Studies on the specificity of Agrotis segetum Granulosis Virus fed to larvae of 17 species of noctuoids

Undersøgelser af specificiteten af ageruglens kapselvirus (Agrotis segetum GV) over for larver af 17 arter af Noctuoidea

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Summary

Cross-transmission studies with the larvae of 17 species of *Noctuoidea* were used to examine the host range of a granulosis virus of *Agrotis segetum* Schiffm. (AsGV). The identity of the virus observed in apparent cross-infection was checked by the enzyme-linked immunosorbent assay (ELISA). The virus replicated in, and killed larvae of *A. exclamationis* and *A. ipsilon* as well as *A. segetum*. In some tests with *Noctua pronuba* and *Lacanobia oleracea* high levels of mortality of larvae exposed to virus may have resulted from virus infection, although no inclusion bodies were observed. The virus did not appear to infect 12 other species.

Key words: Granulosis virus, *Agrotis segetum*, cross-transmission, specificity.

Resumé

Der er tidligere udviklet en opformeringsmetode for ageruglens kapselvirus (*Agrotis segetum* GV = AsGV) i laboratorieopdrættede knoporme i Danmark. Dette virus blev senere afprøvet med lovende resultater i små markforsøg, i Danmark over for larver af *A. segetum* (rødbede, gulerod og kartoffel) og i Pakistan over for *A. ipsilon* (tobak).

I denne afhandling redegøres der for en række forsøg til belysning af AsGV's specificitet. Larver af 17 uglearter blev fodret med AsGV opslemmet i vand. Døde larver blev undersøgt for indhold af AsGV, dels ved mikroskopi, dels ved hjælp af immunologiske metoder (ELISA).

Undersøgelserne viste, at AsGV blev opformeret i og dræbte larver af *A. segetum, A. exclamationis* (udråbstegnuglen) og *A. ipsilon*. AsGV har muligvis forårsaget mortalitet i larver af to uglearter tilhørende andre slægter, nemlig haveuglen *(Lacanobia oleracea)* og smutuglen *(Noctua pronuba)*. I ingen af disse tilfælde blev der dog fundet viruskapsler i de døde larver. Forsøgene med disse to arter bør gentages.

I 12 andre uglearter fra forskellige slægter blev der ikke fundet tegn på virusinfektion.

Sammenfattende synes undersøgelserne at vise en så høj grad af specificitet af *Agrotis segetum* kapselvirus, at virusets værtspektrum kan være begrænset til kun at omfatte arter af slægten *Agrotis*.

Nøgleord: Kapselvirus, *Agrotis segetum*, specificitet.

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Introduction

Cutworms belonging to the genus *Agrotis* are important pests of a great number of crops in agriculture, horticulture and forestry all over the world. In Denmark and in other parts of Western Europe the turnip moth, *A. segetum* (Syn. *Scotia segetum* Schiff.) is the major species involved.

Studies on the use of granulosis virus for the control of *A. segetum* in Denmark were initiated in 1974. A propagation method was developed, and control experiments in fields of carrots, beetroots and potatoes were carried out during the period 1975–1978 (*Zethner*, 1980). Those experiments and similar trials with tobacco seedlings in Northern Pakistan (*Shah et al.*, 1979) showed *A. segetum* granulosis virus (AsGV) to be an effective biological control agent against *A. segetum* as well as against *A. ipsilon*.

Investigations of the specificity of AsGV were run simultaneously, testing 17 different species of moths (*Noctuoidea*) in laboratory bioassays. The results from those tests supported by immunological investigations of diseased larvae are presented in this paper.

Materials and methods

The 17 species tested are presented in Table 1.

Two species belong to the family *Lymantriidae* and 15 species to 4 subfamilies of the family *Noctuidae*. Names are reported according to *Karsholt* and *Nielsen* (1977). The two species of *Lymantria* were obtained as eggs: *L. monacha* from Danish forests, *L. dispar* from a laboratory culture at Institute of Virology, The Natural Resources Research Council, Oxford, United Kingdom. *Spodoptera frugiperda* were obtained from the same British Institute. All other species were caught as adults in a light trap placed at the National Research Centre for Plant Protection, Kgs. Lyngby, Denmark.

The moths were fed on 5–10% honey solution in water. They laid their eggs on either muslin cloth, crepe paper or on the sides of plastic boxes in which they were placed.

Larvae were reared on an artificial diet similar to that used by *Harrap et al.* (1977).

Table 1. List of species tested
Oversigt over de undersøgte arter

Family/Subfamily	Species
Lymantriidae	*Lymantria dispar*
	Lymantria monacha
Noctuidae:	*Noctuinae*
	Agrotis exclamationis
	Agrotis ipsilon
	Agrotis segetum
	Axylia putris
	Noctua (Triphaena) pronuba
	Xestia (Diarsia) triangulum
	Hadeninae
	Discestra (Scotogramma) trifolii
	Lacanobia (Diataraxia, Mamestra) oleracea
	Mamestra (Barathra) brassicae
	Melanchra persicariae
	Mythimna (Leucania) comma
	Amphipyrinae
	Apamea (Hadena) monoglypha
	Caradrina morpheus
	Spodoptera frugiperda
	Plusiinae
	Autographa gamma

Bioassays

In most tests second instar larvae were used for the cross-transmission studies carried out in 1975 and 1977. In 1981 some tests were repeated with first instar larvae of three species (see Table 2).

The number of test insects varied between 25 and 250 per bioassay (normally 50–100). Two groups of insects were fed simultaneously for 48 hours: One group on diet soaked with AsGV-suspension containing approximately 10^7 capsules per ml water, the other group on diet soaked with pure water only. All diet was dried to the same degree before start of bioassays.

After 48 hours both virus-treated and non-treated larvae were reared on diet without AsGV, according to methods described by *Hansen* and *Zethner* (1979).

Enzyme-linked immunosorbent assay (ELISA)

The double antibody sandwich ELISA method as described by *Crook* and *Payne* (1980) was used to examine the larvae which had died during the cross-transmission experiments for the presence of AsGV. Antisera to capsules of AsGV and

Table 2. Summary of cross-transmission experiments with *Agrotis segetum* GV
Resultater fra infektionsforsøg med Agrotis segetum *kapselvirus*

Species	Corrected mortality[1]) in test no: 1	2	3	4	5	6	significance virus-/ non treated	GV present in test larvae[2])
L. dispar	–2[6])	0					NS[3])	
L. monacha	3	–8					NS[3])	
A. exclamationis	83	58	60	75			$p<0.001$[3])	+
A. ipsilon	98						–[5])	+
A. segetum	92	70	89	100	82	70	$p<0.001$[3])	+
Ax. putris	14	27	21				NS[3])	
N. pronuba	29	76	58				NS[3])	(+)
X. triangulum	–14	–2	2				NS[3])	
D. trifolii	13						–[4])	
L. oleracea	–3	96					NS[3])	(+)
M. brassicae	–30						–[4])	
M. persicariae	93						–[5])	
My. comma	–242	–47					NS[3])	
A. monoglypha	18						–[4])	
C. morpheus	8	4	6	–8	41		NS[3])	
S. frugiperda	–42	–134	–10	50	–5	10	NS[3])	
A. gamma	–8						–[4])	
Repeated cross-transmission experiments:								
N. pronuba	100	80	82	50	–20	–30	NS[3])	
M. persicariae	38	–4	–4	–4	–4	–67	NS[3])	
S. frugiperda	6	0	0	0	–5	–11	NS[3])	

[1]) Mortality of virus treated larvae. Corrected for mortality of control larvae by the method of *Abbott* (1925)
[2]) Elisa-test
[3]) Student's t-test
[4]) No reliable test; a X^2 test would indicate NS (no significance)
[5]) No reliable test; a X^2 test would indicate $p<0,001$
[6]) Negative values for corrected mortality were obtained when mortality of control larvae exceeded mortality of virus-treated larvae

Pieris brassicae GV and purified γ-globulin fractions of these sera were prepared as described earlier *(Crook & Payne,* 1980). Larval extracts were prepared by homogenising larvae in destilled water. Virus inclusion bodies in the extracts were dissolved by adding sodium carbonate to a concentration of 0.1 M and leaving it for at least 15 minutes at room temperature. The extracts were then diluted tenfold by the addition of 0.02 M Na_2PO_4 (pH 7.4), 0.15 M NaCl + 0.05 per cent Tween 20. Further 10^{-1} and 10^{-2} dilutions of the larval extracts were also prepared in the same buffer.

The wells of polystyrene microtitre plates (129A, Dynatech Laboratories Ltd., Billingshurst, Sussex) were coated with specific antibody (200 μl/well) at a concentration of 4 μg/ml. After washing, duplicates of 3 dilutions of each larval sample were added to the wells. After incubation and washing, specific antibody conjugated with alkaline phosphatase, at a dilution of 1:800 or 1:1000 was added to each well. After further incubation and washing, enzyme substrate was added. The reaction was terminated by the addition of sodium hydroxide, and the colour intensity measured by reading the absorbance at 405 nm *(Crook & Payne,* 1980). Colour intensities greater than twice the background values were regarded as positive. Most samples were tested on at least two occasions.

Light and electron microscopy

Larval extracts from specimens which had been shown to be positive for the presence of virus by ELISA and those for which bioassay results had suggested that they might be infected, were examined by light and electron microscopy. For light microscopy, wet-mounted preparations were examined using phase contrast optics or dark field illumination. For electron microscopy samples were negatively stained with 2% uranyl acetate.

Results and discussion

Bioassays

The data are summarized in Table 2. Mortalities in virus-treated larvae were corrected for mortalities in the non-treated larvae by the method described by *Abbott* (1925).

High mortalities of approximately 60% or higher in *all* tests were only observed with *A. segetum* and *A. exclamationis*. Mortalities of virus-treated larvae were significantly higher than mortalities of non-treated larvae. In the one test with *Agrotis ipsilon*, 98% mortality was observed.

High levels of mortality were observed in most of the original tests with *S. frugiperda, L. oleracea, M. persicariae* and *N. pronuba*. For *N. pronuba* the mortality of the virus-treated larvae was close to be significantly different (5% level) from the mortality of non-treated larvae level. For the other three species there were great variations in either virus-treated or non-treated larvae, and the differences were not significant.

Inconsistencies in these results may have arisen as a consequence of a generally high level of mortality in some species, which can probably be attributed to difficulties in defining specific rearing requirements and conditions.

An attempt was made to repeat the cross-transmission tests with the above mentioned four species. However, larvae of *L. oleracea* could not be obtained.

The repeated tests showed no significant virusinduced mortality of *M. persicariae* and *S. frugiperda*, while the mortality in virus-treated larvae of *N. pronuba* again was close to be significant at the 5% level. Therefore it seems that additional bioassay-tests with *N. pronuba* and *L. oleracea* must be performed before these two species can finally be stated sensitive or insensitive to AsGV.

Serological and microscopic investigations

The ELISA test was used to check the presence of viral antigen in all 17 species exposed to AsGV (Table 2).

Strong positive reactions were only observed when extracts from larvae of *A. segetum, A. ipsilon* and *A. exclamationis* were used. These samples were also found to contain characteristic GV-capsules when examined by light and electron microscopy.

Earlier studies had demonstrated that the GV of *A. segetum* reacted to a much lower level with antibodies of *P. brassicae*-GV than to its homologous antibody, using the double antibody sandwich method *(Crook & Payne,* 1980). When the reactions of virus-infected larval extracts of *A. segetum, A. ipsilon* and *A. exclamationis* were compared by ELISA, using heterologous and homologous antibodies, the extracts consistently gave the strongest reaction with the antibody prepared to *A. segetum* GV. This suggested that the GV infection of *A. ipsilon* and *A. exclamationis* represented a true cross-transmission of AsGV.

Of other tests which gave high levels of mortality in the virus-treated larvae *(L. oleracea* and *N. pronuba)* larval extracts were examined by light and electron microscopy, but no viral inclusion bodies were observed.

However, since ELISA tests of *N. pronuba* and *L. oleracea* gave reactions which were marginally positive, the absorbance values in some experiments being approximately double the background levels, we cannot rule out the possibility, that AsGV may replicate at least to a low level in these two species, or replicate incompletely without production of capsules.

Recently a restriction endonuclease technique for testing specific content of DNA in viruses has been developed *(Miller & Dawes,* 1978). In future investigations on the specificity of Granulosis

Viruses this new technique should be used to support the ELISA analysis.

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