# CHAPTER 6

## Evaluation of a granulosis virus against the potato tuber moth *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) under laboratory conditions

### ABSTRACT

A local strain of the potato tuber moth granulosis virus (PTM GV) was isolated, propagated and evaluated against larvae of the potato tuber moth on stored tubers. It was found that the virus stayed virulent for at least nine years when stored at –20 °C and at least two weeks when kept in suspension at 25-28 °C. The virus stayed virulent in cadavers, even when exposed to the sun, but the virus in water suspensions started to break down when kept in the sun for six hours. A concentration of one to five pulverized diseased larvae per liter of water resulted in the mortality of nearly all larvae that fed on treated potato tubers. However, damage always occurred to tubers before the larvae died in their fourth instar. Despite the damage, the life cycle of the moth is broken just before pupation. It is concluded that the PTM GV has potential as a control agent in a market where limited damage to tubers is tolerated, like the small-scale farmers.

**Key words**: *Phthorimaea operculella*, potato tuber moth, potatoes, granulosis virus, microbial control.

## INTRODUCTION

The potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), is an important insect pest of potatoes in South Africa (Visser & Steyn 1999). The larva of this moth is a miner that tunnels into leaves and potato tubers. Mining in leaves during the season does not normally result in yield loss (Fuglie *et al.* 1991), but when tubers are damaged, it becomes unmarketable. Tubers are attacked before harvest under the soil as well as in storage. Tuber moth control in the field is aimed at keeping numbers low during the season by insecticidal spraying programs. However, the treating of tubers in stores is not permitted in South Africa due to the potential of toxic residues on an edible crop. For control of potato pests, the International Potato Center (CIP) emphasizes the value of using and adopting integrated pest management procedures that provide adequate control while reducing dependence on insecticides (Raman *et al.* 1987). There is thus a pressing need for safer methods to control the potato tuber moth in store environments in South Africa.

The genus *Baculovirus* contains two well-known groups of insect viruses, namely the granulosis baculoviruses and the nuclear polyhedrosis viruses (Whitten & Oakeshott 1991). The potato tuber moth virus is an occluded granulosis baculovirus (Burges 1981). This virus was first discovered in 1964 in Australia (Reed 1969) and first reported in South Africa ten years later (Broodryk & Pretorius 1974). It is species specific, will only attack the larvae of the potato tuber moth (Reed 1971), and does not infect vertebrates (Hails 2001). The potato tuber moth granulosis virus (PTM GV) was probably introduced with the pest itself when it spread from its origin in South America to the rest of the world (Briese & Mende 1981).

The PTM GV has been tested on foliage under field conditions (Reed 1971; Reed & Springett 1971; Ben Salah & Aalbu 1992) as well as on tubers in storage (Amonkar *et al.* 1979; Raman & Alcazar 1988; Von Arx & Gebhardt 1990). In all these tests relative to excellent control was achieved. Sporleder *et al.* (2001) developed a bioassay method to assess inactivation time of PTM GV at different intensities of natural irradiation. Briese & Mende (1981) found differences in susceptibility between different field populations of the

tuber moth in Australia while Vickers *et al.* (1991) found distinct geographical genotypes (or strains) of the PTM GV across continents.

The present study is mainly concerned with the evaluation of the activity of the local strain of granuloses virus against the potato tuber moth under controlled environments. The aim of the study was to tests crude suspensions with virus-infected cadavers and its stability under certain conditions over time. It did not intend to establish lethal concentrations ($LC_{50}$), but rather to find rough estimates of the number of cadavers needed to use in self-made suspensions. The aim was therefore firstly to establish the potential of the virus for future research and secondly to be able to give guidelines for small farmers who wish to cultivate the virus themselves.

## METHODS

*Finding the virus*

Diseased larvae are easily identified by their milky white appearance in contrast to the translucent green or brown color of healthy larvae (Matthiessen *et al.* 1978). Fully-grown, diseased larvae also fail to orient to pupation substrates such as dry soil, but they will cluster around food material such as leaves, green stems and tubers (Reed & Springett 1971). A survey of experimental fields at ARC-Roodeplaat failed to find any larvae adhering to the above descriptions. Keeping in mind the worldwide endemic nature of the PTM GV (Briese & Mende 1981) and the naturally low incidence of the disease in fields (Reed 1971), it was then decided to induce the disease in an insectary population. High numbers of first instar larvae from the rearing facilities at ARC-Roodeplaat were put on potato tubers after which they were incubated at 30 – 32 °C in plastic buckets (300 x 300 x 300 mm). The lids of these buckets were put loosely over the opening, allowing just enough air to prevent moisture from forming on the inside. This was to increase the humidity to above 80%. These stress conditions of overcrowding, high temperature and high humidity resulted in the appearance of lethargic milky white larvae that appeared on the surfaces of the tubers after three weeks incubation. To test whether these were the virus-infected larvae, they were collected, pulverized in water and made up in one-liter suspensions. The suspensions was used to dip clean tubers after which new infestations

with healthy larvae were made. A control with tubers dipped in distilled water was added and incubated at 26 °C in well-aerated containers. The control produced healthy larvae and pupae, while the tubers dipped with the suspension produced only milky white larvae that failed to pupate. These white larvae were stored in Petri dishes in a deep freezer at –20 °C for future use. All literature cited regarding the use of PTM GV gives vivid descriptions of symptoms of infected larvae. It was always identical and potential confusion with any other disease or disorder was never mentioned in any literature. This, plus the fact that the virus was already isolated and described in South Africa (Broodryk & Pretorius 1974), made taxonomic identifications by electron microscopy unnecessary.

The initial study started in 1992, but was terminated after inconclusive results were obtained under field conditions. Diseased larvae from that year were kept in storage at –20 °C and used again to start new stock solutions for the present study that started in 2001. The virus in this study was thus stored for nine years at –20 °C and proved to be still virulent.

*Choosing the most practical formulation*

Different formulations have been used in studies with PTM GV. These include; number of viral capsules per milliliter (Briese & Mende 1983), larval equivalents per milliliter (Von Arx & Gebhardt 1990), macerated diseased larvae (cadavers) per milliliter (Reed & Springett 1971) and mg/ℓ dried flakes (Broodryk & Pretorius 1974). However, Raman & Alcazar (1988) stressed the fact that there is no difference in efficacy between purified and crude virus and recommended the use of pulverized diseased larvae. For practical reasons and ease of use in the future by small-scale farmers, it was decided to follow the recommendations of Raman & Alcazar (1988).

*Inoculations*

The handling of diseased larvae was according to Reed & Springett (1971). Larvae were stored as intact diseased cadavers at –20 °C in Petri dishes. From here they were collected, pulverized and filtered through a fine sieve. Depending on the test, between one and 20 cadavers were pulverized per one liter of distilled water. One of two additives was

added for wetting and sticking purposes. They were the pH buffer/wetter Bludbuff® and the oil, BP Agripon Super®.

Tubers of the cultivar BP1 were used as substrate in all tests. Medium sized tubers, approximately 100 to 150g, were dipped in the virus suspensions for two minutes. They were left to dry at room temperature for approximately two hours before inoculations with larvae were conducted. All treatments, including those with insecticides and the water control were handled in the same manner. The virus suspension was vigorously agitated before tubers were dipped and special precautions were taken not to contaminate the other treatments with the virus, e.g. handling the virus treatments last.

Two types of tests were conducted. Firstly, a no choice test with a suspension of five diseased larvae per liter was used to test the stability of the suspension, the treated tubers and also the virus inside the cadavers. Ten neonate larvae were put on a treated tuber by means of a fine camel hairbrush. These tubers were isolated in 500 ml plastic containers with white sand supplied at the bottom inside of the container. This was to ensure that the fourth instar larvae that survived pupated in the sand in the relative container. These containers with tubers were incubated at 26 °C for five weeks before counting of the pupae. A complete randomized design was followed with one tuber per container and four replicates.

Secondly, two free choice experiments were conducted where tuber moths were allowed to lay eggs on treated tubers. These tests followed a randomized block design with four replicates. Small crates, containing 10 tubers each, were placed on the floor of an insectary. Tuber moths, 20 moths per crate for the first and 30 moths per crate for the second experiment, were released inside each crate. The room was kept dark at 26 °C for four weeks after which the number of tubers that showed any sign of damage in each treatment was counted. All tubers in each crate were then placed in individual plastic buckets with white sand around the tubers. All surviving larvae left the tubers to pupate in the sand. Pupae were counted three weeks later. The tubers were inspected daily for white larvae straying around or on top of the tubers. These diseased larvae were collected,

counted and placed in separate containers with white sand in case they could still pupate – they never did, confirming mortality.

## RESULTS

PTM GV stayed active inside cadavers for at least nine years at –20 °C and in suspension for at least 14 days at 25 to 28 °C (Table 1). However, when the suspension was exposed to the sun for six hours, the virus started to break down, as indicated in the number of larvae succeeding to pupate in treatments. When treated tubers or intact cadavers were left in the sun for a few hours, however, the virus were still effective against the tuber moth larvae. The virus stayed effective on treated tubers for at least four days when stored in the dark.

A suspension of as little as one diseased larva per liter of water resulted in the eventual death of nearly all larvae tested, as indicated by unsuccessful pupation (Tables 2 and 3). Adding one of two wetting agents did not make any difference to the treatments. However, damaged to tubers always occurred, even at the highest dosage of 20 diseased larvae per liter. Diseased larvae were harvested from virus treated tubers, while no diseased larvae were found on the untreated controls, indicating that the virus did not spread through the air.

## DISCUSSION

From the results it is clear that the South African strain of PTM GV is very virulent and kills nearly all larvae that come in contact with it. It is stable and stayed effective for nine years in cadavers at -20 °C. It seems that the sun is able to break down the virus when in suspension. A dosage of one to five pulverized cadavers per liter always resulted in more than 90% mortality of larvae (expressed as unsuccessful pupation), while a tested dosage of eight or more pulverized cadavers did not give rise to any progeny. However, damage to tubers was always observed irrespective of survival to pupae. The virus only kills the larvae just before the pupal stage and infected larvae are still able to inflict damage similar

to healthy ones. This was clear in the results where the highest dosage of 20 diseased larvae per liter still resulted in 100% damage of tested tubers.

Significantly less ($P < 0.001$) diseased larvae were harvested where a higher dosage of virus was used. This corresponds with the finding of an overdose effect that may kill some larvae instantly at a very young age (Reed 1971). He found that the PTM GV could affect larvae in one of two ways. Firstly when low dosages of the virus are ingested, the virus multiplies inside the larvae during the rest of the larval development and eventually kills it. Secondly, when higher dosages of the virus are ingested, a toxicosis effect is obtained. This normally does not allow the virus to multiply inside the larva, but kills the larva within 48 hours. According to Reed (1971), the overdose of larvae is not preferred because natural multiplication and spread of the virus plays an important role in the epizootic under field conditions. However, because of the stagnant conditions in stores with no wind or rain, the chances of an epizootic are less likely. This is also evident from the results of this study where no contamination was observed from tubers treated with the virus to untreated tubers in crates next to each other. The potential of overdose of tuber moth larvae with the PTM GV should therefore be investigated for storage conditions. This would eliminate the critical issue of the slow rate of kill, thus preventing any damage to tubers from occurring. However, it is clear that this dosage would be more than 20 cadavers per liter of water (Table 2): 20 cadavers/liter of water still resulted in a 100% damage of tubers. A dosage of twenty cadavers per liter of water is relatively high from a production viewpoint, and when large quantities of potatoes have to be treated, these numbers may be difficult to produce. However, further research into improving the efficacy of the virus in suspension and better application methods may results in higher mortalities at lower dosages.

This study has shown that a dosage of one larva (cadaver) per liter kills nearly all larvae that fed on treated tubers. Other researchers have used varying dosages of between one and 15 larvae per liter, depending on whether it was used in the field or on potato tubers in storage. Lacey & Goettel (1995) found that generally 200 to 400 larval equivalents of virus per hectare are required to achieve effective control. This is more or less in line with the finding of this study of a relative effective larval equivalent of 1 larva per liter (potato farmers spray approximately 400 liters of water mixtures per hectare). Reed & Springett

(1971) found excellent control under field conditions with a single application of 15 cadavers per liter of water and maintained a residual effect of 12 weeks under field conditions. Raman & Alcazar (1988) found five cadavers per liter of water to be effective in storage. Five larvae were also found to be effective in this study, mostly giving rise to no progeny (Table 1). However, a dosage of even one larva per liter of water, although not killing all larvae, was effective. It is therefore concluded that crude water extracts of one to five diseased larvae per liter of water will be adequate to break the life cycle of the potato tuber moth. Higher dosages did not increase its effectiveness.

Although propagation of PTM GV has been done under field conditions (Matthiessen *et al.* 1978), the method in the insectary in this study was adequate to supply reasonable numbers of diseased larvae for small storage conditions. Approximately one to five diseased larvae were harvested per 150-gram tuber, less than that reported by Reed & Springett (1971), who harvested approximately 20 diseased larvae per 150-gram tuber. Reed (1971) found that a dosage of one macerated diseased larvae per 10 liters of water was sufficient for multiplication purposes of the virus. It is clear that different dosages are warranted for purposes of control and multiplication of the virus respectively. The low rate of propagation found in this study (Table 3) could be due to the fact that relative high dosages were used. Using lower dosages closer to one larva per 10 liters, as prescribed by Reed (1971) may result in a higher propagation rate than that found in this study.

Two of the limitations of baculoviruses are the slow rate of kill and their poor stability in the field (Whitten & Oakeshott 1991). Attempts thus far to enhance the potency and speed of kill by biotechnological techniques have not succeeded (Casida & Quistad 1998). Another concern is the discovery of very high levels of resistance against the virus in some tuber moth populations (Briese & Mende 1983). Environmental, ecological and production factors in potato fields may have adverse negative effects on the effectiveness and spread of the PTM GV (Reed 1971). However, under storage environments, the conditions are vastly different. Environmental correlates in stores are much more stable and may favour the development of the virus (Von Arx & Gebhardt 1990). The common notion is that diseases are more prevalent in dark, warm and moist environments. Such conditions are most common in potato stores, especially those used by small-scale farmers.

The potential of the PTM GV may be in its usage by small-scale farmers rather than by commercial farmers. Agrochemical companies generally prefer to develop broader spectrum biological control agents like *Bacillus thuringiensis* (Whitten & Oakeshott 1991). The issue of already proven resistance (Briese & Mende 1983) and slow action may also play an important part in a companies' decision to spend millions of rands in developing an insecticide. In a market where some degree of damage to tubers is tolerated, and where the virus can be manually propagated without any input costs, this control agent has potential. Such a market is the small-scale farmer in developing countries (Fuglie *et al.* 1991). In several cases in developing countries, farmer's cooperatives and individual farmers already participate in virus production, either supplying central facilities with dead infected larvae or producing their own virus (Whitten & Oakeshott 1991). Raman & Alcazar (1988) found that the virus stays effective for more than 60 days in storage. Such potatoes will therefore be protected for at least two months, and reapplying of treatments will therefore be limited. The inexpensive nature of this control agent, its safety aspect as well as the long residual action makes it an ideal control agent for use by the small-scale farmer.

The results reported here indicate that the South African strain of PTM GV has huge potential as an effective control agent against potato tuber moth larvae in stores, especially for the small-scale farmer that cannot afford to use cold storage. The farmers can easily handle the propagation and production of the virus themselves, thereby eliminating expenses. The characteristics of baculoviruses, such as specificity and safety to non-target organisms make them desirable agents in integrated pest management programs (Moscardi 1999). The PTM GV has therefore definite potential as a microbial control agent for integrated pest management in rustic store environments, a conclusion shared by Von Arx & Gebhardt (1990).

## REFERENCES

AMONKAR, S.V., PAL, A.K., VIJAYALAKSHMI, L. & RAO, A.S. 1979. Microbial control of potato tuber moth (*Phthorimaea operculella* Zell.) *Indian Journal of Experimental Biology* **17**: 1127-1133.

BEN-SALAH, H. & AALBU, R. 1992. Field use of granulosis virus to reduce initial storage infestation of the potato tuber moth, *Phthorimaea operculella* (Zeller), in North Africa. *Agriculture, Ecosystems and Environment* **38**: 119-126.

BRIESE, D.T. & MENDE, H.A. 1981. Differences in susceptibility to a granulosis virus between field populations of the potato moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae). *Bulletin of Entomological Research* **71:** 11-18.

BRIESE, D.T. & MENDE, H.A. 1983. Selection for increased resistance to a granulosis virus in the potato moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae). *Bulletin of Entomological Research* **73**: 1-9.

BROODRYK, S.W. & PRETORIUS, L.M. 1974. Occurrence in South Africa of a granulosis virus attacking potato tuber moth, *Phthorimaea operculella* (Zeller). *Journal of the Entomological Society of Southern Africa* **37**: 125-128.

BURGES, H.D. 1981. Microbial Control of Pests and Plant Diseases. Academic Press, London.

CASIDA, J.E. & QUISTAD, G.B. 1998. Golden age of insecticide research: past, present and future. *Annual Review of Entomology* **43**: 1-16.

FUGLIE, K., SALAH, H.B., ESSAMET, M., TEMIME, A.B. & RAHMOUNI, A. 1991. The Development and adoption of Integrated Pest Management of the Potato Tuber Moth, *Phthorimaea operculella* (Zeller) in Tunisia. *Social Science Department, Working paper* Series No. 1991-7, CIP Peru.

HAILS, R. 2001. Natural and genetically modified baculoviruses: environmentally friendly pest control or an ecological threat? *Outlook on Agriculture* **30**: 171-178.

LACEY, L.A. & GOETTEL, M.S. 1995. Current developments in microbial control of insect pests and prospects for the early 21st century. *Entomophaga* **40**: 3-27.

MATTHIESSEN, J.N., CRHISTIAN, R.L., GRACE, T.D.C. & FILSHIE, B.K. 1978. Large scale field propagation and the purification of the granulosis virus of the potato moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae). *Bulletin of Entomological Research* **68**: 385-391.

MOSCARDI, F. 1999. Assessment of the application of Baculoviruses for control of Lepidoptera. *Annual Review of Entomology* **44**: 257-289.

RAMAN, K.V. & ALCAZAR, J. 1988. Biological control of potato tuber moth, *Phthorimaea operculella* (Zeller) using a granulosis virus in Peru. In: *Asian Potato Association (APA), Proceedings*, June 16-24 1988, Kuming, China, 173-174.

RAMAN, K.V., BOOTH, R. H, & PALACIOS, M. 1987. Control of potato tuber moth *Phthorimaea operculella* (Zeller) in rustic potato stores. *Tropical Science*. **27**: 175-194.

REED, E.M. 1969. A granulosis virus of potato moth. *Australian Journal of Science* **31**: 300-301.

REED. E.M. 1971. Factors effecting the status of a virus as a control agent for the potato moth (*Phthorimaea operculella*) (Lep., Gelechiidae). *Bulletin of Entomological Research* **61**: 207-222.

REED, E.M. & SPRINGETT, B.P. 1971. Large field testing of a granulosis virus for the control of the potato moth (*Phthorimaea operculella*) (Lep., Gelechiidae). *Bulletin of Entomological Research* **61**: 223-233.

SPORLEDER, M., ZEGARRA, O., KROSCHEL, J., HUBER, J. & LAGNAOUI, A. 2001. Assessment of the inactivation time of *Phthorimaea operculella* granulovirus (PoGV) at different intensities of natural irradiation. CIP Program Report 1999-2000 (2001): 123-128.

VON ARX, R. & GEBHARDT, F. 1990. Effects of a granulosis virus, and *Bacillus thuringiensis* on life-parameters of the potato tubermoth, *Phthorimaea operculella*. *Entomophaga* **35**: 151-159.

VISSER, D & STEYN, P.J. 1999. Voorkoms en beheer van peste en plae. In: Steyn, P.J. (ed.) Handleiding vir Aartappelverbouing in Suid-Afrika. 133-152. Agricultural Research Council, Roodeplaat.

VICKERS, J.M., CORY, J.S. & ENTWISTLE, P.F. 1991. DNA characterization of eight geographic isolates of granulosis virus from the potato tuber moth (*Phthorimaea operculella*) (Lepidoptera, Gelechiidae). *Journal of Invertebrate Pathology* **57**: 334-342.

WHITTEN, M.M. & OAKESHOTT, J.G. 1991. Opportunities for modern biotechnology in control of insect pests and weeds, with special references to developing countries. *FAO Plant Protection Bulletin* **39**: 155-195.

**Table 1.** The mean number of pupae that were harvested from treated potato tubers. n =10

| Handling of treatments with five pulverized larvae in suspension per liter of water | No. of pupae |
|---|---|
| Untreated (water) control | 8.8a |
| Virus suspension | 0.5c |
| **Suspension in translucent glass bottle** | |
| six hours in the sun | 3.3b |
| two days in dark 25-28 °C | 0.5c |
| four days in dark 25-28 °C | 0c |
| 14 days in dark 25-28 °C | 0c |
| four days in dark 4 °C | 0c |
| **Tubers after treatment, before infestation** | |
| two days in dark 25-28 °C | 0.3c |
| four days in dark 25-28 °C | 0c |
| three hours in sun | 0.5c |
| **Cadavers, before pulverizing and dipping** | |
| one day in dark at room temperature | 0.3c |
| six hours in petri dish in sun | 0.3c |
| nine years at -20 °C | 0.5c |

Means followed by different letters were significantly different ($P < 0.001$), SEM = 0.27, LSD(5%) = 0.79 using Fisher's protected t-test.

**Table 2.** The number of pupae harvested from treated tubers (n = 10) where moths were allowed to lay eggs on tubers

| Treatment (cadavers per liter of water) | Pupae harvested |
|---|---|
| Untreated control | 72.5a |
| Control + 1ml/ℓ BPAS | 65.5a |
| Control + 2 drops BB | 63.8a |
| 0,2 + 2 drops BB | 2.8b |
| 1 | 0.5b |
| 1 + 2 drops BB | 0.3b |
| 1 + 1ml/ℓ BPAS | 0.8b |
| 20 + 2 drops BB | 0b |

Means followed by different letters within columns were significantly different ($P < 0.001$), SEM = 6.1, LSD(5%) = 17.9 using Fisher's protected t-test.
BB = Bludbuff wetting agent
BPAS = BP Agripon Super wetting agent

**Table 3.** The number of healthy pupae and diseased larvae harvested from treated tubers (n = 10) where moths were allowed to lay eggs

| Treatment | Pupae harvested | PTM GV larvae harvested |
|---|---|---|
| Untreated control | 132.3a | 0a |
| Methamidophos 1ml/ℓ | 110b | 0a |
| Lufenuron 1,6 ml/ℓ | 0.75c | 0a |
| 1 cadaver/ℓ | 0.5c | 19b |
| 8 cadavers/ℓ | 0c | 12.3c |
| SEM | 2.4 | 2.2 |
| LSD (5%) | 7.2 | 6.6 |
| F Prob. | <0.001 | <0.001 |

Means followed by different letters in the same column indicate significant difference at the 5% level, using Fisher's protected t-test.

# CHAPTER 7

## The potential of crude aqueous extracts of the syringa tree as a bio-insecticide against the potato tuber moth, *Phthorimaea operculella*, (Zeller) (Lepidoptera: Gelechiidae), on potatoes under laboratory and field conditions

### ABSTRACT

Crude plant extracts from the exotic invasive tree, *Melia azedarach*, also known as the syringa tree, were tested against first instar larvae of the tuber moth in the laboratory and against tuber moth infestations in the field. Laboratory bioassays showed that pupal weight was negatively influenced at high concentrations of syringa extracts and that growth of tuber moth larvae was retarded after feeding on potato leaves dipped in syringa extracts. Extracts from fresh syringa leaves were more effective than those from dried leaves. A concentration of 10 gram shredded syringa leaves per litre of water resulted in a mortality of 72% in the laboratory. At 40 gram shredded leaves per litre, control in the field was 70% in relation to the untreated control. Control was not satisfactory when potato tuber moth numbers were too high in the field (48%). However, syringa extracts always faired better than that of the reference insecticide, methamidophos. It became evident from the experimental data that extracts from the syringa tree have good potential to control the potato tuber moth in both field and under storage conditions.

**Key words:** *Phthorimaea operculella*, potato tuber moth, syringa, *Melia azedarach*, plant extracts.

## INTRODUCTION

The potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), is a cosmopolitan pest of potato. It is both a field and post harvest problem. The larvae tunnel into potato leaves and attack tubers under the soil when they are formed later in the season. Moths also lay their eggs on or near potatoes when they are stored in non-refrigerated environments. In South Africa it is responsible for an estimated R40 million damage to potato yields per annum. Although the status of the potato tuber moth varies from area to area, all commercial farmers use insecticides to control it. There were 23 insecticides registered in South Africa against the potato tuber moth on potatoes in 2002 (Nel *et al.* 2002). Preliminary tests, however, confirmed serious tuber moth resistance to the pyrethroids and organophosphates in some areas of South Africa (D. Visser, unpublished data). The newer groups of insecticides that were developed during the late 20$^{th}$ century are too expensive for the non-commercial potato grower in South Africa. In most of the developing world, these farmers can also not afford the cost of storing potatoes in cold stores where they are relatively safe from tuber moth attacks (Foot 1979; Das 1995). Most small-scale farmers use self-made diffused light stores (Raman *et al.* 1987) or rustic shelters (Roux *et al.* 1992) to store potatoes for consumption or seed. Potatoes in such stores are open to attacks by the potato tuber moth originating from dumping sites (Daiber 1989a) and can destroy the entire contents of stores within a short period (Fuglie *et al.* 1991). No chemicals are registered for use in a store environment on potatoes in South Africa (Nel *et al.* 2002). Some research on control methods in potato stores has been done (Raman *et al.* 1987; Daiber 1989a,b; Roux *et al.* 1992). However, simple, sustainable and environmentally friendly control measures are still lacking.

Interest in natural pest control agents has been renewed in recent years due to public concern over health and environmental safety of many products currently in use (Powel 1989). Plant materials as natural pesticides have been used for much longer than any other group of pesticides (Powel 1989; Berger & Mugoya 1995). They are usually locally available, cheap to harvest, sustainable, relatively safe to users and biodegradable (Mwamfuli 1995). One such plant is the exotic tree, *Melia azedarach*, also known as the sering, syringa or Persian Lilac tree. It was introduced into South Africa from India as an ornamental tree, but has become naturalised in southern Africa (Palgrave 1983). It can

grow up to 7 meters in height and is today considered an invasive plant in South Africa (Bromilow 1996). The syringa tree occurs everywhere where potatoes are grown in South Africa and are absent only in the northwestern parts of the Cape Province of South Africa (van Wyk & van Wyk 1997). It is known to have antifeedant properties in insects (van Wyk & van Wyk 1997; van Wyk & Gericke 2000). This tree was therefore a perfect candidate to test as a bio-insecticide against the potato tuber moth.

The study of natural occurring plants as insecticides against agricultural pests is highly warranted (Powell 1989; Das 1995). Das (1995) lists 35 plant species that have been studied for their effects against the potato tuber moth. However, the antifeedant and insecticidal properties of the syringa tree have never been studied against the potato tuber moth.

The aim of this project was to evaluate the potential of syringa as a bio-insecticide for the small-scale farmer who can do the extractions themselves. Although some studies have shown that certain plants may act as repellents against the potato tuber moth when dried leaves or branches were used to cover potatoes in stores (Lal 1987), this study was done to evaluate the antifeedant or insecticidal properties of extracts of the syringa tree leaves. Because the seeds of the syringa tree are known to be toxic to humans and livestock (van Wyk & van Wyk 1997), it was decided to work with leaf extracts only. A secondary aim was to study the influence of the syringa extracts against parasitoids of the potato tuber moth.

## METHODS

### *Preparation of extracts*

All leaves used in this study were picked from one of three syringa trees on the Roodeplaat research farm, 30 km north east of Pretoria (25°35'S, 28°21'E). Extracts were made from freshly picked leaves and also from leaves left to dry for six weeks. Leaves were detached from their stems and shredded into a pulp form by using a commercial Waring® blender. After weighing the desired quantity, the shredded leaves were placed in a plastic bucket filled with hot water (±50°C). The water containing the shredded leaves

was stirred and then left for the extraction process to continue naturally while the water was cooling. After approximately 24 hours, the extract (green water) was filtered off into a clean container.

*Laboratory trials*

Two substrates were used to do the bioassays, i.e. potato leaves and tubers. Potato leaves were collected from plants grown in a greenhouse and tubers were collected from a cool storage facility. The apical three leaves of a potato plant stem were used as a unit for the leaf substrate tests and medium sized tubers (100 to 150 g) were used for the tuber substrate tests. The substrates were dipped into the syringa extracts for two minutes and then left to dry for approximately 2 to 3 hours. During this time a wet piece of cotton wool was supplied to the stem end of each leaf unit to prevent it from wilting. After drying, each leaf unit was put into a Petri dish on a round piece of filter paper (Watman no. 2, 70mm) covering the bottom of the Petri dish. The filter paper was kept moist by adding 2 ml distilled water daily with a micropipette. All Petri dishes were kept closed for the duration of the tests, except when adding water. The leaves in the Petri dishes started yellowing and wilted after about 12 days, before all the larvae could reach maturity and their surviving potential were thus not tested. The tubers were placed in ½ liter plastic containers, containing approximately 30 grams fine building sand for pupation purposes of the larvae. Each container was covered with the rim of its lid (cut open), keeping a piece of tight fitting gauze material in place. This was to prevent any fourth instar larvae from escaping when they were searching for pupation locations.

Fresh first instar larvae (younger than 12 hours) were transferred to the dipped substrates by means of a fine camel hair brush. Five larvae were placed on each leaf unit and ten larvae were used for each tuber. All treatments were incubated at 26 °C. The leaves were dissected after 12 days under a stereomicroscope to determine the number and developmental stages of the larvae found. The larvae emerged from the tubers after about two weeks and constructed cocoons inside the sand in which they pupated. Cocoons were collected from the sand and the pupae removed for weighing purposes. Tubers were therefore used to evaluate mortality (unsuccessful pupation) and potato leaves to determine

whether syringa had a negative influence on the development of the larvae (anti-feeding properties).

*Field trial*

The field trial was conducted at Roodeplaat Agricultural Research Institute near Pretoria (25°35'S, 28°21'E). A randomized block design with six replicates was used. Each plot consisted of three rows containing 15 potato plants each. Only the middle row was evaluated. The cultivar, BP1, was planted and normal agricultural practices implemented to maintain the trial. The trial was under overhead irrigation. Applications with syringa extracts were done with a hand operated knapsack sprayer using a concentration of 40 grams fresh shredded leaves per liter of water. A reference insecticide, methamidophos, was applied at the recommended field dose of one milliliter per liter of water. Applications started when the first tuber moths were observed in the field; approximately 46 days after plant emergence.

The syringa and methamidophos treatments were applied eight times during the growing season with 7-day intervals. Three evaluations were done; 79, 93 and 108 days after plant emergence. All leaf mines in all plots were removed by hand and stored in labeled brown paper bags. These leaf mines were counted and dissected in the laboratory to detect any larvae present in the leaf mines. The larvae collected with this process in the field were put on tubers and reared to adults to determine the percentage parasitism. All tubers were examined for tuber moth damage at harvest.

*Statistical analyses*

For comparisons between the efficiency of dry *vs.* fresh leaf extracts, a factorial ANOVA was used to test for differences between concentrations, type of leaf extract and the concentration-by-type interactions. For all other tests, a one-way ANOVA was used to test for differences between treatments and the control. Means were separated using Fisher's protected least significant difference test.

## RESULTS

### Laboratory bioassays

In the untreated control treatment with potato leaves as substrates, all the surviving larvae were in the final instar (instar iv) after 12 days (Table 1). In the syringa treatments, the percentage of final instars decreased with the increase in concentration, while the percentage lower instars (ii and iii) increased (Fig. 1). Tubers treated with fresh leaf syringa extracts gave better control than with the dried leaves (Table 2). There were no significant differences between any of the concentrations with the fresh leaves, while the dried leaf extracts only showed effective control with the two highest concentrations. The weight of potato tuber moth pupae was significantly lower when they were fed on tubers treated with 40 g/ℓ and 80 g/ℓ fresh leaf syringa extracts (Table 3). However, the pupal weights of those larvae fed on tubers treated with dried out syringa leaves only decreased significantly at 80 g/ℓ.

### Field trial

Field treatments with fresh syringa extracts gave significantly better control than that of methamidophos during the first two evaluation dates when leaf mines were considered and during the first and last evaluation when live larvae were considered (Table 4). However, the good control achieved by the syringa treatment (73%) could not be maintained to the end of the season.

### Parasitoids

The parasitoids found in the field trial were *Copidosoma koehleri* and *Apanteles subandinus*. There were no differences in the percentage parasitism between the syringa treatment and the control ($P = 0.723$ for 13 Nov, 0.753 for 27 Nov and 0.502 for 12 Dec.). The initial percentage parasitism was 12%, increased to 39% and reached 78% at the end of the season (Fig. 2).

### Damage to tubers at the end of the season

The total yield in the untreated control plots reached 39.2 ton/ha, the syringa plots 49.5 ton/ha and in the methamidophos plots 40.0 ton/ha. However, no significant differences

were found between any of the three treatments (Wilcoxon, $P>0.05$). The damage to tubers in the different treatments varied between 2 and 3%, also without significant differences (Wilcoxon test, $P>0.05$).

## DISCUSSION

It was shown that crude aqueous extracts from both fresh and dried syringa leaves had a negative influence on the larvae of the potato tuber moth. It was also shown that the extracts from the fresh syringa leaves were more effective than that of the extracts from dried leaves. The lowest concentration of 10 g/ℓ fresh leaf extracts had a marked influence on the growth tempo of larvae as indicated by their growth stages after 12 days. This negative influence increased with the increase of syringa concentration. Pupal weights of larvae that survived to adults were significantly reduced at concentrations of 40 g/ℓ (or higher) fresh leave extracts and 80 g/ℓ dried leaf extracts.

Extracts from fresh syringa leaves were relatively effective under field conditions. Control relating to live larvae found in the field stayed between 44 and 67%. This was much better than the organophosphate, methamidophos, which could only maintain a control figure of between 20 and 27%. The poor performance of methamidophos was expected due to resistance found in some populations (D. Visser, unpublished data). The good performance by syringa extracts in the field is complimented by its apparent non-lethal effect on parasitoids. No differences could be found in percentage parasitism between the untreated control and the syringa treatment.

Although the control figures of field applied syringa (at 40 g/ℓ) are not as impressive as those of the new generations of insecticides (D. Visser, unpublished data), better control could be achieved if parasitoids could play a more important role. This is achievable in a small-scale farmer environment where more emphasis is put on sustainable and organic farming. The syringa tree fits perfectly into such an environment where it could be used to protect vegetables produced on a limited and small-scale.

The results with tubers treated with syringa extracts showed that it can effectively be used to protect stored potatoes against the potato tuber moth. The fact that there are no insecticides registered to protect potatoes under storage conditions, and that the potato tuber moth can destroy the contents of a potato store in a short period of time, makes this an important tool in the hands of the small-scale farmer. It can be used in a self-sustainable manner with no extra costs to the farmer and no negative influences to the environment. It is therefore well suited to be used in any non-commercial environment where potatoes are grown in small plots and where it is stored in non-refrigerated store environments.

## REFERENCES

BERGER, A & MUGOYA, C.F. 1995. Natural Plant Products as Pesticides. *Proceedings from the First National Symposium.* Lusaka, Zambia, 2nd - 5th August, 1994. Swedish University of Agricultural Sciences, Department of Plant Protection Sciences Report 4, 1995.

BROMILOW, C. 1996. Probleemplante van Suid-Afrika. Briza Publikasies Bk. Arcadia, Pretoria.

DAIBER, K.C. 1989a. A preliminary survey of the male flight of potato tuber moth, *Gnorimoschema operculella* inside and outside potato stores. *Phytophylactica*: **21**: 95-96.

DAIBER, K.C. 1989b. The potato tuber moth *Phthorimaea operculella*, in potato stores. Proceedings of the Potato Research Symposium, 1-2 August, Pretoria.

DAS, G.P. 1995. Plants used in controlling the potato tuber moth, *Phthorimaea operculella* (Zeller). *Crop Protection* **14**: 631-636.

FOOT, M.A. 1979 .Bionomics of the potato tuber moth *Phthorimaea operculella* (Lepidoptera: Gelechiidae) at Pukekohe. *New Zealand Journal of Zoology* **6**: 623-636

FUGLIE, K., SALAH, H.B., ESSAMET, M., TEMIME, A.B. & RAHMOUNI, A. 1991. The Development and adoption of Integrated Pest Management of the Potato Tuber Moth, *Phthorimaea operculella* (Zellar) in Tunisia. Social Science Department, Working paper Series No. 1991-7, CIP Peru.

LAL, L. 1987. Studies on natural repellents against potato tuber moth (*Phthorimaea operculella*) in country stores. *Potato Research* **30**: 329-334.

MWAMFULI, P.L. 1995. Opening address. In: Berger, A & Mugoya, C.F. (Eds.) Natural Plant Products as Pesticides. Proceedings from the First National Symposium. Lusaka, Zambia, 2nd - 5th August, 1994. Swedish University of Agricultural Sciences Department of Plant Protection Sciences Report 4.

NEL, A., KRAUSE, M. & KHELAWANLALL, N. 2002. A guide for the control of plant pests (thirty ninth edition). Directorate: Agricultural Productions Inputs. National Department of Agriculture, Republic of South Africa, Pretoria.

PALGRAVE, K.C. 1983. Trees of Southern Africa, second revised edition. C Struik, (Pty) Ltd, Cape Town.

POWELL, R.G. 1989. Higher plants as a source of new insecticidal compounds. *Pesticide Science,* **27**: 228-229.

RAMAN, K.V., BOOTH, R. H, & PALACIOS, M. 1987. Control of potato tuber moth *Phthorimaea operculella* (Zeller) in rustic potato stores. *Tropical Science.* **27**: 175-194.

ROUX, O., VON ARX, R. & BAUMGARTNER, J. 1992. Estimating potato tuberworm (Lepidoptera: Gelechiidae) damage in stored potatoes in Tunisia. *Journal of Economic Entomology* **85:** 2246-2250.

VAN WYK, B & VAN WYK P. 1997. Field Guide to Trees of Southern Africa. Struik Publishers, Cape Town.

VAN WYK, B & GERICKE, N. 2000. People's Plants, A Guide to Useful Plants of Southern Africa. Briza Publications, Arcadia, Pretoria.

**Table 1.** The number of surviving potato tuber moth larvae in potato leaves (treated with syringa extracts) after 12 days. The instars of the larvae (ii to iv) are indicated. n = 5

| Treatment | Instar | Replicates a | b | c | d | Av. |
|---|---|---|---|---|---|---|
| Control | iv | 4 | 4 | 5 | 5 | 4.5a |
| | iii | 0 | 0 | 0 | 0 | 0 |
| | ii | 0 | 0 | 0 | 0 | 0 |
| 10 g/l | iv | 1 | 1 | 4 | 3 | 2.3b |
| | iii | 3 | 2 | 0 | 1 | 1.5 |
| | ii | 1 | 0 | 0 | 0 | 0.3 |
| 20g/l | iv | 3 | 2 | 0 | 0 | 1.3bc |
| | iii | 0 | 2 | 4 | 3 | 2.3 |
| | ii | 0 | 0 | 0 | 2 | 0.5 |
| 30 g/l | iv | 2 | 1 | 1 | 1 | 1.3bc |
| | iii | 2 | 1 | 0 | 3 | 1.5 |
| | ii | 0 | 0 | 0 | 0 | 0 |
| 40 g/ℓ | iv | 0 | 1 | 1 | 0 | 0.5c |
| | iii | 3 | 1 | 2 | 3 | 2.3 |
| | ii | 0 | 0 | 0 | 0 | 0 |
| 80 g/ℓ | iv | 0 | 0 | 1 | 0 | 0.3c |
| | iii | 2 | 3 | 1 | 1 | 1.8 |
| | ii | 0 | 0 | 0 | 0 | 0 |

Means of instar iv, followed by different letters, indicate significant difference at the 5% level (Fisher's protected least significant difference test SEM = 0.49, LSD(5%) = 1.4).
Note: Total survival rates to adults could not be determined with this test.

**Table 2.** The mean number of first instar larvae (n = 10) that survived to adults on syringa treated potato tubers

| Treatments | No. of first instars | |
|---|---|---|
| | Dried leaves | Fresh leaves |
| Control | 9.8a | 9.2ab |
| 10g/ℓ | 9.8a | 2.6d |
| 20g/ℓ | 9.4a | 2.4d |
| 40g/ℓ | 7.6b | 2.8d |
| 80g/ℓ | 5.2c | 2.2d |

Means followed by different letters within and across columns were significant different ($P < 0.001$), SEM = 0.56, LSD(5%) = 1.6

**Table 3.** The mean weight (mg) of pupae collected from tubers treated with syringa leaf extracts. (n = 10)

| Treatments | Pupal weight (mg) | |
|---|---|---|
| | Dried leaves | Fresh leaves |
| Control | 12.27a | 11.95a |
| 10g/ℓ | 12.38a | 12.19a |
| 20g/ℓ | 12.69a | 12.38a |
| 40g/ℓ | 11.41a | 9.25b |
| 80g/ℓ | 9.96b | 8.95b |

Means followed by different letters within and across columns were significant different ($P = 0.044$),
SEM = 0.36, LSD(5%) = 1.02

**Table 4.** Mean number of potato tuber moth leaf mines and live larvae found per 15 plants for three different time intervals. The percentage control is given in parentheses.

| Treatments | Number of leaf mines | | | Number of live larvae | | |
|---|---|---|---|---|---|---|
| | Days after plant emergence | | | Days after plant emergence | | |
| | 79 | 93 | 108 | 79 | 93 | 108 |
| Untreated control | 84.3 (-)a | 303 (-)a | 119.0 (-)a | 36.2 (-)a | 147.2 (-)a | 38.3 (-)a |
| Syringa (40g/ℓ) | 22.2 (73)b | 159 (48)b | 76.7 (36)b | 11.8 (67)b | 84.2 (44)b | 17.8 (54)b |
| Methamidophos (1ml/ℓ) | 69.2 (18)c | 233 (23)c | 83.2 (30)b | 27.3 (25)a | 117.3 (20)ab | 28.3 (27)c |
| SEM | 4.3 | 17.8 | 8.5 | 2.9 | 11.9 | 2.8 |
| F Prob. | P< 0.001 | P< 0.001 | P= 0.011 | P< 0.001 | P = 0.012 | P = 0.001 |
| LSD (5%) | 13.5 | 56.0 | 26.7 | 9.0 | 37.4 | 8.8 |

Means followed by different letters in the same column indicate significant difference at the 5% level, using Fisher's protected t-test.

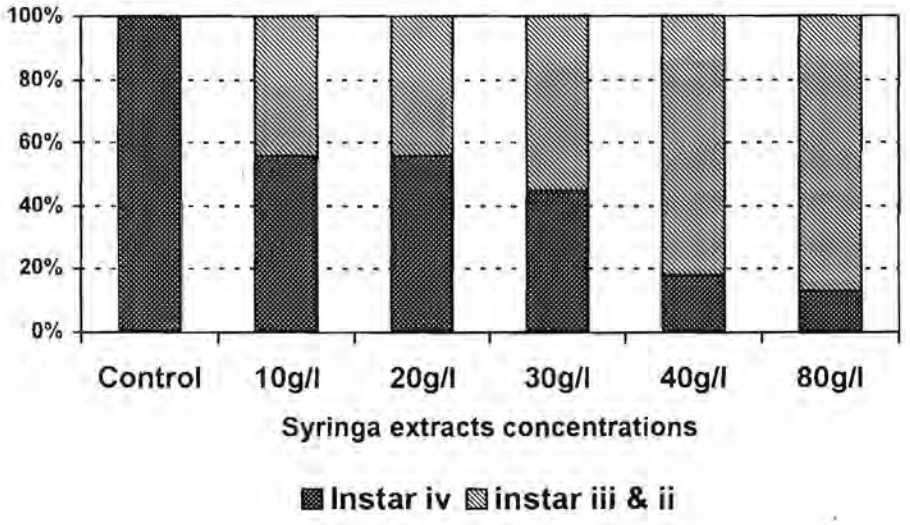


**Figure 1.** The percentage of surviving potato tuber moth instars after 12 days on syringa treated potato leaves (data recompiled from Table 1).

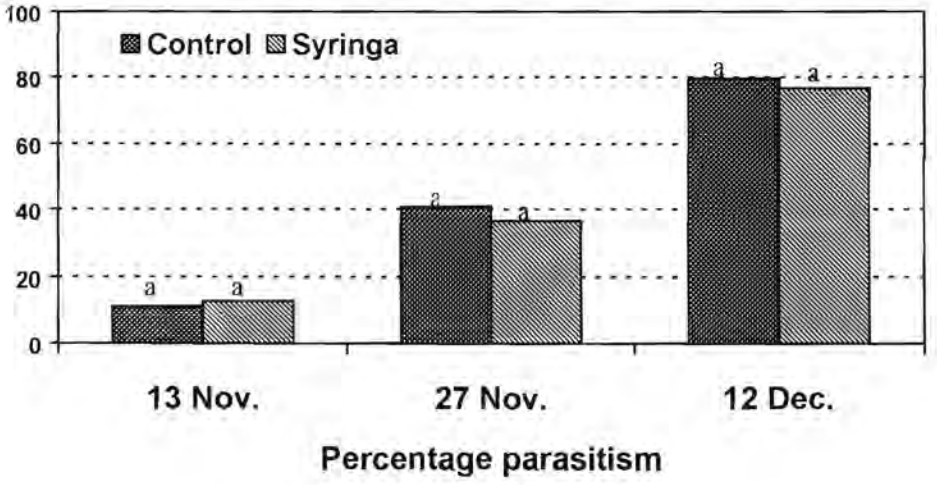


**Figure 2.** Mean percentage parasitism of potato tuber moth larvae in the syringa treated plots and controls. Means with the same letters were not significant at the 5% level for each monitor date.

# CHAPTER 8

## The potential of mating disruption and attract-and-kill techniques with pheromones against the potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), in a non-refrigerated store environment


### ABSTRACT

Potato tuber moth pheromones were evaluated for their efficacy against attacks of the potato tuber moth *Phthorimaea operculella* in store environments. Two formulations of synthetic pheromones were used; impregnated rubber capsules and an attract-and-kill formulation with permethrin in a sticky grease solution (attracticide). Both formulations contained the potato tuber moth pheromone E,Z,Z-4,7,10-tridecatrienyl acetate and E,Z-4,7-tridecatrienyl acetate (0.4/0.6 mg). One pheromone rubber capsule and two drops of the attracticide, *Last Call™ PTM*, were used per crate of potatoes. The aim of the tests with the pheromone rubber capsules was mating disruption and the aim with *Last Call™ PTM* was attract-and-kill. Efficacy was estimated by the inability of released moths to infest the potatoes. The pheromone rubber capsules were only effective when high numbers of tuber moths were released (> 10 moths per $m^2$). The attracticide, on the other hand, was only effective when low numbers of tuber moths were released (< 10 moths per $m^2$), and did not give any control when 20 moths per $m^2$ were released. The results are discussed relating to factors such as the pheromone formulations, techniques used, experimental layout and tuber moth behavior.


**Key words**: Potato tuber moth, *Phthorimaea operculella*, mating disruption, attract-and-kill, attracticide.

## INTRODUCTION

Potatoes in non-refrigerated stores are always prone to attacks by the potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) (Das *et al.* 1992). Non-commercial farmers often have to store seed tubers for up to nine months in self-made diffused light structures (Potts 1983) because planting times are seasonal (Hanafi 1999). These farmers use any available places or often old buildings where implements are also stored (Acasio *et al.* 1986). The main origin of the moths that infest tubers in such stores are the larvae already inside the tubers when they are stored (Ben Salah & Aalbu 1992; Kroschel & Koch 1994). When matured, these larvae exit the tubers to pupate in any place in the store (University Of California 1986) after which the moths eclose, mate, lay eggs and thereby infest the tubers in the same store again. Tuber moths that appear in stores in this way will therefore always be non-mated. If males find these females and mating take place, the result can be the total destruction of the contents of a potato store in just two moths (Fuglie *et al.* 1991). Potato tuber moths usually only mate once (Ono 1993), and can lay up to 236 eggs after mating (Fenemore 1977). The potential for damage of only a few individuals in a potato store are therefore huge.

Since the inception of gas chromatography in the 1950's, knowledge of insect pheromones has dramatically increased (Chapman 2000). Pheromones are today not only used for monitoring, but also mating disruption, mass trapping and as attracticides (Fields & White 2002). On potatoes it has been used extensively for monitoring potato tuber moth populations (Raman 1988; Daiber 1989; Raman 1994; Chandramohan 1995) and also to a lesser extend, mass trapping (Raman 1984; Raman *et al.* 1987). The success of mating disruption with various insect pests has resulted in commercially available pheromone formulations specifically for this purpose (Carde & Minks 1995). However, mating disruption with pheromones against the potato tuber moth has only been mentioned in the literature (Raman 1988), but no published scientific research has followed.

The reason for the lack of research on mating disruption with pheromones against the potato tuber moth is the unavailability of correctly formulated pheromones. Carde & Minks (1995) listed all the lepidopteran pests for which mating disruption pheromone

formulations were available, of which the potato tuber moth was not one. The only pheromones available thus far are the rubber-encapsulated formulation for monitoring purposes and *Last Call™ PTM* for attract-and-kill purposes. Mating disruption with rubber capsule pheromones against the potato tuber moth in the field has been evaluated (Visser 1992 & D Visser unpublished data), but these results were inconclusive. Because the store environment is vastly different from a large open field, it was decided to test the same pheromone in store environments. The attracticide, *Last Call™ PTM,* has successfully been tested against the potato tuber moth in tomato fields (G Booysens, unpublished data) and was registered on potatoes (Nel *et al.* 2002). This pheromone based insecticide is therefore known to control the potato tuber moth in fields, but studies in potato stores have not been conducted. Control strategies against the potato tuber moth in non-refrigerated stores are limited and the use of mating disruption and attract-and-kill techniques in potato stores could be a cheap and environmentally sound way to protect potatoes in storage.

## METHODS

*Rubber capsule pheromones*

One hundred medium sized (100 to 150 g) potatoes were placed in five crates (20 per crate). The crates were placed next to each other in a row on the floor of a room. One pheromone capsule was attached to an inner side of each crate approximately 200 mm above the potatoes. The potato tuber moth pheromones were obtained from Agribiol® cc. These were rubber capsules impregnated with two potato tuber moth pheromone components, namely E,Z-4,7-tridecatrienyl acetate and E,Z,Z-4,7,10-tridecatrienyl acetate (0.4/0.6 mg). This pheromone was formulated for monitoring only.

Potato tuber moth pupae were obtained from an insectary and were placed individually in glass vials with cotton stoppers for eclosion purposes. This was to prevent mating before releases. The tuber moths were released simultaneously next to the tuber containing crates. This was done by removing the cotton stoppers and by shaking of the vials to dislodge all moths in a one-meter border around the crates. The moths that landed on the floor next to the crates immediately fluttered away, seeking protection in the surroundings or between the crates and potatoes.

All tests were done in abandoned outbuildings, representing self made structures of small-scale farmers. The buildings varied in size and were semi-enclosed or had their windows and doors removed. Tuber moths could therefore have escaped if they wanted to. The buildings were not in close approximation to each other and "contamination" of the controls with the pheromone treatments was thus less likely. Because eight buildings were used for one test (four replicates for the treatment and four for the control), tests where different numbers of moths were used had to be done over time. The experimental design was therefore a complete random design with the treatments done over time. Approximately four weeks after releasing the moths, all tubers in the treatment and control were examined for tuber moth damage. All tubers were replaced with uninfested ones before a second test with different number of moths was started.

*Attract-and-kill pheromone*

One hundred medium sized (100 to 150 g) potatoes were placed in five crates (20 in each crate). The crates were placed next to each other in a row on the floor of a room. The attract-and-kill potato tuber moth pheromone, *Last Call™ PTM*, was supplied by IPM Technologies® as an experimental research product, Lot. No. 103000/1-3. The formulation was a sticky grease solution with active ingredients permethrin (60 g/kg) and potato tuber moth pheromone (1.6 g/kg). It functioned as an attracticide; attracting the moths with the inert pheromone and then killing them when they came into contact with the permethrin. The composition of the pheromone was the same as for the rubber capsule pheromones. The product was supplied in a ready to use hand pump-type applicator designed to produce one drop per pump action. Individual drops of this solution, weighing 50 mg, were applied to pieces of tin foil 100 x 50 mm. Two pieces of *Last Call™ PTM* containing tin foil were attached to the inner sides of each crate, 100 mm above the tubers. A total of 500 mg *Last Call™ PTM* was therefore administered to a treatment, equaling to 0.8 mg potato tuber moth pheromone.

All tests were done in closed air-conditioned insectary rooms (3.5 m x 2.6 m) with temperatures kept at 21 – 26 °C and uncontrolled humidity. There were no escape holes present and all tuber moths that were released were thus trapped inside the rooms. The air in the rooms were not circulated, but were continuously extracted by a separate extractor

fan connected to six extractor hoses with a diameter of 40 mm each. The extractor hoses were hung from the ceiling with their inlets half way down at approximately eye level. The openings of the hoses were covered with gauze material to prevent moths from being sucked into the extractor fan. Releases of tuber moths and evaluations were similar as to the rubber capsules pheromone tests.

A two-way analysis of variance was performed to test for differences between treatments (number of moths released), the number of tubers infested and the interactions. Fisher's protected least significant deferent (LSD) test was used to separate interaction means.

## RESULTS

### Rubber capsule pheromones

When 80 tuber moths were released, there was no difference in damage between the pheromone and untreated control treatments (Table 1). However, the number of tubers attacked decreased in the pheromone treatment when more tuber moths were released, with the lowest infestation level of only 3% when 300 moths were released. The opposite was found in the control; when more moths were released, more tubers were damaged with 83% of tubers attacked when 300 moths were released.

### Attract-and-kill pheromone

The number of tubers attacked increased for both treatments when the number of tuber moths were increased (Table 2). At low tuber moth numbers, the *Last Call™ PTM* treatment gave good control, but where the maximum of 200 moths were released, no control resulted. When 100 tuber moths were released, 46% of tubers were attacked in the *Last Call™ PTM* treatment.

## DISCUSSION

*Mating disruption*

It was found that the presence of pheromones, formulated for monitoring, might have a mating disruption effect on potato tuber moths when used in a store environment. Damage

in the untreated control increased when an increasing number of tuber moths were released. However, this was not found were the pheromones were present. Meaningful disruption (in relation to damage in the untreated control) only occurred when tuber moth numbers were more than 200, while no disruption occurred when numbers were less than 100. Usually control is easier when pest numbers are low and sometimes impossible when their numbers reach very high numbers. This was also true for mating disruption (Felland *et al.* 1995). Raman (1988) also noted that mating disruption with pheromones in potato stores may have potential when infestations are low. The poor control at low tuber moth numbers in this study was therefore unexpected.

The reason for the unexpected results could be a combination of the pheromone formulation, the environment and the pheromone orientated biology of the moth. The nature of a pheromone's release matrix differs for the different purposes they are used for (Carde & Minks 1995). For instance, a mating disruption pheromone formulation will release its pheromone much slower than the formulation used for monitoring (B Barnes, personal communication). Raman (1984) also noted that the composition of tuber moth pheromone blends is crucial for the effectiveness of its different uses. Ono & Ito (1989) state that very high doses of potato tuber moth pheromone disrupt the behavior of male tuber moths. The latter study found that most male tuber moths flew away on arriving within two meters of a pheromone capsule loaded with extremely high doses of pheromone. The moths are therefore not only unable to find the pheromone source, but are possibly also irritated and/or disrupted and move away from the source.

The attractiveness of one pheromone capsule is roughly equal to 17 virgin female tuber moths (Raman 1988). The sex ratio of potato tuber moths is roughly 1:1 (Kroschel & Koch 1994). Therefore, where 200 tuber moths were released, and where 100 are presumably females, the amount of pheromone produced by them would theoretically be equal to 5.8 pheromone capsules. The amount of pheromone volatiles in the air where 200 pheromone producing tuber moths were released should therefore theoretically be twice as much as where only the five pheromone capsules were placed. With the findings of Ono & Ito (1989) in mind, it is expected that the five capsules pheromone plus the added pheromone produced by the high numbers of females, could have exceeded the threshold at which the

male tuber moths were able to function normally. The semi-closed environment in which the experiments were done allowed the moths to move out of the building if they wanted to.

*Attract-and-kill*

*Last Call™ PTM* is not formulated for mating disruption, but as an attracticide. However, it only attracts the male moths after which they are killed when they come into contact with the pheromone drop containing the pyrethroid, permethrin. It therefore indirectly acts as a mating disruption agent by removing all male moths.

The results with *Last Call™ PTM* attracticide showed that when more moths were released, more damage was inflicted to tubers. When 50 tuber moths were released, all males were presumably able to reach the attracticide droplets and were killed, preventing mating. However, 200 tuber moths proved to be too much and a 100% infestation resulted. It is therefore clear that *Last Call™ PTM* were effective under low population pressure of less than 100 moths in this study.

The results with increasing numbers of tuber moths differed between the *Last Call™ PTM* and monitoring pheromone tests. Possible reasons for this discrepancy could be the different formulations of the pheromones and differences in the environments used for these tests. The *Last Call™ PTM* attracticide was applied in small drops and contained much less pheromone than that of the monitoring pheromones (0.08 mg per drop *vs.*1 mg per capsule). *Last Call™ PTM* were applied at 0.8 mg (10 drops) and the rubber capsule pheromone at 5 mg (5 capsules) per treatment. It is thus possible that the volatile pheromones in the *Last Call™ PTM* environment were much less than that in the pheromone environment and that the male moths had no problem in reaching the *Last Call™ PTM* drops because no "search disruption" occurred. However, when the moth numbers were very high, other factors must have come into play, because only then were all males not killed but did they manage to find females (as expressed in the damaged that followed). Carde & Minks (1995) found that when high populations of moth pests are present, "mate finding may be a less formidable task because of the on-average diminished distance between males and females". The moths in the rubber capsule pheromone tests

could fly away, but the *Last Call™ PTM* tests were done in closed insectary rooms where the moths could not fly away. Thus, even if they were irritated by too high pheromone concentrations, they were still trapped in the same rooms with the females. Mating therefore must have taken place even in the presence of the attracticide.

The results of this study are very preliminary, but give an idea of what may be expected when pheromones are used as mating disruption agents or as attracticides. More work is needed with different concentrations of pheromones, different store environments and a broader range of moth numbers released. However, when such work is undertaken, it must be taken into account what the situations may be in real potato stores. The number of moths may be the most important factor. The area in which the moths were released in this study (the crates plus the one meter border area around the crates), was approximately 9.5 $m^2$. This represents an approximate number of 20 and 30 moths per $m^2$ where 200 and 300 moths were released respectively, which is extremely high. Moth numbers will most probably never reach such high numbers in a real store environment at any given time. Daiber (1989) caught a maximum of 30 moths in a pheromone trap in a potato store per week, which calculates to only four moths per day for an entire store. This illustrates that future tests must be done with lower numbers of tuber moths than used in this study.

When experimenting with mating disruption, it is also important to differentiate between moths in fields and moths in stores. It is known that some lepidopteran pests, e.g. some *Heliothis* spp. females never "call" with pheromones in the absence of suitable host plant volatiles and that some moths (including other gelechiids) are more receptive when different plant parts are present near the pheromones (McNiel 1991). Potato tuber moths may thus behave differently in fields (where only foliage are present above ground) and in stores (where only tubers are available). In this study growing potato plants were not present near the stores. The potato tubers in the crates were the only source of suitable plant material available for females. Whether tuber moths differ in their calling and receiving behavior when only foliage or only tubers are present needs further investigation.

The preliminary findings of this study with pheromones showed that mating disruption in stores might be dependent on the number of moths released at once. A more realistic

scenario may be to release low numbers of moths over a longer period. Other factors, such as the environment (closed *vs*. open stores) may also influence the results. The results with an attracticide in a closed room showed that it might be effective against the potato tuber moth when tuber moth numbers are low.

## REFERENCES

ACASIO, R.F., DE LOS SANTOS, A. & VANDER ZAAG, P. 1986. Acceptance of diffused light storage technology by potato farmers in the Benguet and Mountain provinces of the Philippines. *American Potato Journal* **63:** 162-170.

BEN ALAH, H. & AALBU, R. 1992. Field use of granulosis virus to reduce initial storage infestation of the potato tuber moth, *Phthorimaea operculella* (Zeller), in North Africa. *Agriculture, Ecosystems and Environment* **38:** 119-126.

CARDE, R.T. & MINKS, A.K. 1995. Control of moth pests by mating disruption: successes and constraints. *Annual Review of Entomology* **40:** 559-585.

CHANDRAMOHAN, N. 1995. Influence of weather factors on potato tuber moth catches in pheromone traps. *Madras Agriculture Journal* **82**: 9-10.

CHAPMAN, R.F. 2000. Entomology in the twentieth century. *Annual Review of Entomology* **45:** 261-285.

DAIBER, K.C. 1989. A preliminary survey of the male flight of potato tuber moth, *Gnorimoschema operculella* inside and outside potato stores. *Phytophylactica*: **21:** 95-96.

DAS. G.P., MAGALLONA, E.D., RAMAN, K.V. & ADALLA, C.B. 1992. Effects of different components of IPM in the management of the potato tuber moth, in storage. *Agriculture, Ecosystems and Environment* **41:** 321-325.

FELLAND, C.M., HULL, L.A., BARRETT, B.A., KNIGHT, A.L., JENKINS, J.W., KIRSH, P.A. & THOMSON, D.R. 1995. Small plot mating disruption trials for tufted apple bud moth, *Platynota idaeusalis*, in Pennsylvania apple orchards. *Entomologia Experimentalis et Applicata* **74:** 105-114.

FENEMORE, P.G. 1977. Oviposition of potato tuber moth, *Phthormaea operculella* Zell. (Lepidoptera: Gelechiidae); fecundity in relation to mated state, age and pupal weight. *New Zealand Journal of Zoology* **4:** 187-191.

FIELDS, P.G. & WHITE, N.D.G. 2002. Alternatives to methyl bromide treatments for stored-product and quarantine insects. *Annual Review of Entomology* **47:** 331-359.

FUGLIE, K., SALAH, H.B., ESSAMET, M., TEMIME, A.B. & RAHMOUNI, A. 1991. The development and adoption of integrated pest management of the potato tuber moth, *Phthorimaea operculella* (Zellar) in Tunisia. Social Science Department, Working paper Series No. 1991-7, CIP, Peru.

HANAFI, A. 1999. Integrated pest management of potato tuber moth in field and storage. *Potato Research* **42**: 373-380.

KROSCHEL, J. & KOCH. W. 1994. Studies on the population dynamics of the potato tuber moth in the republic of Yemen. *Journal of Applied Entomology* **118:** 327-341.

McNIEL, J.N. 1991. Behavioral ecology of pheromone-mediated communication in moths and its importance in the use of pheromone traps. *Annual Review of Entomology*: **36:** 407-430.

NEL, A., KRAUSE, M. & KHELAWANLALL, N. 2002. A guide for the control of plant pests (thirty ninth edition). Directorate: Agricultural Productions Inputs. National Department of Agriculture, Republic of South Africa. Pretoria.

ONO, T. 1993. Effect of JHA on pheromone production in the potato tuberworm moth, *Phthorimaea operculella* (Lepidoptera: Gelechiidae). *Applied Entomology and Zoology*. **28:** 121-124.

ONO, T. & ITO, M. 1989. Pattern of pheromone-oriented flight in male potato tuberworm moths. *Journal of Chemical Ecology* **15**: 2357-2368.

POTTS, M. J. 1983. Diffused light potato seed storage as an example of technology transfer: A case study. *American Potato Journal* **60:** 217-226.

RAMAN, K.V. 1984. Evaluation of a synthetic sex pheromone funnel trap for potato tuberworm moths (Lepidoptera: Gelechiidae). *Environmental Entomology* **13:** 61-64.

RAMAN, K.V. 1988. Control of potato tuber moth, *Phthorimaea operculella*, with sex pheromones in Peru. *Agriculture, Ecosystems and Environment* **21:** 85-99.

RAMAN, K.V. 1994. Potato pest management in developing countries. In: Zehnder, G.W., Powelson, M.L., Jansson, R.K. & Raman, K.V. *Advances in potato pest biology and management.* 583-598. APS Press, The American Phytopathological Society, St. Paul, Minnesota.

RAMAN, K.V., BOOTH, R. H, & PALACIOS, M. 1987. Control of potato tuber moth *Phthorimaea operculella* (Zeller) in rustic potato stores. *Tropical Science*. **27:** 175-194.

UNIVERSITY OF CALIFORNIA, 1986. Integrated pest management for potatoes in the western United States. Division of Agriculture and Natural Resources Publication No.3316. University of California: Berkeley, CA, U.S.A., California.

VISSER, D. 1992. Slaan 'n motmannetjie waar dit seermaak. *Chips* Oct/Nov/Dec: 20-21.

**Table 1.** The number of tubers infested out of 100 where a varying number of tuber moths were released.

| No. of moths released | Five pheromone capsules present | Untreated Control | % control in relation to the untreated control |
|---|---|---|---|
| 80 | 44c | 44c | 0 |
| 130 | 41c | 70d | 41 |
| 200 | 22b | 74de | 70 |
| 300 | 3a | 83e | 96 |

Means followed by different letters within and across columns were significant different ($P < 0.001$), SEM = 4.16, LSD(5%) = 12.1

**Table 2.** The number of tubers infested out of 100 where a varying number of tuber moths were released.

| No. of moths released | Ten drops *Last Call™ PTM* | Untreated Control | % control in relation to the untreated control |
|---|---|---|---|
| 50 | 0a | 17b | 100 |
| 100 | 46c | 93d | 50 |
| 200 | 100d | 100d | 0 |

Means followed by different letters within and across columns were significant different ($P < 0.001$), SEM = 3.1, LSD(5%) = 9.2

# CHAPTER 9

## Evaluation of genetically modified potatoes against the potato tuber moth *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) under laboratory and non-refrigerated store conditions in South Africa

**ABSTRACT**

Potatoes containing one of two *Bacillus thuringiensis* (*Bt*) genes were evaluated against the potato tuber moth under laboratory and storage conditions. The two genes were *Bt-cry1c* and *Bt-cry5* (synonym *Bt-cry11a1*). The transformed potato cultivars were Desiree, Shepody, O'Maya and Lady Rosetta (all containing the *Bt-cry1c* gene) as well as Spunta and a glandular trichome line (both containing the *Bt-cry5* gene). The *Bt-cry1c* transgenes were transformed by Vitality Biotechnologies in Israel and the *Bt-cry5* transgenes by the Michigan State University in the U.S.A. Both no-choice and free-choice experiments were carried out in an insectary and a diffused light store with artificial tuber moth infestations. In all these tests the transgenic lines always yielded 100% control, except for one *Bt-cry5* line (Spunta-S4) which yielded 97% control. It is concluded that the resistant *Bt*-transgenic potatoes will result in excellent control (if not absolute) against the potato tuber moth under storage conditions in South Africa.

**Key words**: *Phthorimaea operculella*, potato tuber moth, *Solanum tuberosum, Bacillus thuringiensis*, transgenic potato, genetic engineering, *Bt-cry5*, *Bt-cry1c, Bt-cry11a1*.

## INTRODUCTION

The potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), is the most important insect pest of potatoes in South Africa. It attacks the foliage and tubers in the field, as well as tubers in non-refrigerated stores. Between five and 20% (depending on the production region) of tubers are discarded on the sorting tables after harvest, but in extreme cases this may be as high as 80%. Damage figures for stored potatoes in South Africa are not easily obtainable, but situations where infested batches of potato contaminated new clean batches in the same store are reported regularly. However, damage figures of potatoes in non-refrigerated stores in especially third world countries may reach 100% (Fuglie *et al.* 1991; Ferro & Boiteau 1993). The potato tuber moth is regarded as a serious post harvest pest problem for both the commercial and the small-scale farmer.

The potato tuber moth attacks at least 40 plant species in the family Solanaceae (Foot 1976). This extensive host range reduces the likelihood of a breeding program producing potato cultivars that are resistant to the tuber moth (Foot 1976). Breeding for insect resistance in potato has been attempted since 1967 by the University of Minnesota (Flanders *et al.* 1992) and since 1978 by the International Potato Center in Lima, Peru (Raman & Palacios 1982). Because resistance in already improved cultivars is very unlikely (Khalil *et al.* 1987), researchers usually experiment with crosses between original wild potato species and improved cultivars (Chavez *et al.* 1988). Some of the wild potato species were shown to be resistant or tolerant to potato tuber moth attacks (Raman & Palacios 1982; Malakar & Tingey 1999). However, no commercial non-transgenic cultivar has ever been shown to express appreciable levels of resistance against the potato tuber moth (Lagnaoui *et al.* 2001). This is disappointing in the light of all the research that showed the huge potential of breeding for resistance against the potato tuber moth (Chavez *et al.* 1988; Oritz *et al.* 1990; Arnone *et al.* 1998). The closest that certain commercial cultivars came to be labeled "resistant" is where they were shown to be less preferred by the potato tuber moth than other cultivars (Gyawali 1989).

The common soil bacterium *Bacillus thuringiensis* (*Bt*) produces insecticidal crystal proteins that are harmless to mammals, including man (Raman *et al.* 1987). The proteins derived from *Bt* are called δ (delta)-endotoxins (Tabashnik 1994), or insecticidal crystal proteins and

sometimes protoxins (Ebora & Sticklen 1994), while the genes that code for these proteins in transgenic plants are called *cry* genes (Ferre & van Rie 2002). Some literature refers to these proteins as *cry* proteins (Honée& Visser 1993). Five cry proteins (*Cry1* to *Cry5*) are known to have highly potent and specific insecticidal activity (Beuning *et al.* 2001). These *cry* proteins bind to specific receptors in the midgut after ingestion, causing the death of the insect larva (Gill *et al.* 1992). Plants that express these *cry* genes are therefore protected from those insects that are affected by these proteins.

Previous research on genetically engineered (GE) potatoes include; protein-rich genotypes (Gahukar 2002), the production of edible vaccines against various animal diseases (Mason *et al.* 1999), resistance against plant viruses (Palucha *et al.* 1998; Grieco *et al.* 1999), disease resistance (Gusui *et al.* 1995; Lorito *et al.* 1999), resistance against the bollworm *Helicoverpa armigera* (Chakrabarti *et al.* 2000) and resistance against the Colorado potato beetle (Haffani *et al.* 2000). The use of genetically engineered crops against the potato tuber moth always included the *cry1* and *cry5* genes. Van Rie *et al.* (1994) could not find control against the potato tuber moth using potato plants with the *Cry1B* gene, but noted that further research was needed to amplify the expression of the gene in the plant. Ebora *et al.* (1994) only found limited mortality (10%) in potatoes engineered with the *Cry1Ac* gene. The *Cry1Ab* gene gave 100% mortality in stored potatoes for up to seven months (Jansens *et al.* 1995; Canedo *et al.* 1999). *Cry1Ac9* genes in modified tobacco plants were effective against the potato tuber moth (Beuning *et al.* 2001). Potatoes with the *Bt-cry5* gene showed 100% mortality against potato tuber moth larvae (Mohammed *et al.* 2000).

The objective of this study was to evaluate transgenic potato tubers containing the *Bt-cry1c* and *Bt-cry5* genes under laboratory and storage conditions against the South African strain of potato tuber moth. The main criterion was whether tubers were damaged or not, and not mortality of individuals feeding on the transgenic tubers. The results are thus directly indicative of what the farmer who uses the GE potatoes can expect when potatoes are stored in the presence of potato tuber moths.

The importation, handling and experiments with the genetically modified cultivars in this study was authorized and strictly monitored by The Directorate, Genetic Resources of the

National Department of Agriculture, the regulatory body of transgenics in South Africa. All the cultivars and lines used in this study have been issued with permits for experimental purposes only. Licenses for commercial use have not been issued yet at time of publishing this document.

## METHODS

### *Acquisition of the transgenic plants*

The cultivars with the *Bt-cry5* gene (five *Spunta* modifications and one modified line) were transformed and supplied by Michigan State University, U.S.A. They were received as test-tube plantlets and multiplied by the ARC-Roodeplaat, Pretoria (25°35'S, 28°21'E). The *Bt-cry5* gene is the property of Syngenta and also known under new proposed *Bt*-nomenclature as *Bt-cry11a1* (Crickmore *et al.* 1989). The *Bt-cry1c* gene is owned by and was transferred into potatoes by *Vitality Biotechnologies* (Israel) (Lochner 2000). The four cultivars with this gene were obtained from *First Potato Dynamics* (Durbanville, South Africa). These modified cultivars were *Desiree* (two modifications), *O'Maya* (two modifications), *Shepody* and *Lady Rosetta*. The transgenic plants with the two different genes were not received and evaluated simultaneously and the results will therefore be handled separately. Two types of resistance may influence results with transgenic potatoes, namely antixenosis (non-preference) and antibiosis (affecting feeding) (Arnone *et al.* 1998). To test for both of these types of resistance, two experimental layouts (modified from Ortiz *et al.* 1990) were followed. They were no-choice and free-choice experiments.

### *No choice experiments*

Two types of no-choice experiments were conducted. Moths were allowed to lay their eggs on potatoes in a closed cage (no-choice moths), and first instar larvae were put on tubers (no-choice larvae). The no-choice experiments were all done in small insect proof cages (450 x 450 x 350 mm).

#### Bt-cry1c

Two no-choice evaluations (with moths) were conducted with lines containing the *Bt-cry1c* gene; one week after harvest and 150 days after harvest. Medium sized tubers (100 to 150 g) (15 for the first test and 20 for the second test) of each line and unmodified controls were

placed in separate insect cages after which moths (30 for the first test and 50 for the second test) were released in each cage. Each line was thus represented by 15 and 20 tubers, separated in insect proof cages, without replicates. Moths in the cages had no choice but to lay their eggs on or near the tubers in the same cage. To prevent the possible movement of first instar larvae between cages, each cage was suspended on an inverted plastic bucket with sticky glue spread around its outside. The experiments were incubated at 26 ± 2 °C until the larvae pupated inside the cages after approximately 21 days. For pupation purposes, a layer of white sand (approximately ten millimeters wide) was supplied in each cage around the tubers. The fourth instar larvae that exited the tubers in search for pupation loci pupated in the sand when they reached it. Pupae were collected from the sand, counted and kept until moths appeared.

**Bt-cry5**

Two experiments were conducted using moths on mini-tubers (10 to 20 g) and larvae on medium sized tubers (100 to 150 g). The mini-tubers were used two weeks after harvest and the medium sized tubers 200 days after harvest. The experiment with moths was conducted with 15 mini-tubers and 30 moths for each line and unmodified control. The experiment with larvae was conducted with 10 medium sized tubers (100 to 150 g) and five larvae per tuber. The moths and larvae were collected from a rearing facility at ARC-Roodeplaat. The tubers of both the experiments were handled the same as for *Bt-cry1c*.

*Free choice experiment*

**Bt-cry1c**

The free choice experiment was conducted in a closed air-conditioned insectary room, with no windows and temperature of 20 ± 2 °C. This experiment was conducted at a lower temperature because the objective was also to extend the storage time and to limit the chances of rotting. Twenty medium-sized potatoes (100 to 150 g) of the abovementioned lines and unmodified controls were placed in crates. The crates were not stacked but all were placed on the floor of the room in a randomized block design with four replicates. Potato tuber moths were released in the room by placing a Petri dish in each crate with pupae ready to hatch within 48 hours. Moths were released on two occasions, the first with 12 moths per crate and the second, 30 days later, with 25 moths per crate. The moths that emerged from the pupae had a free choice as to which tubers in which crates they wanted to lay their eggs on. The

tubers were incubated for 30 days before an evaluation was performed. A second control was added before the second release. This was a BP1 control treatment and was meant to be an indicator treatment with no damage to start with in relation with the other controls, which already showed damage after the first evaluation. Fifteen randomly selected tubers from each treatment (across replicates) were selected at the end of the second evaluation and transferred to separate containers with white sand. They were kept until the larvae inside exited and pupated in the sand. After pupation the pupae were counted and kept until moths emerged.

**Bt-cry5**

Two free-choice experiments were conducted, one with mini-tubers in an insect cage and the other with medium sized tubers (100 to 150 g) in a diffused light store. Because of the small size of the mini-tubers, the entire experiment fitted into one insect cage (450 x 450 x 350 mm). The experimental layout was a complete randomized design with five mini-tubers of each line or control in Petri dishes, with four replicates. Two hundred potato tuber moths (as pupae) were place in the middle inside of the cage and allowed to infest any potato in any Petri dish. To prevent first instar larvae that hatched from eggs laid by the moths from moving between treatments, each Petri dish was suspended on a plastic vial stopper. The outer edge of this stopper was treated with sticky glue to prevent larval movement. The tubers were incubated for three weeks before the number of damaged tubers was counted. The tubers were then placed in separate containers with white sand to collect pupae. The pupae were kept until moths emerged.

The construction of the diffused light store was similar to that illustrated in Potts (1983). It was a small thatched roof building 2 x 4 m and 2 m high. The sidewalls were constructed with round split wooden poles twenty to thirty millimetres in diameter. The split poles were spaced approximately one centimetre apart, allowing enough light to enter the building for sprouting purposes of the potatoes. Ten medium-sized tubers (100 to 150 g) of each potato line and unmodified controls were put in individual crates in a randomized block design, with the four replicates. Each replicate was on a separate shelf with a space of approximately 450 mm between the shelves. The test was started two weeks after harvest. Tuber moths were released on two occasions; 30 per crate at the start and another 40 per crate two weeks later. Before the second release, all the damaged tubers were replace by new, uninfested tubers.

## RESULTS

### No choice experiments

When potato tuber moths had no choice as to lay their eggs in the same container than the tubers, or where larvae were put on tubers, no damage was recorded in any of the transgenic lines. (Tables 1, 3 and 5). The *Bt-cry1c* gene stayed active for the tested 150 days after harvest and the *Bt-cry5* gene for the tested 200 days after harvest. All the unmodified controls were always damaged. Healthy progeny (moths) were collected from all the unmodified controls while the transgenic lines did not give rise to any progeny.

### Free choice experiments

All the transgenic lines were free of any tuber moth damage, except for the *Bt-cry5* transgenic Spunta-S4 line (Tables 2, 4 and 5). However, only a mean number of 0.3 out of 10 tubers showed damage in this line in both of the two tests.

## DISCUSSION

Conventional breeding for resistance relating to the potato tuber moth has received attention for more than 30 years. However, it was only with the recent introduction of genetically modified potatoes that high levels of resistance were obtained. This study intended to add to existing knowledge relating to the levels of post harvest resistance in GE potatoes that is crucial when potatoes are stored for prolonged periods outside cool storage facilities. Both the commercial and small-scale farmers have to keep potatoes in non-refrigerated store environments for various reasons at certain times in the production system. Because no insecticides were registered for protection of stored potatoes, and because of the dangers of treating tubers with toxic chemicals, resistance is the only safe option for tuber moth control in stored potatoes.

Both the *Bt-cry1c* and the *Bt-cry5* genes were evaluated for their efficacy against the potato tuber moth under storage conditions. Lines with these two genes provided excellent control in environments where high numbers of potato tuber moths were present. Previous research results obtained with lines containing the *Bt-cry5* gene correspond with the results of this study. These works include (Douches *et al.* 1998; Westedt *et al.* 1998; Li *et al.* 1999;

Mohammed *et al.* 2000; Lagnaoui *et al.* 2001). This study showed that all the *Bt-cry5* transgenic lines, except Spunta-S4, which scored 97% in two of the five tests, would always control potato tuber moth. Even the 97% control observed with the Spunta-S4 is acceptable to label it as resistant. The resistance of lines with this gene lasted for the tested 200 days. Preliminary research conducted with potatoes containing the *Bt-cry1c* gene in South Africa was reported in the popular press (Lochner 2001). The four cultivars with this gene always gave 100% control in all four tests. This absolute resistance lasted for the tested five months of storage. Most developed country markets rejects even slightly damaged potatoes and only a lethal antibiosis effect would therefore be acceptable (Arnone *et al.* 1998). The results with GM potatoes against the potato tuber moth comply with this prerequisite. However, potato production and markets in developing countries follows a different pattern. The economic loss threshold for small-scale farmers cultivating consumer potatoes in Africa is between 20 and 30% (Fuglie *et al.* 1991). These farmers are also known to sell their seed potatoes as soon as the first signs of infestations are noted (Kroschel & Koch 1994). The high resistance that the GM potatoes express will therefore add value to the crop, and will result in much longer storage times of potatoes in the developing countries.

Genetically modified crops will most probably play a more important role in studies for insect resistance than conventional breeding in the future. This is already indicated by the research programs of the International Potato research Center (CIP) in Peru, where their vigorous breeding programs for tuber moth resistance were reduced while new programs for research with *cry* genes were started. It generally takes eight to 11 years to breed a new variety with new resistance (Day-Rubenstein 2000). Potatoes also have a narrow genetic base and conventional breeding schemes are generally inefficient (Douches 1996). This, plus the fact that no conventionally bred potato cultivars have been released with tuber moth resistance (Lagnaoui *et al.* 2001), increases the likelihood that genetic resistance will replace conventional resistance in potato plants in the future.

Geographic variability in the potato tuber moth has been documented (Briese 1986). This variability in potato tuber moth populations was also indicated as the reason for varying results with resistance tests with wild potato species in Peru and Italy (Arnone *et al.* 1998). For a cultivar to be labeled as "resistant" against a certain pest, it therefore has to be evaluated

against a wide range of different geographic populations. This study is the third country outside the U.S.A. to use a local potato tuber moth population in tests for the efficacy of the *Bt-cry5* gene in potatoes (the other being Egypt and Peru). The *Bt-cry1c* gene was also effective against the potato tuber moth in Israel (L Olivier, personnel communication). It was therefore shown that the relevant *cry* genes are potent enough to control geographically removed populations of the potato tuber moth.

Public acceptance aside, the success of GE potatoes will depend on its effectiveness in the field, its agronomic performances and its nutritional compositions relative to conventional cultivars. GE potatoes have now proven its efficacy against the potato tuber moth with control of nearly always 100%. It has also been confirmed that the composition of important nutritional and antinutritional factors in tubers produced by GE insect resistant and conventional potato plants are substantially equivalent (Rogan *et al.* 2000). The only one aspect that has not received adequate attention is the variability in agronomic traits that are sometimes crucial in the acceptance of a new cultivar.

The benefits of GE potatoes fall outside of the scope of this study. However, there are many reviews on the prospects and potential of genetically modified crops in a future agricultural environment, of which Krattiger (1997), Sharma (2000), Gianessi (2002) and Shelton *et al.* (2002) are only a few. All of them demonstrate that *Bt* is merely the beginning of a long series of new and safer technologies to augment productivity, to bring about a more sustainable agriculture, to reduce the use of pesticides and to protect the environment. And all agree that the adoption of current and future *Bt* crops will have a tremendous effect on pest management, but also emphasize that strategies also have to be put in place to prolong the life span of the transgenics.

The use of tuber moth resistant potato cultivars will allow for the reduction or elimination of the use of toxic chemicals on an edible crop, a practice that is still common in some areas of the developing world. It will also possibly result in an increase of seed production of higher quality and will add much value to the table potato market. The high levels of resistance of potatoes containing the *Bt-cry5* and *Bt-cry1c* genes will allow potato growers to lower the status of the potato tuber moth as a post harvest pest of stored potatoes. It is even possible that

growers which use these resistant cultivars may remove the tuber moth from their list of problems to allow them to concentrate on other potential post harvest problems.

## REFERENCES

ARNONE, S., MUSMECI, S., BACCHETTA, L., CORDISCHI, N., PUCCI, E., CRISTOFARO, M. & SONNINO, A. 1998. Research in *Solanum* spp. of sources of resistance to the potato tuber moth *Phthorimaea operculella* (Zeller). *Potato Research* **41**: 39-49.

BRIESE, D.T. 1986. Geographic variability in demographic performance of the potato moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae). *Australia Bulletin of Entomological Research* **67**: 719-726.

BEUNING, L.L., MITRA, D.S., MARKWICK, N.P. & GLEAVE, A.P. 2001. Minor modifications to the cry1Ac9 nucleotide sequence are sufficient to generate transgenic plants resistant to *Phthorimaea operculella*. *Annals of Applied Biology* **138**: 281-292.

CANEDO, V., BENAVIDES, J., GOLMIRZAIE, A., CISNEROS, F., GHISLAIN, M. & LANGNAOUI, A. 1999. Assessing Bt-transformed potatoes for potato tuber moth, *Phthorimaea operculella* (Zeller), management. CIP Program Report 1997-1989 CIP, Lima.

CHAKRABARTI, S.K., MANDAOKAR, A.D., SHUKLA, A., PATTANAYAK, D., NAIK, P.S., SHARMA, R.P. & KUMAR, P.A. 2000. *Bacillus thuringiensis* cry1Ab gene confers resistance to potato against *Helicoverpa armigera* (Hubner). *Potato Research* **43**: 143-152.

CHAVEZ, R., SCHMIEDICHE, P.E., JACKSON, M.T. & RMAMAN, K.V. 1988. The breeding potential of wild potato species resistant to potato tuber moth, *Phthorimaea operculella* (Zeller). *Euphytica* **39**: 123-132.

CRICKMORE, N., ZEIGLER, D.R., FEITELSON, J., SCHNEPF, E., VAN RIE, J., LERECLUS, D., BAUM, J. & DEAN, D.H. 1998. Revision of the nomenclature for the *Bacillus thuringiensis* pesticidal crystal proteins. *Microbial and Molecular Biology Reviews* **62**: 807-813.

DAY-RUBENSTEIN, K. 2000. Agricultural genetic resources: building blocks for future crops. *Agricultural Outlook* AGO-276: 18-21.

DOUCHES, D.S., MAAS, D. JASTRZEBSKI, K & CHASE, R.W. 1996. Assessment of potato breeding progress in the USA over the last century. *Crop Science* **36**: 1544-1552.

DOUCHES, D.S. WESTEDT, A.L., ZARKA, B., SCHROETER, B. & GRAFIUS, E.J. 2000. Potato transformation to combine natural and engineered resistance for controlling tuber moth. *HortScience* **33**: 1053-1056.

EBORA, R.V. & STICKLEN, M.B. 1994. Genetic transformation of potato for insect resistance. In: Zehnder, G.W., Powelson, M.L., Jansson, R.K. & Raman, K.V. Advances in Potato Pest Biology and Management. 509-521. APS Press, The American Phytopathological Society, St. Paul, Minnesota.

EBORA, R.V., EBORA, M.M. & STICKLEN, M.B. 1994. Transgenic potato expressing the *Bacillus thuringiensis* Cry1Ac gene effects on the survival and food consumption of *Phthorimaea operculella* (Lepidoptera: Gelechiidae) and *Ostrinia nubilalis* (Lepidoptera: Noctuidae). *Journal of Economic Entomology* **87**: 1122-1127.

FERRE, J. & VAN RIE, J. 2002. Biochemistry and genetics of insect resistance to *Bacillus thuringiensis*. *Annual Review of Entomology* **47**: 501-533.

FLANDERS, K.L., HAWKES, J.G., RADCLIFFE, E.B. & LAUER, F.I. 1992. Insect resistance in potatoes: sources, evolutionary relationships, morphological and chemical defenses and ecogeographical associations. *Euphytica* **61**: 83-111.

FOOT, M.A. 1976. Susceptibility of twenty potato cultivars to the potato moth at Pukekohe: A preliminary assessment. *New Zealand Journal of Experimental Agriculture* **4**: 239-242.

FUGLIE, K., SALAH, H.B., ESSAMET, M., TEMIME, A.B. & RAHMOUNI, A. 1991. The development and adoption of integrated pest management of the potato tuber moth, *Phthorimaea operculella* (Zellar) in Tunisia. Social Science Department, Working paper Series No. 1991-7, CIP Peru.

FERRO, D. N. & BOITEAU, G. 1993. Management of insect pests. In: Rowe, R.C. (Ed). Potato Health Management. APS Press, Minnesota.

GAHUKAR, R.T. 2002. Status of genetically modified food crops in India. *Outlook on Agriculture* **31**: 43-49.

GIANESSI, L.P., SILVERS, C.S., SANKULA, S. & CARPENTER, J.E. 2002. Plant biotechnology: current and potential impact for improving pest management in U.S. agriculture. An analysis of 40 case studies. National Center for Food and Agricultural Policy (NCFAP), Washington DC.

GILL, S.S., COWLES, E.A. & PIETRANTONIO, P.V. 1992. The mode of action on *Bacillus thuringiensis* endotoxins. *Annual Review of Entomology* **37**: 615-636.

GRIECO, P.D., D'AMBROSIO, C., CELLINI, F., IANNACONE, R. SUNSERI, F. & ARPAIA, S. 1999. Development of transgenic potato clones resistant to potato spindle tuber viroid (PSTVd) and Lepidoptera. Proceedings of the 14th Triennial Conference of the European Association for Potato Research 287-288, 2-7 May, Sorrento, Italy.

GYAWALI, B.K. 1989. Ovipositional preference of tuber moth *Phthorimaea (Gnorimoshema) operculella* Zeller on five varieties of potatoes in Nepal. *Tropical. Pest Management* **35**: 106-107.

HAFFANI, Y.Z., OVERNEY, S., YELLE, G., BELLEMARE, G. & BEZILE, F.J. 2000. Premature polyadenylation contributes to the poor expression of the *Bacillus thuringiensis* cry3Ca1 gene in transgenic potato plants. *Molecular and General Genetis*: **264**: 82-88.

HONÉE, G. & VISSER, B. 1993. The mode of action of *Bacillus thuringiensis* crystal proteins. *Entomologia Experimentalis et Applicata* **69**: 145-155.

JANSENS, S., CORNELISSEN, M., DE CLERCQ, R., REYNAERTS, A. & PEFEROEN, M. 1995. *Phthorimaea operculella* (Lepidoptera: Gelechiidae) resistance in potato by expression of the *Bacillus thuringiensis* Cry1A(b) insecticidal crystal protein. *Journal of Economic Entomology* **88**: 1469-1476.

KHALIL, F.M., ABDEL-GALIL, F.A. & ALI, A.M. 1987. Susceptibility of certain potato varieties to aphids and tuberworm. *Assiut Journal of Agricultural Sciences* **18**: 217-234.

KRATTIGER, A.F. 1997. Insect Resistance in Crops: A case study of *Bacillus thuringiensis* (*Bt*) and its transfer to developing countries. ISAAA Briefs No. 2. ISAAA: Ithaca, NY.

KROSCHEL, J. & KOCH, W. 1994. Studies on the population dynamics of the potato tuber moth in the Republic of Yemen. *Journal of Applied Entomology* **118**:327-341.

LAGNAOUI, A., CANEDO, V. & DOUCHES, D.S. 2001. Evaluation of Bt-cry1la1 (cryV) transgenic potatoes on two species of potato tuber moth, *Phthorimaea operculella* and *Symmetrischema tangolias* (Lepidoptera: Gelechiidae) in Peru. CIP Program Report 1999-2000 (2001). CIP Lima.

LI, W., ZARKA, K.A., DOUCHES, D.S., COOMBS, J.J., PETT, W.L. & GRAFIUS, E.J. 1999. Coexpression of potato $PVY^0$ coat protein and cryV-Bt genes in potato. *Journal of the American Society for Horticultural Science* **124**: 218-223.

LOCHNER, H. 2000. Motbestande aartappels toon groot belofte. *Landbouweekblad* 22 September: 36-40.

LOCHNER, H. 2001. GMO-kultivar tem aartappelmot. *Landbouweekblad* 1186: 8-11

LORITO, M., WOO, S.L., ZOINA, A., FILIPPONE, E., COLUCCI, G., ESPOSITO, S. & SCALA, F. 1999. Biocontrol genes from fungi confer complete resistance to transgenic potato against *Rhizoctonia solani* and *Altenaria* spp. Proceedings of the 14th Triennial Conference of the European Association for Potato Research, 285-286. 2-7 May, Sorrento, Italy.

MASON, H.S., RICHTER, L., MOR, T.S. & ARNTZEN, C.J. 1999. Edible vaccines produced and delivered in transgenic potatoes. Proceedings of the 14th Triennial Conference of the European Association for Potato Research, 207-208. 2-7 May, Sorrento, Italy.

MOHAMMED, A., DOUCHES, D.S., PETT, W. GRAFIUS, E. COOMBS, J. LISWIDOWATI, LI, W. & MADKOUR, M.A. 2000. Evaluation of potato tuber moth (Lepidoptera: Gelechiidae) resistance in tubers of Bt-cry5 transgenic potato lines. *Journal of Economic Entomology* **93**: 472-476.

MALAKAR, R. & TINGEY, W. 1999. Resistance of *Solanum berthaultii* foliage to potato tuberworm (Lepidoptera: Gelehciidae). *Journal of Economic Entomology*: **92**: 497-502.

ORITZ, R., IWANAGA, M., RAMAN, K.V. & PALACIOS, M. 1990. Breeding for resistance to potato tuber moth, *Phthorimaea operculella* (Zeller), in diploid potatoes. *Euphytica* **50**: 119-125.

PALUCHA, A., ZAGORSKI, W., CHRZANOWSKA M. & HULANICKA, D. 1998. An antisense coat protein gene confers immunity to potato leafroll virus in a genetically engineered potato. *European Journal of Plant Pathology* **104**: 287-293.

POTTS, M. J. 1983. Diffused light potato seed storage as an example of technology transfer: A case study. *American Potato Journal* **60**: 217-226.

RAMAN, K.V. & PALACIOS, M. 1982. Screening potato for resistance to potato tuberworm. *Journal of Economic Entomology* **75**: 47-49.

RAMAN, K.V., BOOTH, R. H, & PALACIOS, M. 1987. Control of potato tuber moth *Phthorimaea operculella* (Zeller) in rustic potato stores. *Tropical Science*. **27**: 175-194.

ROGAN, G.J., BOOKOUT, J.T., DUNCAN, D.R., FUCHS, R.L., LAVRIK, P.B., LOVE, S.L., MUETH, M., OLSON, T., OWENS, E.D., RAYMOND, P.J. & ZALEWSKI, J. 2000. Compositional analysis of tubers from insect and virus resistant potato plants. *Journal of Agricultural and Food Chemistry* **48**: 5936-5945.

SHARMA., H.C., SHARMA, K.K., SEETHARAMA, N. & ORTIZ, R. 2000. Prospects or using transgenic resistance to insects in crop improvement. *EJB Electronic Journal of Biotechnology* 24 May 2000, vol. 3 no. 2.

SHELTON, A.M., ZHAO, J.Z. & ROUSH, R.T. 2002. Economic, ecological, food safety, and social consequences of the deployment of *Bt* transgenic plants. *Annual Review of Entomology* **47**: 845-881.

TABASHNIK, B.E. 1994. Evolution of resistance to *Bacillus thuringiensis*. *Annual Review of Entomology* **39**: 47-79.

VAN RIE, J., JANSENS, S. & REYNAERTS, A. 1994. Engineered resistance against potato tuber moth. In: Zehnder, G.W., Powelson, M.L., Jansson, R.K. & Raman, K.V. Advances in Potato Pest Biology and Management. 499-508 APS Press, The American Phytopathological Society, St. Paul, Minnesota.

WU, G., SHORTT, B.J., LAWRENCE, E.B., LEVINE, E.B., FITZSIMMONS, K.C. & SHAH, D.M. 1995. Disease resistance conferred by expression of a gene encoding $H_2O_2$-generating glucose oxidase in transgenic potato plants. *The Plant Cell* **7**: 1357-1368.

WESTEDT, A.L., DOUCHES, D.S., PETT, W. & GRAFIUS, E.J. 1998. Evaluation of natural and engineered resistance mechanisms in *Solanum tuberosum* for resistance to *Phthorimaea operculella* (Lepidoptera: Gelechiidae). *Journal of Economic Entomology* **91**: 552-556.

**Table 1.** The number of tubers from lines containing the *Bt-cry1c* gene infested with potato tuber moth larvae, the number of healthy tuber moths that emerged from them and the number of tubers attacked 150 days post harvest (no-choice, moths).

| Lines | Infested one week after harvest | | 150 days of storage |
|---|---|---|---|
| | No. of tubers attacked (n = 15) | No. of healthy moths that appeared | No. of tubers attacked (n = 20) |
| Desiree (GE-1) | 0 | 0 | 0 |
| Desiree (GE-2) | 0 | 0 | 0 |
| Shepody (GE) | 0 | 0 | 0 |
| Lady Rosetta (GE) | 0 | 0 | 0 |
| O'Maya (GE-1) | 0 | 0 | 0 |
| O'Maya (GE-2) | 0 | 0 | 0 |
| Vanderplank | 14 | 7 | - |
| O'Maya | 15 | 65 | - |
| BP13 | 15 | 24 | - |
| BP1 | 15 | 77 | 20* |
| Up-To-Date | 15 | 72 | - |
| Shepody | 15 | 102 | - |
| Desiree | 15 | 24 | - |
| Lady Rosetta | 15 | 103 | - |

*fresh uninfested tubers were used
GE: Genetically engineered

**Table 2.** The mean number of tubers from lines containing the *Bt-cry1c* gene infested with potato tuber moth larvae 60 and 90 days after harvest and the number of healthy moths appearing from 15 randomly selected tubers after the 60 day interval (free choice moths, n = 20)

| Lines | 60 days of storage* | 90 days of storage | No. of moths from 15 tubers |
|---|---|---|---|
| Desiree (GE-1) | 0 | 0 | 0 |
| Desiree (GE-2) | 0 | 0 | 0 |
| Shepody (GE) | 0 | 0 | 0 |
| Lady Rosetta (GE) | 0 | 0 | 0 |
| O'Maya (GE-1) | 0 | 0 | 0 |
| O'Maya (GE-2) | 0 | 0 | 0 |
| Vanderplank | 7 | 18 | 9 |
| O'Maya | 6.8 | 20 | 17 |
| BP13 | 4.8 | 19.8 | 16 |
| Up-To-Date | 8.3 | 20 | 18 |
| Shepody | 6.3 | 19 | 14 |
| Desiree | 7.8 | 19.3 | 21 |
| Lady Rosetta | 5.5 | 19 | 28 |
| BP1 | 11.3 | 19.5 | 18 |
| BP1(b)** | - | 19 | - |

*Infested tubers were not replaced but kept in the same crates for the 90-day evaluation
**new fresh tubers (extra treatment added after the 60 day evaluation)
GE: Genetically engineered

**Table 3.** The number of mini-tubers (10 to 20 g) from lines containing the *Bt-cry5* gene infested with potato tuber moth larvae and the number of healthy moths that appeared from them (no- choice moths, n = 15)

| Lines | No. of tubers attacked | No. of healthy moths that appeared |
|---|---|---|
| Spunta Control | 12 | 15 |
| BP1 Control* | 15 | 440 |
| Spunta-G2 (GE) | 0 | 0 |
| Spunta-G3 (GE) | 0 | 0 |
| L235.4.13 (GE) | 0 | 0 |
| Spunta-S1 (GE) | 0 | 0 |
| Spunta-S4 (GE) | 0 | 0 |
| Spunta-6a-3 (GE) | 0 | 0 |

* Medium sized tubers (100 to 150 g); More moths appeared in relation to Spunta because tubers size was much bigger.
GE: Genetically engineered

**Table 4.** The mean number of mini-tubers (10 to 20g) from lines containing the *Bt-cry5* gene infested with potato tuber moth larvae and the mean number of healthy moths that appeared from them (free-choice moths, n = 5)

| Lines | No. of tubers attacked | No. of healthy moths that appeared |
|---|---|---|
| Spunta control | 4 | 8.8 |
| Spunta-G2 (GE) | 0 | 0 |
| Spunta-G3 (GE) | 0 | 0 |
| L235.4.13 (GE) | 0 | 0 |
| Spunta-S1 (GE) | 0 | 0 |
| Spunta-S4 (GE) | 0 | 0 |
| Spunta-6a-3 (GE) | 0 | 0 |

GE: Genetically engineered

**Table 5.** The mean number of tubers from lines containing the *Bt-cry5* gene infested with potato tuber moth larvae after 42, 72 and 200 days of storage in a diffused light store. The number of tubers that started to rot is also indicated (n = 10)

| Lines | Free choice (moths) | | No choice (larvae) | Rotting |
|---|---|---|---|---|
| | 42 days after harvest* | 72 days after harvest | 200 days after harvest | 200 days after harvest |
| Spunta Control | 5.5 | 8.5 | 10 | NA |
| BP1 Control** | 6.0 | 9.8 | 10 | NA |
| Spunta-G2 (GE) | 0 | 0 | 0 | 0 |
| Spunta-G3 (GE) | 0 | 0 | 0 | 0 |
| L235.4.13 (GE) | 0 | 0 | 0 | 0 |
| Spunta-S1 (GE) | 0 | 0 | 0 | 0 |
| Spunta-S4 (GE) | 0.3 | 0.3 | 0 | 0 |
| Spunta-6a-3 (GE) | 0 | 0 | 0 | 0 |

*all infested tubers were removed and replaced before the second evaluation was started
**fresh seed tubers, not stored for the mentioned days
GE: Genetically engineered
NA Not applicable because all infested tubers were replaced after every evaluation

# GENERAL DISCUSSION

The potato tuber moth is a serious post harvest pest of potatoes and may destroy the contents of a potato store completely if left unprotected (Fuglie *et al.* 1991). To help address this problem, it is important to understand from where the first infestations originate. There are four possible origins, namely;

- tuber moths flying in from dumping sites
- tuber moths flying in from fields
- tuber moths arriving in stores as eggs or larvae on or inside tubers
- tuber moths originating inside stores as progeny of previous store infestations

To address the problem of moths that fly in from other areas, identifying the source will be the first step in preventing infestations. However, when moths are already inside stores, other strategies have to be utilized to either prevent further damage or to rid the entire store of all stages of the potato tuber moth.

Daiber (1989) has studied the relation between tuber moth numbers and dumping sites. However, most farmers manage dumping sites by covering them with soil in the winter while some farmers dispose of unmarketable tubers by feeding it to their animals (Van Rooyen 1991). By studying the flight phenology of the tuber moth with pheromones, this study has shown another important origin of the first tuber moths of the season. Previously it was thought that the potato tuber moth was unable to survive in cull potatoes in the ground during the winters in South Africa (Whiteside 1985). However, this study has shown that tuber moths were present throughout the winter in harvested fields, even when temperatures dropped to minus 4 °C. No other crop or weeds that could have given rise to moths were present in the vicinity. The only source of newly emerging moths was cull potatoes beneath the ground. Harvested potato fields containing cull potatoes are known to harbor as much as 24 tuber moth larvae per square meter (Shelton & Wyman 1980). Although Broodryk (1971) mentioned this possible source of the first moths of the season for the South African region, this study was the first to monitor the number of moths appearing in harvested fields over an extended period (three years). These moths that

appear throughout the winter months may find their way to storage areas where higher temperatures and the protected environments are much more favorable for faster development of the potato tuber moth (Hanafi 1999).

To prevent tuber moths from reaching storage areas, it will therefore be important to reduce the emergence of moths in cull potato fields. During the growing season farmers apply insecticide treatments to keep moth numbers low, while newly emerging moths that escape these treatments normally attack the same crop in the field again. However, after harvest all insecticidal treatments are normally stopped. Because it is now known that harvested potato fields keep on producing moths, even during the coldest months, it may be necessary to give attention to cull potatoes again. For the commercial farmer it may be difficult, because of the large areas planted. However, the small-scale farmer can easily rework his soil by hand to make sure that all tubers, even the smallest ones, are removed. This aspect will also be more relevant to the small-scale farmer, because these farmers normally utilize smaller areas of land (Abate *et al.* 2000) on which they also may store potatoes in close proximity. Removing all cull potatoes from harvested fields will be an effective preventative strategy to stop tuber moths from reaching potato stores.

When prevention fails and tuber moths succeed in reaching potato tubers in stores, the first wall of defense will be to treat tubers to protect them from attacks. However, because of the possibility of transmitting plant diseases, treating seed potato tubers with any form of liquid is highly undesirable (Dean 1994). One option is to use insecticidal powders. This study has shown that powder formulations of carbaryl and *Bacillus thuringiensis* (*Bt*) will be able to protect potatoes for at least 60 days. If a re-application can be administered after 60 days, this will be sufficient to protect seed tubers until the next planting season. In the case of the small-scale farmer who can not afford these insecticides, this study has shown that normal baby powder will provide reasonable protection for at least one moth.

Application of potential toxic insecticides on table potatoes is undesirable (Raman *et al.* 1987). The use of insecticides is therefore only recommended on seed potatoes that are not destined for consumption. However, seed potatoes may be consumed for various reasons, and the danger of human poisoning is real. The fact that at least two non-toxic powders

gave reasonable to excellent protection against the potato tuber moth indicates that control without dangerous chemicals is viable. *Bt* is harmless to mammals including man, and high residues on potatoes therefore, do not pose any environmental or health hazards (Raman *et al.* 1987). Powder formulations administered to potatoes prevent infestations by moths already inside stores. However, these formulations will not kill larvae already inside tubers. Damage to infested tubers may therefore continue to escalate, despite the use of insecticidal powders. The only way to rid infested tubers from all stages of the potato tuber moth is by fumigation.

Aluminium phosphide is used as a fumigant in tobacco stores in South Africa (Nel *et al.* 2002). However, its effectiveness against the different stages of the potato tuber moth in potato stores has never been evaluated. All stages of the potato tuber moth may be found in potato stores. The only stage that directly damages the tubers is the larva. Once inside the tuber, the larvae are relatively safe until the fourth instar larva have to exit the tuber again for pupation purposes. In storage, larvae may crawl a considerable distance before pupating in crevices among building materials, in potato sacks, or at a similar protected site (University of California 1986). The fourth instar larva pupates inside a strong silken cocoon it makes by impregnating sand or any debris it can find into the silken lining (Visser & Steyn 1999). This cocoon acts as a safe hiding place from natural enemies, but may also protect it from substances like insecticides until moths appear. Moths fly around after dark and hide during the day (Annecke & Moran 1982) and are relatively exposed. Eggs may enter the potato store on freshly harvested tubers (Kroschel. & Koch 1994). The potato tuber moth is known to lay its eggs on substrates near their host plant (van Vuuren *et al.* 1998), and does not require the presence of host-plant material for oviposition (Fenemore, 1978). Eggs may thus be laid anywhere in a store, on tubers, but also on crates, packing material and on walls and floors. There are therefore a lot of different stages at different ages hiding in different places in a potato store. It is therefore important for a farmer to realize that the entire store environment, and not just the tubers, is a source for potential infestations of new potatoes in storage. The following scenarios were taken into account when aluminium phosphide was evaluated;

- eggs of four different ages (some laid in inaccessible places)
- first instar larvae not inside tubers (searching for food)
- first to fourth instar larvae inside tubers
- fourth instar larvae not inside tubers (searching for pupation locations)
- fourth instar larvae inside cocoons (waiting to pupate)
- pupae inside cocoons
- pupae not inside cocoons (sometimes a larva does not make a cocoon)
- moths

Aluminium phosphide killed all above stages in their relevant environments when administered in an airtight container for 48 hours at a dose of four grams phosphine/$m^3$. It was thus shown that fumigation with aluminium phosphide do not only have potential to control potato tuber moth in infested tubers, but also to sterilise a store from any stage of the tuber moth. Although a 100% mortality of all stages was achieved in all tests, phytotoxicity of sprouted eyes on tubers occurred. More research is thus needed to find the correct combination between dosage and exposure time that will still kill all the stages, but without phytotoxicity.

Traditional control methods of potato pests are still widely used today in rural areas where insecticides are too expensive or unobtainable (Abate *et al.* 2000). The small-scale farmer in these areas still use self made insecticides or repellents to protect stored potatoes against the potato tuber moth (Raman *et al.* 1987). This study showed that extracts from two naturally occurring substances, the syringa tree and an insect virus, could also effectively be used to protect potatoes in stores. The syringa tree occurs throughout South Africa, except the northwestern parts of the Cape Province (van Wyk & van Wyk 1997). It is considered an invasive plant in South Africa (Bromilow 1996) and may therefore be utilized for subsistence. The virus is a granulosis virus, specific to the potato tuber moth (Reed 1971) and occurs naturally in wild populations. This study showed that both these natural substances could effectively protect potatoes when dipped in aqueous suspensions of their extracts. These extracts can easily be self-made without the need for expensive equipment. They are also safe to the environment and will not harm natural enemies. It

will therefore fit perfectly into a small-scale environment where potatoes are stored in limited quantities and where protection against the potato tuber moth is needed.

Another strategy to protect stored potato tubers is to prevent tuber moths from reaching them. Non-refrigerated potato stores are rarely insect proof, and tuber moths will always be able to reach the tubers inside if special screens are not installed over potential entry point (Hanafi 1999). Moths often originate inside stores from previous infestations (Ferro & Boiteau 1993). This study has evaluated the potential of two methods in an attempt to prevent moths from reaching the tubers, whether they originate form outside or inside the store. These methods were the use of UV light-assisted insect electrocuter traps and mating disruption/attract and kill by means of pheromones. Although it was shown that the UV light-assisted insect electrocuter trap does attract and kill various insects, the results were inconclusive relating to the protection of potato tubers in stores. The tests with the pheromones showed that the attract and kill technique with *Last Call PTM* could be effective under low population pressures. This pheromone/permethrin combination is applied as sticky droplets to crates containing potato tubers. In tests where 50 tuber moths were release, no damage occurred in relation to the control. However, when the population pressure was increased to 200 moths, no control was achieved. This technique has potential in potato stores where population pressures are low. However, moth populations normally build up from lower levels, and effective control could therefore be achieved if the pheromone is administered from the beginning of storage.

Control methods to protect potato tuber moths in stores often involve either extensive labor or expensive chemicals/equipment. However, one strategy that negates this assumption is the use of genetically modified potatoes. This study has shown that two different genes, both coding for the *Bacillus thuringiensis* (*Bt*) toxin, are very effective against attacks of the potato tuber moth when used in several potato cultivars. Control was nearly always absolute, protecting tubers for up to the tested five months in storage. This is the only option for tuber moth control where no inputs are needed after the storage phase has begun. The use of genetically modified potato cultivars will therefore allow for the elimination of the use of toxic chemicals on an edible crop, a practice that is still common in some areas of the developing world. It will also possibly result in an increase of seed

production of higher quality and will add much value to the table potato market in developing countries. The high levels of resistance of potatoes containing the *Bt* genes will allow potato growers to lower the status of the potato tuber moth as a post harvest pest of stored potatoes. It is even possible that growers that use these resistant cultivars may remove the tuber moth from their list of problems to allow them to concentrate on other potential post harvest problems.

## SUMMARY OF CONTROL STRATEGIES

This study has shown that the following strategies can effectively be used to prevent damage to stored potato tubers from occurring;

- *Remove cull potatoes from harvested fields*

  It was shown that cull potatoes beneath the ground could give rise to potato tuber moths throughout the winter months. These moths can move to store environments where the conditions are much more favorable for fast reproduction and subsequent damage to tubers. This strategy is important for both the small-scale farmer and the commercial farmer.

- *Treatment of tubers with insecticidal powders*

  Two insecticidal powders, *Bacillus thuringiensis* (*Bt*) and carbaryl were shown to protect tubers for at least 60 days. This strategy can be used when tuber moths are already present in a store and when risks of infestations are high. Because these insecticides are often unobtainable in rural areas and are expensive, especially *Bt,* normal baby powder can be used to give short-term (one month) protection against tuber moths. This strategy is recommended for both the small-scale farmer and the commercial farmer.

- *Fumigation with aluminium phosphide*

  The fumigant, aluminium phosphide (Phostoxin), was shown to kill the potato tuber moth larvae inside potatoes. Because all stages, also those that may be hiding in other areas of a store, are also killed, this fumigant can be used to "sterilize" a store infested

with tuber moth. However, care must be taken to prevent phytotoxicity of sprouted eyes when seed tubers are treated. This strategy can be used by both the small-scale farmer and commercial farmer, especially when an already infested store needs to be treated.

- *Self made insecticides*

  This study has shown that aqueous extracts of syringa tree leaves and the potato tuber moth virus can be used to kill the larvae of the potato tuber moth. Using syringa leaves of trees growing in the wild is sustainable and the virus can be propagated by means of a simple rearing program of the tuber moth. Simplified rearing techniques for the potato tuber moth are described in this study. Because the treatment of large quantities of potatoes will be impracticable using self made insecticides, this strategy is mainly aimed at the small-scale potato farmer. The use of these self made insecticides is safe, environmentally friendly and will not kill natural enemies. It can therefore form an integral part of an IPM program.

- *Pheromones*

  Pheromones can be used to monitor potato tuber moth, but also to control them by means of mating disruption and attract and kill formulations. This study has shown that using the attract and kill pheromone *Last Call PTM*, damage by tuber moths can be prevented. This formulation is a sticky droplet containing permethrin that is applied in crates containing potatoes. It is easy to apply and can be used by both the small-scale farmer and the commercial potato farmer when tuber moth population pressure is relatively low.

- *Genetically modified potatoes*

  Genetically modified (or engineered) potatoes were tested extensively under storage conditions. Two different genes, both coding for the *Bacillus thuringiensis* toxin, using various cultivars, were evaluated for up to six months in storage. Control was nearly always absolute. The use of genetically engineered potatoes will without a doubt be the future preference of farmers who have severe potato tuber moth problems. This strategy may even lower the status of the potato tuber moth from a serious post harvest

pest to an irrelevant insect in potato stores. Using this strategy does not require any inputs other than obtaining the modified seed potatoes.

## REFERENCES

ABATE, T., VAN HUIS, A. & AMPOFO J.K.O. 2000. Pest management strategies in traditional agriculture: An African Perspective. *Annual Review of Entomology* **45**: 631-659.

ANNECKE, D.P. & MORAN, V.C. 1982. Insects and mites of cultivated plants in South Africa. Butterworths, Durban/Pretoria.

BROMILOW, C. 1996. Probleemplante van Suid-Afrika. Briza Publikasies Bk. Arcadia, Pretoria.

BROODRYK, S.W. 1971. Ecological investigations on the potato tuber moth, *Phthorimaea operculella* (Zeller). *Phytophylactica* **3**: 73-84.

DAIBER, K.C. 1989. A preliminary survey of the male flight of potato tuber moth, *Gnorimoschema operculella* inside and outside potato stores. *Phytophylactica*: **21**: 95-96.

DEAN, B.B. 1994. Managing the Potato Production System. Food Products Press, New York.

FENEMORE, P.G. 1978. Oviposition of potato tuber moth *Phthorimaea operculella* Zell. (Lepidoptera: Gelechiidae); the physical nature of the oviposition substrate. *New Zealand Journal of Zoology* **5**: 591-599.

FERRO, D. N. & BOITEAU, G. 1993. Management of Insect Pests. In: Rowe, R.C. (Ed). Potato Health Management. APS Press, Minnesota.

FUGLIE, K., SALAH, H.B., ESSAMET, M., TEMIME, A.B. & RAHMOUNI, A. 1991. The Development and adoption of Integrated Pest Management of the Potato Tuber Moth, *Phthorimaea operculella* (Zellar) in Tunisia. Social Science Department, Working paper Series No. 1991-7, CIP Peru.

HANAFI, A. 1999. Integrated pest management of potato tuber moth in field and storage. *Potato Research* **42**: 373-380.

KROSCHEL, J. & KOCH, W. 1994. Studies on the population dynamics of the potato tuber moth in the Republic of Yemen. *Journal of Applied Entomology* **118**: 327-341.

NEL, A., KRAUSE, M. & KHELAWANLALL, N. 2002. A guide for the control of plant pests (thirty ninth edition). Directorate: Agricultural Productions Inputs. National Department of Agriculture, Republic of South Africa, Pretoria.

RAMAN, K.V., BOOTH, R. H, & PALACIOS, M. 1987. Control of potato tuber moth *Phthorimaea operculella* (Zeller) in rustic potato stores. *Tropical Science*. **27**: 175-194.

REED. E.M. 1971. Factors effecting the status of a virus as a control agent for the potato moth (*Phthorimaea operculella*) (Lep., Gelechiidae). *Bulletin of Entomological Research* **61**: 207-222.

SHELTON, A.M. & WYMAN, J.A. 1980. Postharvest potato tuberworm populations in cull and volunteer potatoes, and means of control. *Journal of Economic Entomology* **73**: 8-11.

UNIVERSITY OF CALIFORNIA, 1986. Integrated Pest Management for Potatoes in the Western United States. Division of Agriculture and Natural Resources Publication No.3316. University of California: Berkeley, California.

VAN ROOYEN, C. 1991. Beeste presteer op aartappels. *Landbouweekblad*, 24 May, 1991.

VAN VUUREN, J.J., BENNETT, A. & BENNETT, A.L. 1998. Oviposition site preferences of potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), a pest on tobacco, *Nicotiana tabacum* L. (Solanaceae). *African Entomology* **6:** 177-183.

VAN WYK, B & VAN WYK P. 1997. Field Guide to Trees of Southern Africa. Struik Publishers, Cape Town.

VISSER, D & STEYN, P.J. 1999. Voorkoms en beheer van peste en plae. In: Steyn, P.J. (ed.) Handleiding vir Aartappelverbouing in Suid-Afrika. 133-152. Agricultural Research Council, Roodeplaat.

WHITESIDE, E.F. 1985. An adaptation to overwintering in the potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae). *Journal of the Entomological Society of Southern Africa*. **48**: 163-167.