the lower numbers of plants in addition to the low mean number of ears per plant as well as.

Infestation with *S. cretica* had affected the weight of both ears and seeds. Consequently the estimated values of corrected yield in ardabs/feddan were 20.11 for the chemical insecticide treated plots, 16.04 for the *Sc*GV treated plots and only 12.79 for the untreated plots.

Finally, the calculated percentage of yield reduction was 36% in the case of the untreated plots and only 20% in the *Sc*GV treatment.

Generally speaking, application of the virus treatment had achieved a considerable degree of control of *S. cretica* that lies near, but under, that obtained from applying the chemical insecticide.

Our results in line with those reported by CABALLERO et al. (1991); who used a Granulosis Virus to control *Agrotis segetum* on corn seedlings in Spain and found that the crop protection was similar to that produced by chemical insecticides. Also *Phthorimaea operculella* GV was tested on the potato tuber moth on potato in Peru by Raman et al., in 1987 and *Pieris brassicae* GV on *Pieris rapae* on cabbage in England by TATCHELL and PAYNE (1984) with success. Moreover, excellent results were recorded with *Cydia pomonella* GV used in apple orchards against the insect host in England (GLEN and PAYNE, 1984) and in Canada (JAQUES et al., 1994).

We concluded that ScGV seems to be a suitable potential biocontrol agent and can be considered as a potential pest control agent. It is worth mentioning that the achieved results are preliminary and further detailed are required, eventually to provide sufficient reliable information on the doses of field application, adjuvants and other expected possible ways of increasing the pathogenicity and the host range by combination with other virus(es).

Table 3. Maize yield/feddan and percentage of yield reduction assessed from five plots in untreated and chemical or biological treated plants, Kafr El-Sheikh region, 1995

Treatment	Mean no. of plants	Mean no. of ears	Mean weight of ears (in kg)	Mean weight of seeds (in kg)	% Water content	Corrected yield (in ardabs)	% yield reduction
Chemical insecticide	22140	21970	3572	2937	16.12	20.11	—
Biological insecticide (*Sc*GV)	19800	18020	2776	2344	16.17	16.04	20.24
Untreated	17690	14460	2291	1859	16.08	12.79	36.40

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Authors' addresses: Dr G. FÉDIÈRE (corresponding author), s/c Pr. Peter Tijssen, Centre de Recherches en Virologie, Institut Armand Frapier, 531 Boulevard des Prairies, CP 100, Laval Montreal, Quebec, Canada; M.A.K. EL-SHEIKH, A. M. SEMEADA, M. MASRI, LEC-ORSTOM, PO Box 26, Giza, Egypt; A. EL-HEFNY and S. EL-SHERIF, Corn Borers Research Laboratory, Department of Economical Entomology and Pesticides, Faculty of Agriculture, Cairo University, Giza, Egypt