THE GRANULOSIS VIRUS OF INDIANMEAL MOTH:

RECENT DEVELOPMENTS

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Abstract

The granulosis virus of the Indianmeal moth (Plodia interpunctella (Hübner)) was first described by Arnott and Smith (1968). Since that time, it has been studied intensively as a microbial agent for this insect by numerous investigators. Major impediments to the use of this virus were the relatively labor intensive production and the unavailability of a formulated product.

Between 1980 and 1986, production was simplified to reduce costs and a formulation developed. The formulation is in the process of being patented by USDA-ARS, and we expect exclusive rights to be awarded shortly. Tests with the latest formulation show a high degree of efficacy and reductions in damage to a noneconomic level. This dry formulation can be applied either as a spray or a dust in processing lines where the commodity is in a thin layer and agitation occurs subsequent to treatment and prior to packaging. The commodity entering the marketing channels has assumed most of its value added costs. At the present time, there are few materials that can be used to protect a commodity after entering marketing channels and eventual consumption (a period estimated to be six months for dried fruits and nuts). We propose the virus be used for processed (finished) dried fruits and nuts as a protectant after processing, packaging and in the marketing channels.

Data are presented on formulation activity, efficacy, persistence as related to commodity temperature, control of incipient populations and efficacy of the formulation against six populations of Indianmeal moth from geographic areas throughout the United States.

Introduction

The dried fruit and nut industry contributes substantially to the economic well-being of California agriculture. This industry alone produces an annual average (1983-87) of over 983,000 tons of dried fruits and nuts which had an average farm value of over $1 billion. These high priced commodities are currently treated with chemical fumigants upon which the industry is dependent for protection of the commodity, not only in storage,

but also as it is processed and passes through marketing channels. We at the Fresno laboratory are researching alternatives to the chemical fumigants and one of those alternatives is the development of the granulosis virus (IMMGV) infectious to the Indianmeal moth (Plodia interpunctella Hübner) as a protectant for these commodities primarily in marketing channels.

The first studies of this virus by Arnott and Smith (1968) determined the ultrastructure and development of the granulosis in the insects' cells. They reported that the virus appeared to infect primarily the fat cells, although there was some evidence of its occurrence in the trachea. Later, laboratory studies by Hunter (1970) quantified the virulence of this organism to the Indianmeal moth. These studies prompted Hunter (1973) to conduct the first efficacy tests of Indianmeal moth granulosis virus on inshell almonds, peanuts and walnuts (Table 1). He obtained 95% to 99% control of the Indianmeal moth in these tests. In 1975 McGaughey conducted similar tests with corn and wheat and obtained 72% to 100% control. Studies by Kinsinger and McGaughey (1976) confirmed the previous results and also showed that the Indianmeal moth granulosis virus could probably be expected to protect these commodities for at least 1 year under normal storage conditions. Hunter (1977, 1979) further extended his studies to inshell almonds (97% to 100% control) and raisins (81% to 99% control) as commodities that could be protected by adequate concentrations of the Indianmeal moth granulosis virus. Together these studies indicate a high degree of Indianmeal moth control on a wide variety of commodity groups. The above tests were conducted with fresh aqueous diseased larval homogenates or dried powders of the homogenates produced by lactose coprecipitation with acetone. Diseased larvae were extracted by hand from the diets prior to use.

Table 1. Efficacy of IMMGV as a protectant against Indianmeal moth on various commodities.

Source	Formulation	Commodity	Control obtained (%)
Hunter et al., 1973	aqueous*, dust	inshell almonds inshell peanuts inshell walnuts	99.3 99.7 95.1
McGaughey, 1975	aqueous*, dust	corn, wheat	72-100
Kinsinger and McGaughey, 1976	aqueous*, dust	wheat	100
Hunter et al., 1977	aqueous	inshell almonds	97-100
Hunter et al., 1979	aqueous	raisins	81-99
Cowan et al., 1986	formulated with bran	inshell almonds and meats	100

*Coprecipitation with lactose.

Although these earlier studies showed that the virus was effective for stored grains and dried fruits and nuts, we decided that the best place to fit

this organism into the dried fruit and nut industry's processing and marketing scheme was to use it in the period between the time the product is processed and the time it is eventually consumed. Presently, once packaged and in the marketing channels the commodity is not protected from infestation by Indianmeal moth. The objective of the present research was to develop a more useable formulation of this granulosis virus and further define the activity limits of the virus.

Materials and methods

All the Indianmeal moth larvae were reared on the diet described by Tebbets et al. (1978) as modified from Kinney and Brinkman (1967). The diet and components include raw wheat bran, Brewer's yeast, methylparahydroxybenzoate, sorbic acid, 10% Vanderzant's vitamin mix, glycerol, water and honey. The two components complicating the formulation of this virus were, as mentioned before, glycerol and honey. Therefore we removed the glycerol and honey for the virus production. It was initially thought that this possibly would not work because they may be key components for larval development. However, as we found out, at least for one generation, Indianmeal moth larvae can survive and develop on this diet. Therefore we incubated larvae for approximately 10 days at 26.7°C on the diet not containing honey and glycerol, inoculated them with the granulosis virus and 10 days later, approximately 20 days after hatch, the diseased larvae and diet which was to be used as a carrier, was harvested. Fortunately this system is very close to being sterile with the exception of the Brewer's yeast. All the components have been sterilized and unless a microorganism is added by contamination the formulation is very clean.

Once the mixture was harvested it was homogenized in sterile distilled water, the homogenate was then freeze-dried and after freeze-drying then milled. After milling the formulated product was then passed through a series of sieves in order to get the particle size down to a level that it could be applied through spray nozzels or as a dust. These formulations are very potent and the problem with consistency observed with the previous formulations was eliminated by the removal of glycerol and honey. After milling and sieving the dust formulation is bioassayed per os and typical results of one of these bioassays can be seen in Table 2. The LC_{99} for one of these formulations is approximately 14 milligrams of the granulosis formulation per kilogram of diet (14 μg/g). We believe the granulosis should be applied to the commodity at least at the LC_{95} concentration, preferably the LC_{99}, and actually recommend using the upper 95% confidence limits of the LC_{99} which would cost on the order of $4.08 per ton treated.

Table 2. Potency of IMMGV dust formulation.

Estimate of potency	Dose (μg/g)	Number of tons	Cost per ton[1]
Lower 95% CL	5	44	$0.82[2]
LC_{99}	14	15	2.45[2]
Upper 95% CL	25	9	4.08[2]
10 x LC_{99}	150	1.5	24.50

[1]Does not include overhead or application costs.
[2]Economically feasible application rates.

As shown in Table 3 the production costs for the granulosis virus formulation are estimated to be $36.00 for 200 grams. This includes 50¢ for the larval diet which in turn is the carrier for the virus, Indianmeal moth eggs $4.00, inoculum $1.50 and labor $30.00 which totals $36.00 for a 200 gram batch.

Table 3. Estimated costs[1] for producing 200 g of the IMMGV formulation.

Item	Cost
Larval diet/GV carrier	$0.50
IMM eggs	4.00
Inoculum	1.50
Labor	30.00
TOTAL	$36.00

[1]Does not include overhead.

Using the LC_{99} as a basis for treatment at 14 micrograms of formulation per gram of commodity, it is estimated that a 200 gram production lot of the Indianmeal moth granulosis virus will treat more than 16 tons or over 14,000 kilograms of commodity applied as a spray suspension (Table 4). I might also mention that the technical granulosis virus can be applied as a dust by dilution with ground bran or some other inert material (Cowan 1986).

Table 4. Potency of IMMGV dust formulation, USDA-ARS Fresno test batch F-12-12-20.

LC_{99} = 14 μg/g = 14 mg/Kg
200 g will treat 14,286 Kg as a spray.

Using doses ranging from the lower confidence limits of the LC_{99} and the upper confidence of the LC_{99} we can arrive at a cost per ton varying from 82¢ to $4.08 (Table 5). Comparing these costs to the various methods used per disinfestation at the present time, which are primarily fumigation, methyl bromide fumigation may cost 32¢ to 86¢ per ton, phosphine may cost $2.66 to $5.00 per ton, and the Indianmeal moth granulosis virus used as a protectant ranges from 82¢ to $4.08 per ton depending upon what dosage is used. Modified atmospheres also being studied at Fresno cost between $4.40 to $6.80 per ton. In addition the formulation will provide extended protection to the commodities in marketing channels.

The potency of these formulations can probably be further improved by adjustments in the time of inoculation and the larval densities used for production. However, at this time we feel that we have conducted enough research on the production method/formulation for it to be taken over by industry if they are so inclined.

Table 5. Relative costs of fumigation, modified atmospheres and the granulosis virus formulation.

Treatment	Cost per ton
Methyl bromide fumigation	$0.32 - 0.86[1]
IMMGV protectant	0.82 - 4.08[2]
Phosphine fumigation	2.58 - 4.98[1]
Modified atmosphere	4.40 - 6.80[1]

[1]From report DE-A104-83AL24327, 1986.
[2]Does not include overhead or application costs.

We have conducted tests with this virus on various populations of Plodia interpunctella obtained from various geographical areas in the United States. These populations included the Fresno laboratory strain which had been in culture for 400+ generations, a Florida lab strain, a Fresno, California wild strain collected on figs, a wild strain collected in Indio, California on dates, a wild strain collected in San Jose, California in a household and a strain from South Carolina collected on corn. We found that there are basically two susceptibility groups within these populations (Table 6). One group had LC_{50}s ranging from 0.17 to 0.25 micrograms per gram of diet. Another population (San Jose) which was intermediate in susceptibility having an LC_{50} of 0.48 micrograms per gram of diet and the South Carolina strain was the least susceptible having an LC_{50} of approximately 0.69 micrograms of formulation per gram of diet. However, the differences between the San Jose and South Carolina strains were not significant. These differences are not that much different from those found by Hunter and Hoffmann (1973) in which a 7-fold difference between two laboratory strains of Indianmeal moth were obtained. They suggested the difference in susceptibility may be due to either genetic differences or the length of time under colonization. Our data show the length of time under colonization, i.e., 1 year versus 30 years, had little or no influence on susceptibility.

Table 6. Susceptibility of 6 populations on Indianmeal moth larvae to formulated granulosis virus.

Location	Number of generations	Commodity	Susceptibility[1] ($LC_{50}^{\cdot}$) µg/g
Fresno, CA lab	400+	unknown	0.22 I
Florida lab	18	peanuts	0.25 I
Fresno, CA	3	figs	0.18 I
Indio, CA	2	dates	0.17 I
San Jose, CA	1	household	0.48 II
South Carolina	2	corn	0.69 II

[1]N = a minimum of 3,300 larvae/population.

Biological investigations associated with these studies suggested that the slower growing populations were less susceptible to the granulosis virus. The South Carolina strain or population of Indianmeal moth was the least

susceptible to the formulation but also had the longest rate of development. The next least susceptible population also had the next to longest rate of development. The other four populations developed faster and were more susceptible. Thus, population differences may have to be considered when developing not only the granulosis virus but also other types of control measures.

In further tests with this new formulation we determined the formulation's sensitivity to 3 different storage temperatures (27°, 32° and 38°C) for storage periods of 0, 1, 3, 6 and 12 months. Applications were made to raisins in a commercial packing line and were automatically packed in 1 pound cartons. These tests confirmed the previous studies of Kinsinger and McGaughey (1976). Control could be obtained for up to 6 months at 27°C while sustained temperatures of 32° and 38°C caused reductions in efficacy and increased damage between 1 and 3 months exposure. It should be kept in mind that exceedingly high infestation rates were used in these tests. Raisins would rarely be subjected to temperatures of 32° and 38°C for extended periods of time without having quality adversely affected.

Other studies have been conducted to determine the influence of the formulation on raisins already infested with Indianmeal moth. Good control of infestations less than 2 weeks old (held at 26.7°C) was attained (Table 7). Although mortality of older infestations is reduced and reproduction may occur, residues from the applied granulosis virus or that in infected larvae will provide effective control of the next generation. At normal temperatures and considering the increase in granulosis virus titre in the parent population it is reasonable to assume that little or no increase in damage would occur from the progeny.

Table 7. Control of incipient Indianmeal moth populations on raisins with the granulosis virus (IMMGV).

Post infestation inoculation time (days)	Doses tested (mg/Kg)	Range of mortality due to treatment	
		Parental generation	Progeny
0	100, 500 or 1,000	99.5 - 100	99.7 - 100
14	100, 500 or 1,000	72.1 - 91.6	84.0 - 93.3
28	100, 500 or 1,000	47.0 - 66.4	57.3 - 85.3
42	100, 500 or 1,000	25.4 - 29.1	72.2 - 75.6

Conclusions

Studies during the last 20 years have indicated a high degree of efficacy of the Indianmeal moth granulosis virus for control of the Indianmeal moth on dried fruits and nuts as well as grains. However, up until the present time, commodities have been treated with wet preparations made just prior to use or with preparations that were prepared by acetone coprecipitation with lactose. One of the problems involved in production of the Indianmeal moth virus was that it was tedious and labor intensive, because larvae were removed by hand from the media.

The new formulation of the granulosis virus developed at the Fresno laboratory provides at least the same and probably higher activity than previous preparations and in addition is stable, is in a dry form, and is easy to use either as an aqueous spray or as a dust. Efficacy testing of this formulation on dried fruits and nuts has shown that we can expect a high level of control from these formulations and that persistence is probably long enough to provide extended protection from populations of Indianmeal moths naturally infesting these commodities. Studies on incipient populations show that although the formulation cannot provide complete control of later instar larvae already infesting a commodity, the persistence of the applied virus coupled with that produced in infected larvae can provide a ready source for control of the progeny of the original populations. For these reasons the Agricultural Research Service has instigated patent procedures on this formulation and it is hoped in the future that it will become commercially available.

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LE VIRUS DE LA GRANULOSE DE LA PYRALE DES FRUITS SECS

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RESUME

Le virus de la granulose de la mite de la pyrale des fruits secs a été décrit pour la première fois par Arnott & Smith (1968). Depuis cette date, de nombreux chercheurs l'ont étudié intensivement en tant qu'agent microbien de lutte contre cet insecte. Le faible rendement de sa production et l'absence de formulation convenable du produit avaient jusque là empêché son utilisation.

Entre 1980 et 1986, on a simplifié sa production pour en réduire les coûts et une formule a pu être développée. Cette formule a été brevetée, et nous nous attendons à en acquérir prochainement les droits. Des études entreprises avec cette dernière formule démontrent sa haute efficacité sur la réduction des ravages. Cette formule sèche peut être appliquée soit comme aérosol soit comme poudre dans la chaîne de conditionnement, là où la marchandise arrive en couche mince et où elle a été remuée à la suite de son conditionnement avant l'emballage. En pénétrant dans le circuit commercial, le produit a intégré la plus grande partie de sa valeur ajoutée. Actuellement, il existe peu de substances pouvant protéger les marchandises après leur mise sur le marché et jusqu'au moment de leur consommation éventuelle (une durée estimée à 6 mois pour les fruits secs et les noix). Nous suggérons d'utiliser le virus sur les noix et les fruits secs conditionnés (terminés) comme agent de protection après conditionnement, emballage et dans le circuit commercial.

Nous donnons des précisions sur l'action de la formule, son efficacité, sa persistance en rapport avec la température du produit, l'élimination des populations naissantes ainsi que son efficacité contre 6 populations de pyrale des fruits secs (IMM) originaires de différentes régions des Etats Unis.