# The potato tuber moth, *Phthorimaea operculella* (Zeller), in South Africa: potential control measures in non-refrigerated store environments

by

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# DECLARATION

I the undersigned hereby declare that the dissertation submitted herewith to the University of Pretoria contains my own original work and has not previously in its entirety or part been submitted for any degree at any other university.

Diedrich Visser
February 2004

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# CONTENTS

# SUMMARY

**The potato tuber moth, *Phthorimaea operculella* (Zeller), in South Africa: potential control measures in non-refrigerated store environments**

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Cull potatoes beneath the soil could give rise to potato tuber moths throughout the winter months. These moths may move to store environments where the conditions are favorable for fast reproduction and subsequent damage to tubers in storage.

Carbaryl, permethrin, gamma-BHC, *Bacillus thuringiensis* (*Bt*), and a domestic baby powder (as the control) were tested against the potato tuber moth on stored potatoes. All the treatments protected the tubers from moth attacks immediately after treatment. However, it was only carbaryl and *Bt* that protected the tubers 60 days after treatment.

Aluminium phosphide (Phostoxin®) was tested against all stages of the potato tuber moth at a dosage of four grams phosphine/$m^3$. Exposure time was 48 hours inside an airtight plastic container. In all tests, aluminium phosphide was lethal to all stages of the potato tuber moth. Aluminium phosphide can therefore effectively be used to rid infested potatoes and/or potato stores of any stage of the potato tuber moth.

It was found that a local strain of the tuber moth virus remained virulent for at least nine years when stored at -20 °C and at least two weeks when kept in suspension at 25-28 °C. 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.

Crude plant extracts from the exotic invasive tree, *Melia azedarach*, (syringa), were tested against first instar larvae of the tuber moth in the laboratory. Bioassays showed that pupal weight was negatively influenced and that growth of tuber moth larvae was retarded after feeding on potato leaves dipped in 10g/ℓ syringa leaf extracts.

Two pheromone formulations (monitor capsules and an attracticide), both containing 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), were evaluated. 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.

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*). 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 nearly always yielded 100% 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.

Rearing methods for the potato tuber moth are described. These include a program for small-scale rearing where as little as 500 moths are reared and a medium scale rearing where 10 000 moths are produced per month. An overview of the literature on rearing techniques and the biology of the tuber moth under laboratory conditions is given.

# GENERAL INTRODUCTION

## Background

The potato, (*Solanum tuberosum* L.) is the most widely planted vegetable crop in South Africa. Between 55 000 and 60 000 hectares are planted annually in all nine provinces of which 16% is utilized for seed production (Potatoes South Africa 1999). Nearly 80% of all potato plantings are irrigated with an average yield of approximately 40 tons per hectare (A. Visser, personnel communication). The total average yield (including non-irrigated farms), is approximately 30 tons per hectare. Seventy percent of all potatoes are planted with seed certified to be disease and virus free (Steyn 1999).

The potato plant is adapted to a cool climate, originating from the Andes Mountains in South America. However, new cultivars were bred to adapt to warmer agricultural environments. These warmer areas where potatoes are cultivated are ideal for most pests and diseases (Steyn 1999). The most important biotic constraints for the potato plant during the growing season in South Africa are fungal diseases like early and late blight, virus diseases, and insect pests like potato leafminer and the potato tuber moth.

The most important part of a potato plant is the tuber. Potatoes are planted, harvested, stored and consumed in the vegetative state, the tuber. Except for the first two weeks after planting, progeny tubers are always present whenever the plants are growing. When tubers are harvested, they are removed from the relatively protected environment (soil) and are then especially vulnerable during storage to insect attack. The only insect that can do significant damage to stored potato tubers is the potato tuber moth.

The potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), is a ubiquitous pest on all continents, except the arctic. It is most serious on potatoes, but has also become increasingly important on tobacco (Van Vuuren *et al.* 1998) and tomato (Gilboa & Podoler 1994). Damage has also been reported on eggplant and other Solanaceous crops and weeds (Rahalkar *et al.* 1985). The potato tuber moth larvae are

miners or tunnelers. They mine in potato leaves during the growing season, but also move down cracks in the soil to reach tubers under the ground when the foliage start dying off naturally at the end of the season. They tunnel through the tubers, filling the tunnels with frass and webbing and allowing the entry of pathogens (Ferro & Boiteau 1993). These damaged tubers are only noticed at harvest time. Potatoes to the value of approximately R40 million are lost every year by South African potato farmers due to tuber moth damage in the field (D. Visser, unpublished data).

A third stage in potato production where potatoes are vulnerable to tuber moth attack is during storage. Potato tubers are stored in various ways, depending on their destination and whether they are utilized by commercial or small-scale farmers. Most commercial farmers plant large areas (more than 30 hectares) per season, and are well equipped with expensive infrastructure, including large cold storage facilities. Those farmers without cold storage facilities collaborate with co-operatives that provide these facilities as part of their seed distribution channels. However, small-scale farmers usually plant for subsistence only, do not have expensive equipment or infrastructure and rely on traditional methods of storing. The inputs of small-scale farmers are therefore significantly lower, but the overall quality of their product is also lower compared to that of commercial potato farmers.

Commercial farmers rarely store table potatoes because they have the infrastructure to send potatoes to the market directly after harvest and sorting. They only store seed potatoes, and then only in cold storage facilities. The temperature inside these facilities normally runs at 2 to 3 °C while the tuber moth larvae need temperatures above 10 °C to develop (Ferro & Boiteau 1993). Seed tubers are therefore protected from potato tuber moth attacks and damage in such facilities (Raman *et al.* 1987). There are, however, times when seed potato tubers have to be left in open stores for lengthy periods. This include the in-transit scenarios when seed tubers are send from co-operatives to farmers, the two to four week period that seed tubers have to be taken out of cool storage for sprouting or "reconditioning" purposes (Dean 1994), and the time tubers have to wait for the sorting process after harvest. At all of these stages the tubers are vulnerable to attacks from the potato tuber moth. It has also happened that a seemingly uninfested batch of potatoes suddenly started to show infestation symptoms while no tuber moths were present

(J. van Vuuren, personal communication). This is sometimes due to "latent" infestations – eggs or first instar larvae were present on or inside the tubers when the seed were bought, but not noted until the damage became more visual days later (Kroschel & Koch 1994).

The potato tuber moth is the major pest of potatoes stored under traditional storage systems in Africa (Roux *et al.* 1992). These small-scale farmers store both seed and table potatoes. They use self-made diffused light stores (Raman *et al.* 1987) or rustic shelters (Roux *et al.* 1992) to store potatoes for consumption or seed. Some farmers cultivate their own seed, which they store for a few months until planting commences (Kroschel & Koch 1994). Potatoes in such stores are vulnerable to attacks by the potato tuber moth originating from dumping sites (Daiber 1989) or from infested tubers stored unknowingly with healthy tubers (Kroschel & Koch 1994). Seed bought from such sources will almost certainly be infested by various stages of the potato tuber moth. If left untreated, the contents of a potato store may be completely destroyed by potato tuber moth larvae (Fuglie *et al.* 1991; Ferro & Boiteau 1993).

Pesticides are often used on seed tubers to protect them from diseases (Dillard *et al.* 1993; Dover & Ingram 1999) and insects (Roux *et al.* 1992). The efficacy of these pesticides is mostly based on unsupported information and little research has been done to evaluate them under controlled conditions. Twenty-three insecticides were registered in South Africa for tuber moth control in the field in 2002 (Nel *et al.* 2002), but no insecticide has been registered for post harvest applications. Because of long storage times, any insecticide that can potentially protect potatoes in a non-refrigerated store has to have a long residual action. Chemical companies are therefore reluctant to test their chemicals on a potential edible crop because of the dangers of human poisoning.

Concern for the lack of research for the protection of seed potatoes against the potato tuber moth in stores has been expressed by Daiber (1989), and is still one aspect of potato production that has received no attention in South Africa. The potato farmers in South Africa have expressed their concerns about the lack of control measures to protect tubers in storage. Control measures used by small-scale farmers to protect stored potatoes are

inefficient and sometimes huge losses still occur. 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 alternative methods to control the potato tuber moth in store environments. This was therefore an important aspect of potato production that needed urgent attention to the benefit of the commercial potato industry and small-scale potato farmers in South Africa.

**Motivation and Objectives**

The main objective of this study was to evaluate different control strategies against the potato tuber moth attacking potatoes under storage conditions. The aim was to find those control strategies that could be used by the small-scale as well as the commercial potato farmer. Depending on the situation, the following scenarios can be expected relating to stored tubers:

- infested potato tubers arrive in the store after severe field infestations during the previous season
- a batch of freshly harvested potato tubers have to be stored temporarily before sorting facilities become available
- tubers have to be transported to remote destinations in containers that are not insect proof
- already sprouted seed tubers have to be taken out of cool storage and kept at room temperature for at least three days for reconditioning purposes before planting
- dormant seed tubers have to be taken out of cool storage and kept at room temperature for two to four weeks for sprouting purposes
- subsistence farmers have to store potato tubers for lengthy periods without the convenience of cold storage facilities
- sometimes a farmer have to use an infested batch of seed tubers and needs to kill the larvae inside tubers before planting

The following objectives were pursued in an effort to expand our understanding of the pest and to address the problems the potato tuber moth may cause relating to the above scenarios.

1. to monitor potato tuber moth numbers with synthetic pheromones to quantify the relationship between temperature, rainfall and seasonal flight activity
2. to evaluate potential insecticidal powders to be used to prevent potato tuber moth attacks in those situations where no other methods are effective, e.g. severe tuber moth pressure
3. to evaluate fumigation with aluminium phosphide for those situations when a large batch of seed tubers became infected and needs to be cleaned from potato tuber moth larvae infection before planting proceeds
4. to evaluate the potential of UV light-assisted insect electrocuter traps to control newly emerging tuber moths in a potato store
5. to evaluate the potential of crude extracts of a potent insect virus for protection of stored potato tubers
6. to evaluate the potential of crude aqueous extracts of syringa tree leaves for protection of stored potato tubers
7. to evaluate the potential of mating disruption and attract-and-kill techniques with pheromones in store environments
8. to evaluate the potential of genetically modified potatoes against severe potato tuber moth attacks in potatoes stored for lengthy periods
9. to report on alternative and simplified rearing techniques of the potato tuber moth

**Aim and rationale of this study**

The aim of this study was to address the lack of control strategies relating to the control of the potato tuber moth in non-refrigerated store environments. The control measures currently used by most farmers are ineffective. This study attempts to address these shortcomings by providing alternative and novel strategies that can be used by both the commercial and small-scale potato farmer to control the potato tuber moth in potato stores.

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# CHAPTER 1

## Flight activity patterns of the potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae)

### ABSTRACT

The synthetic pheromone of the potato tuber moth, *Phthorimaea operculella*, was tested for the first time in South African against potato tuber moth populations under field conditions. Moth activity in two adjacent potato farms north east of Pretoria was monitored. Potato tuber moths were present in traps all year round. There was a sudden increase in moth numbers after the winters of 1993 and 1994, while a gradual increase was recorded for 1995. The highest moth numbers were recorded during or immediately after harvest at the end of summer. During winter, when no plants were available as food for larvae and when minimum temperatures declined to below freezing point on some days, moths were still caught in traps. There was a positive correlation between mean monthly temperatures and the number of moths caught in traps. Trap catches on two nearby potato farms showed similar variation in seasonal tuber moth numbers. The population pressure, however, was not the same at both farms. Tubers left in the soil after harvest may be the main inoculum of tuber moths for the new season after the winter.

**Key words**: *Phthorimaea operculella*, potato tuber moth, potatoes, pheromone, flight activity.

## INTRODUCTION

The potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), is a serious insect pest of potatoes in South Africa (Visser & Steyn 1999). The larvae attack potato plants and tubers under the soil and in stores and it is responsible for losses of up to R 40 million per annum to the South African potato industry. All producers rely on insecticide application, generally applied at weekly intervals in combination with a fungicide, for tuber moth control. Applications usually start when the first moths appear and are applied eight to twelve times per season. Control is not always satisfactory and damage levels vary between seasons and years, depending largely on the overwintering survival of moths and their reinfestation of newly planted fields (Lal 1987).

The pheromone glands in tuber moth females were first described by Adeesan *et al.* (1969) and the first synthesis of the pheromone was by Voerman *et al.* (1977). The potato tuber moth is nocturnal (Annecke & Moran 1982). However, males are strongly attracted to synthetic pheromones, even during the day. Pheromone traps are therefore a potentially very useful method for monitoring population activity as part of an integrated management program for this species (Raman 1988). However, although the pheromone was imported into South Africa in the 1980's, it was only recently registered here for commercial use (Nel *et al.* 1999). Daiber (1989) used pheromones to monitor tuber moths in potato stores, but no field studies using pheromone traps have been conducted in South Africa.

Studies on the occurrence, flight phenology and survival of potato tuber moths in potato fields have shown that their development is mainly determined by climatic factors (Ali 1993; Kroschel & Koch 1994; Trivedi *et al.* 1994, Roux & Baumgartner 1995). Geographic variability in the demography of potato tuber moth and its natural enemies, as well as differences between potato cultivars have been documented (Briese 1986; Vickers & Entwistle 1991; Flanders *et al.* 1997). The effects of different factors on the effectiveness of pheromones vary considerably between different populations of the same insect species (McNiel 1991). Therefore, although the tuber moth pheromone is known to be a potent attractant, little is known about its efficacy under South African conditions. Furthermore, the South African tuber moth population has been isolated for more than 100

years (Broodryk 1967). These two factors (environment and population variability) could thus influence the efficacy of synthetic pheromone traps in South Africa.

The objectives of this study were to investigate the effectiveness of commercially available synthetic potato tuber moth pheromones for monitoring local tuber moth populations, and to quantify the relationship between temperature and rainfall with seasonal flight activity using pheromone traps. Information gained from the study will contribute to the understanding of emergence patterns, seasonal variation in population numbers and assist in the timing of insecticidal applications.

## METHODS

Tuber moth populations were monitored on two farms belonging to the Agricultural Research Council, Roodeplaat and Zeekoegat *ca* 5 km apart, located approximately 30 km northeast of Pretoria (25°35'S, 28°21'E), 1164 m above sea level. On both farms one potato field, two to three ha in size, was planted. They were under the same management, therefore irrigation, fertilizer applications, ridging, spraying programs for insects and diseases, etc. were similar. Three pheromone traps were placed in each field, approximately 100 m apart and 15 m inside the outer edges of the field, in accordance with the recommendations of Wedding *et al.* (1995). Traps were examined weekly throughout the year and all moths were removed and counted. All pheromones in this study were removed and replaced after three months (Raman 1988). The pheromone capsules used were manufactured by the Laboratory for Research on Insecticides, Wageningen, The Netherlands, and obtained from the International Potato Center (C.I.P.) in Peru. These were rubber capsules impregnated with two tuber moth pheromone components, namely, trans-4, cis-7-tridecadien-1-ol acetate and trans-4, cis-7, cis-10-tridecatrien-1-ol acetate (0.4/0.6 mg).

Traps were hand-made and omni-directional (modified from Bacon *et al.* 1976). They consisted of 290 x 210 x 200 mm plastic containers with two 30 mm access holes through two opposite sides and one hole through the two other opposite sides, approximately 100 mm from the bottom of the container. The traps were filled with water up to the holes,

after which half a teaspoon of detergent was added and the water stirred. The traps were then closed with a loose polystyrene lid, held in place by a weight. The pheromone capsule was attached to the lid of the container with a thin wire and hung to the same height as the access holes. The water level was maintained just below the pheromone capsule (in line with the access holes). The traps were placed on the ground between plant rows and later moved to the top of ridges (200 to 300 mm high) when plants were ridged approximately one month after plant emergence (Kennedy 1975). One plant next to the trap on each side was removed to prevent obstruction of airflow through the trap.

The initial study only included the farm Zeekoegat where the traps were maintained for three years (1993 to 1995). The second field (Roodeplaat) was included for one year (1994) for comparison. When a new season's crop was planted, in late August and late February, the traps were moved to the new location as soon as the first plants emerged. This was normally adjacent to the previous season's field.

For the purpose of this study, the seasons were defined as summer, December to February; autumn, March to May; winter, June to August, and spring, September to November. Temperature and rainfall data were supplied by the ARC-Institute for Soil, Climate and Water in Pretoria, using the Roodeplaat-AGR weather station.

Pearson's correlation coefficients (Snedecor & Cochran 1980), were used to quantify relationships between logarithms of the mean number of tuber moths, and temperature and rainfall for each of the three years. A multiple regression model (Genstat 2000) was used including both temperature and rainfall as explanatory variables. Because the weekly trap data were temporally autocorrelated, the intention was not to build a predictive model, but rather to correlate moth numbers with temperature and rainfall during the period under the prevailing climatic conditions. Differences between tuber moth numbers on the two adjacent farms were tested using Students' t-test.

## RESULTS

There was a rapid increase in pheromone trap catches during the warmer summer months. This was followed by a sharp decline in moth numbers during autumn (Fig. 1).

Moth numbers peaked during or just after the summer harvest (January/February). Lower moth numbers were recorded during the colder winter months, although moths were present in traps even during the coldest winter months of 1994 when the minimum temperatures dropped to –2 .4 °C (Table 1). For a given year, moth numbers fluctuated dramatically from one month to the next (Fig. 1). This was most obvious during 1993 when average catches declined from almost 1000 moths per week in March to less than 100 in April. The main decline for 1994 and 1995 occurred during February and during March for 1993. During 1995 the increase in numbers after winter was gradual, taking several months, while a dramatic and sudden increase occurred during November of 1993 and 1994 (Fig. 1). A sharp decline in numbers occurred in December during all three years. At the beginning of 1994 numbers increased to more than 1200 (from approximately 700 during the previous years' December), but during the following year (1995) the numbers stayed lower (500 to 600) for the rest of the summer (Fig. 1). During the winter months of 1995, however, more moths were caught in traps than during the previous two years.

There was a positive correlation between both temperature and rainfall and potato tuber moth numbers during all three years (Table 2). Except for 1994, temperature correlated better with tuber moth numbers than rainfall. Temperature and rainfall were also strongly correlated with each other ($r > 0.8$). The multiple regression model showed that adding rainfall to the model of log counts against temperature ($y = 1.96 + 0.26x$ ; $P < 0.001$, d.f. = 10) did not significantly improve the model during 1993 and 1995 ($P > 0.64$). When rainfall was added during 1994, however, the model was improved ($P = 0.024$).

Depending on the time of the year, tuber moth numbers differed between the two adjacent farms. There was always significantly ($P < 0.05$) higher numbers in traps during the warmer months at Zeekoegat, except for February and October (Fig. 2). Zeekoegat also started and ended the year with significant higher numbers than at Roodeplaat. During the colder months (April to August), tuber moth numbers remained below 100 per week for both farms. For three months (March, April and December), Zeekoegat had twice as much tuber moths in traps than Roodeplaat.

## DISCUSSION

High moth numbers during summer harvest (January/February in this study) were also recorded by Raman (1988), Gilboa & Podoler (1994) and Trivedi et al. (1994). At harvest, the potato season has ended and theoretically tuber moth numbers should be at their highest. Gilboa & Podoler (1994) found that moths present in potato fields that are being harvested are the primary source of infestation for nearby solanaceous crops. The results of the present study demonstrated that high numbers of moths could be sustained for longer than a month after harvest. It is therefore important for producers of potato, tomato and tobacco to consider this when planting solanaceous crops shortly after, or adjacent to, each other.

Although temperature and moth numbers were strongly correlated, dramatic changes in moth numbers never followed dramatic changes in temperatures. For all three years, temperatures only started to decline after moth numbers decreased. Short-term weather changes (weekly) could therefore not always be an indication of an expected change in moth activity in a specific field. Krambias (1976) also found that hourly temperature changes do not affect tuber moth pheromone catches.

Long term temperature changes, e.g. during winter months, and the availability of food sources were probably the most important factors influencing noticeable changes in moth numbers. This conclusion was shared by Kroschel & Koch (1994), Trivedi et al. (1994) and Roux & Baumgartner (1995). Temperature therefore indirectly affects moth catches by regulating the rate of development of the tuber moth larvae. More moths will therefore be caught in warmer months because more moths appear over shorter intervals. The increase in moth numbers in this study followed the increase in mean monthly temperature up until harvest in January/February. This positive correlation between temperature and moth numbers supports findings of Kroschel & Koch (1994) and Trivedi et al. (1994).

Trivedi et al. (1994) found a negative correlation between rainfall and moth numbers caught in pheromone traps, and Whiteside (1980) also showed that rainfall caused mortality in tuber moth populations. The positive correlation found between rainfall and

moth numbers in this study could be attributed to the fact that it was done in a summer rainfall area where temperature and rainfall were strongly correlated. The negative effect that rainfall could have had on tuber moth numbers in this study could have been masked by the positive effect of temperature. The year during which the correlation between moth numbers and temperature was higher (1994), was also the year with the lowest total rainfall. This suggested that rainfall may have influenced the correlation between temperature and moth numbers negatively. The years during which there was low total rainfall resulted in stronger correlations between temperature and moth numbers. The two factors thus worked against each other with temperature seemingly the most important factor regulating moth numbers.

It is well known that high temperatures stimulate shorter generation times of the tuber moth (Kroschel & Koch 1994). This, in addition to the fact that the potato crop is usually grown in the warm months, contributes to the serious damage levels during summer. Farmers seldom complain of damage to potatoes grown during cold months. Relative humidity does not influence the development cycle of the tuber moth (Broodryk 1971), but may negatively influence pheromone catches in the short term (Krambias 1976; Chandramohan 1995). However, humidity was not measured in this study.

The potato tuber moth does not undergo diapause (Broodryk 1971; Mitchell 1978). It therefore has to have continuous access to a host plant for the duration of larval development. All solanaceous weeds that grew in the vicinity of the trial, and which could have acted as alternate hosts, died during the cold winter months. A large number of tubers always escape harvesting (Broodryk 1971), as was the case in this study. The only plant material that could therefore have given rise to the steady supply of male moths throughout the cold months (May to August) was post harvest tubers left on or just beneath the soil surface. Lal (1987) and Kroschel & Koch (1994) came to the same conclusion in their studies in India and Yemen respectively.

Although tuber moth numbers were higher at Zeekoegat than at Roodeplaat during a given year, the temporal pattern was similar at the two sites. Medium-term (monthly) differences between the two sites were noted during the warmer months in particular, but

long term patterns were similar. Differences between moth catches on the two farms for eight of the twelve months indicates that monitoring systems are required on individual farms. Treatment thresholds should therefore be calculated and adjusted according to catches and damage from the previous year on individual farms (University of California 1986).

Temperature has a definitive long-term seasonal effect on catches, while other short-term changes in moth numbers could not be explained. Farmers can therefore always expect high tuber moth numbers during warmer periods when potatoes are in the field. It is, however, critical for farmers to remove all left over tubers after harvest. These tubers are probably the most important, or possibly the only source of inoculum for the new infestation of the season. If all tubers are not removed from a field after harvest, tuber moths will be present all year round, even if temperatures reach freezing in winter.

This study has shown that the commercial potato tuber moth pheromone, developed for overseas tuber moth populations, is very effective against the local moth population and under South African conditions. It can thus accurately be used to monitor South African tuber moth population numbers at any given time or to monitor fluctuations over time. This is yet another invaluable tool to be used in an integrated pest control program where an accurate pest figure is needed for decision making.

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**Table 1.** Minimum and maximum temperatures and the total rainfall per month for three consecutive years for the Roodeplaat Research Farm, Zeekoegat.

| | 1993 | | | 1994 | | | 1995 | | |
|---|---|---|---|---|---|---|---|---|---|
| | Max. temp. | Min. temp. | Tot. rain | Max. temp. | Min. temp. | Tot. rain | Max. temp. | Min. temp. | Tot. rain |
| Jan. | 32.6 | 15.4 | 185 | 28.9 | 16.3 | 131 | 33.3 | 16 | 85 |
| Feb. | 29.9 | 14.2 | 92 | 28.3 | 14.2 | 101 | 33 | 14.7 | 114 |
| Mar. | 28.7 | 12.7 | 51 | 29.6 | 12.8 | 71 | 31.1 | 14.5 | 116 |
| Apr. | 26.4 | 9.4 | 18 | 27.7 | 6.7 | 31 | 25.5 | 10 | 65 |
| May. | 26 | 4.4 | 7 | 26.2 | -0.9 | 0 | 22.6 | 4.4 | 13 |
| Jun. | 21.5 | -1 | 1 | 20.8 | -1.9 | 0 | 22.4 | -0.6 | 0 |
| Jul. | 23.9 | 1.7 | 0 | 20.9 | -2.4 | 0 | 23.1 | -0.6 | 0 |
| Aug. | 24.1 | 2.7 | 0 | 25.8 | 0.9 | 0 | 25.5 | 2.8 | 2 |
| Sept. | 31.3 | 6.4 | 21 | 30.3 | 5.1 | 18 | 31.7 | 4.4 | 6 |
| Oct. | 29.8 | 13.9 | 111 | 29.2 | 8.6 | 33 | 32 | 11.3 | 62 |
| Nov. | 30 | 12.7 | 123 | 31.1 | 13.5 | 72 | 33.1 | 13.9 | 129 |
| Dec. | 30.1 | 16.5 | 113 | 31.8 | 14.2 | 135 | 28.3 | 13.7 | 174 |

**Table 2.** Correlation coefficients of average monthly temperature and total monthly rainfall with logarithms of mean potato tuber moth numbers. The total rainfall for each year is also given for comparisons. n = 12

| | 1993 | 1994 | 1995 |
|---|---|---|---|
| Temp. | 0.872*** | 0.895*** | 0.770** |
| Rainfall | 0.771** | 0.921*** | 0.580* |
| Rainfall (mm) | 722 | 592 | 766 |

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

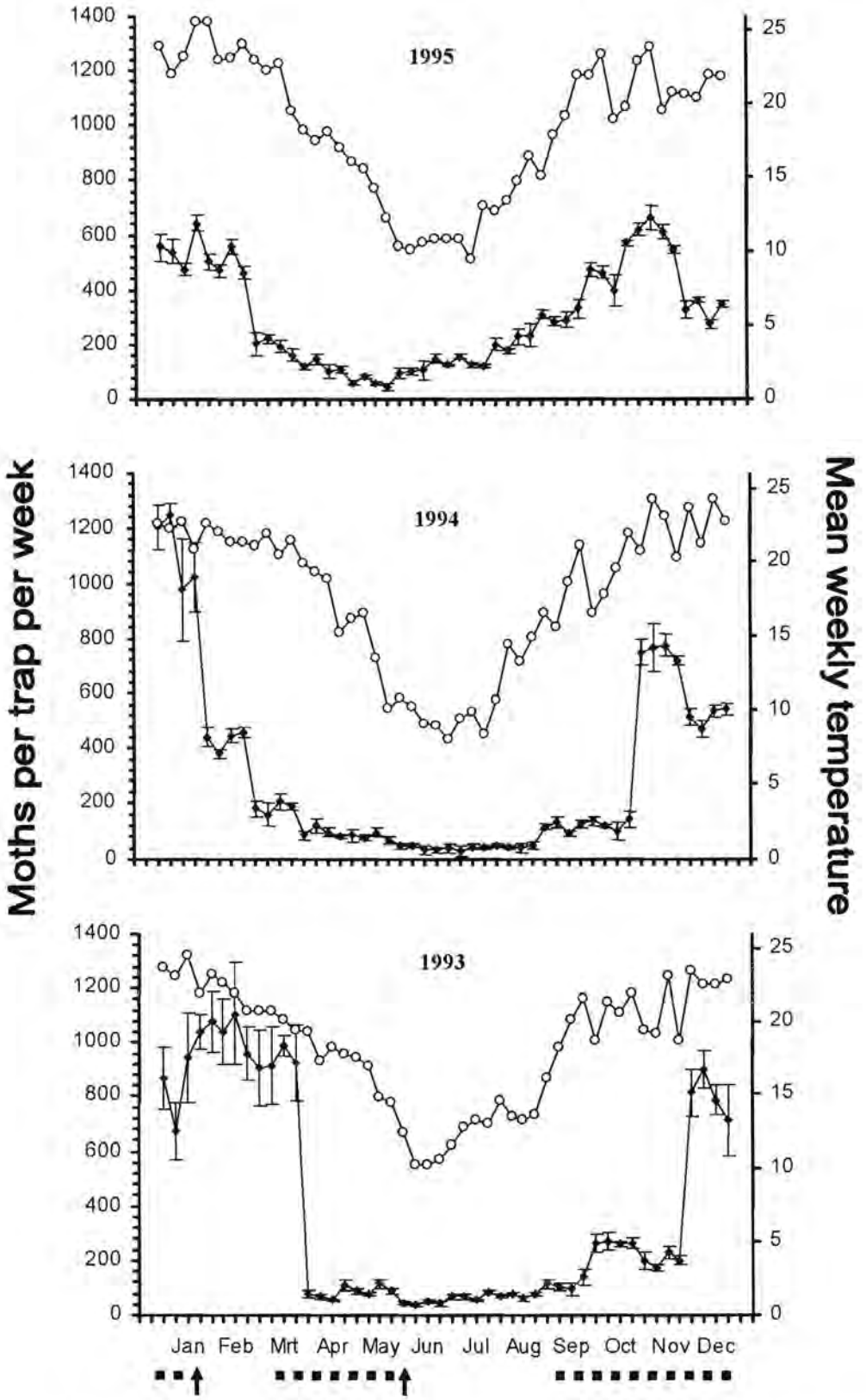


**Fig. 1.** The mean number of tuber moths (solid dots) caught per week with synthetic pheromones traps, from 1993 to 1995 on the farm Zeekoegat. The corresponding average temperatures (open dots) are given (centigrade). Bars represent standard errors (S.D.) of three traps. The dotted lines below the graphs indicate the time potato plants were present in the field and the upward arrows times of harvesting.

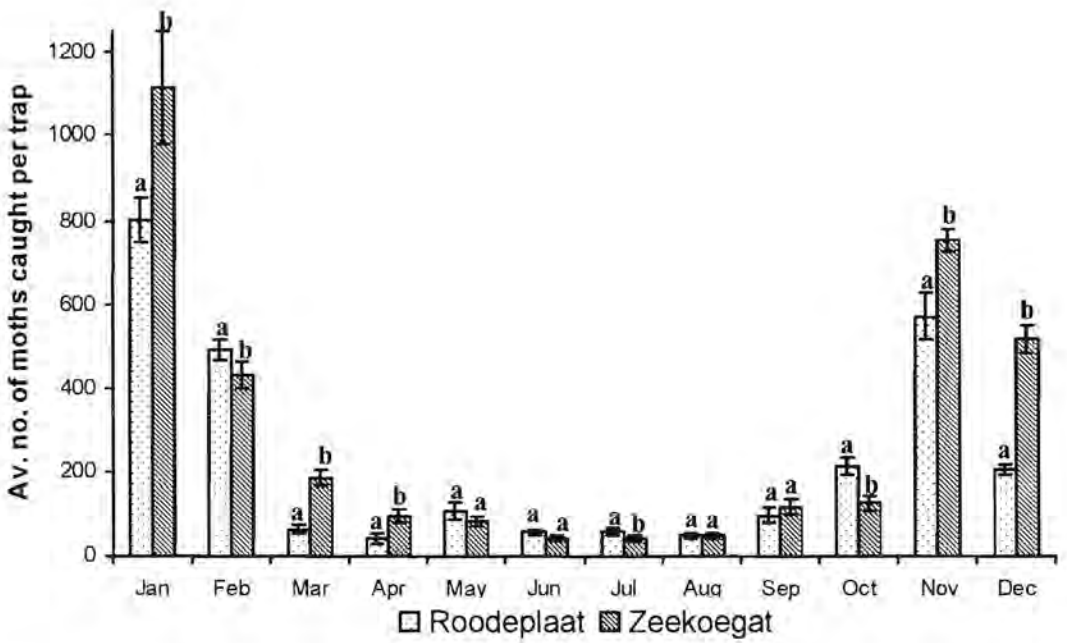


**Fig. 2.** The mean number of potato tuber moths caught per trap during 1994. The numbers are expressed as the weekly averages per trap for each month. Bars represent standard errors (S.D.) of three traps. Different letters (per month) indicate significant difference at the 5% level.

# Chapter 2

## Rearing techniques for small to medium sized rearing programs of the potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), with an overview of the literature on its basic biology under laboratory conditions

### ABSTRACT

Rearing methods for the potato tuber moth are described. These include a program for small-scale rearing where as little as 500 moths are reared and a medium scale rearing where 10 000 moths are produced per month. Different aspects of the rearing techniques are discussed and alternatives are given for some elements of the program. Critical aspects that may result in successes of failures are highlighted. Precautions that must be taken e.g. steps to minimise insect diseases and human health hazards like moth scales are explained. An overview of the literature on rearing techniques and the biology of the tuber moth under laboratory conditions is given.

**Key words:** Potato tuber moth, *Phthorimaea operculella*, rearing techniques, basic biology

## MOTIVATION

This study formed part of the larger study "The potato tuber moth, *Phthorimaea operculella* (Zeller), in South Africa; potential control measures in non-refrigerated store environments". Aspects of that study included research that was conducted with different stages of the potato tuber moth. Before any research in stores could have started, the rearing of the tuber moth had to be studied and mastered to ensure a constant supply of test subjects. A successful rearing program was a prerequisite, without which the larger study would have been impossible.

## INTRODUCTION

The first comprehensive rearing techniques for the potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), was published in 1947 (Finney *et al.* 1947). This was an improved method on their previous brief publication (Finney *et al.* 1944) and formed the basis of most subsequent tuber moth rearing publications. The authors used this mass rearing program for tuber moths to rear 19 million parasitoids per year. Several further publications on rearing techniques followed from various authors, most only briefly describing their rearing techniques as part of research projects. However, through the years a few important findings were made that allowed for more efficient and easier methods to follow. These include the use of NaOCl to free pupae from cocoons (Bartlett & Martin 1945) and the fact that moths will not lay eggs in brightly lit environments (Broodryk 1971). Other work that mainly involved the rearing methods of the potato tuber moth include; Platner & Oatman (1968), Etzel (1985) and Rahalkar *et al.* (1985). Cardona & Oatman (1975) used single tubers in individual holding containers to rear potato tuber moth for experiments. Griffith *et al.* (1979) described a technique for rearing potato moth larvae in isolation. Singh & Charles (1977) described an artificial diet for larvae of the potato tuber moth while Musmeci *et al.* (1999) described a method for *in vitro* rearing of tuber moth larvae on potato calluses.

Before starting a potato tuber moth colony at ARC-Roodeplaat (25°35'S, 28°21'E), in 1990, the literature was searched for previously published techniques. From the literature

it was mainly the recommendations of Finney *et al.* (1947) and Broodryk (1967) that were followed. However, over a period of several years, some of the procedures were found to be cumbersome, time consuming and labour intensive. From the literature it also soon became apparent that most researchers that reared the tuber moths for experiments have altered their rearing programs to suit their own needs. This study reports on the rearing methods that were implemented over a period of 12 years at ARC-Roodeplaat. The aim of this study was to develop a rearing program that was easy to maintain with limited inputs in man-hours and expenses. Some of the techniques and aspects that were introduced were new and never reported on before. This study describes all aspects of the rearing process and can be used by anybody starting a small to medium size rearing program for research purposes. With the techniques described here, one person was able to maintain a medium sized rearing program as well as 10 simultaneous smaller populations taking less than one hour every second day. This study also lists and reviews nearly all tuber moth rearing techniques that were reported in the literature.

## REARING METHODS

When rearing any insect, the most important aspect to consider is the number of individuals that is needed. If tuber moths are only needed to do small laboratory experiments with few individuals, the rearing technique will be considerably simpler than one where thousands are needed. The methods described here for both small and larger rearing programs are not rigid. Nearly every aspect of the rearing program can be altered to the needs of and equipment available to the researcher.

### *The insectary building*

The insectary is housed in a solid corrugate iron roof brick building with four rooms (3,5 x 2,6 meter each) adjacent to a storage area with its own entrance (Fig. 1). The three rearing/experimenting rooms in the insectary are air conditioned by means of a combined central "chilled air" and heating system. Temperature is kept at 26 °C and humidity is not controlled. All rooms are kept dark (0:24) and lights are only switched on when personnel needs to enter. The rooms are also fitted with an extractor fan that is connected to a 150-mm diameter extraction pipe, which runs across the room 500 mm beneath the ceiling (Fig

2a, white arrow). Forty millimeter diameter holes were inserted into the extraction pipe at distances of 400 mm. The extraction fans are always on and serve to remove air and scales from the rooms continuously. In one of the rooms, insect cages (480 x 460 x 380 mm) are connected with the extraction pipe by means of 40 mm diameter swimming pool cleaner pipes (Fig. 2a). In the other rooms the height of the extraction points can be adjusted by hanging different lengths of swimming pool pipes from the extraction pipe downwards to any height. Two of the rooms in the insectary are constantly in use for rearing purposes while a third is a spare room that is used when experiments are carried out (Fig. 1). The fourth is the handling room and is used for handling insects and cleaning of equipment. The handling room is fitted with an extra large extraction fan with a vacuum hood that is used when handling moths.

*Note: Wherever temperatures are given, it is always plus minus one degree centigrade. Relative humidity was never controlled and is not mentioned.*

*Adults and oviposition*

Adult moths are kept in plastic oviposition containers or buckets of various types and sizes. The containers are closed by means of fine gauze material fitted tightly over the opening with a large elastic band (Fig. 2b). Approximately 500 pupae are placed in a container with a 170 mm diameter. A relative reliable method to estimate the number of pupae is to correlate numbers with pupal weight. The weight of the pupae in this study averaged 10 mg per pupa. A clean batch of 500 pupae (with all sand and debris removed) will thus weigh five gram. If the average weight of a pupa is established for a given population, counts can then be replaced by weight, saving considerable time and effort. For rearing purposes, moths are only handled and counted in the pupal stage. The number of moths used in a rearing program will depend on the number of eggs needed and available space.

Thin tissue paper is used as the substrate for egg laying. It is placed flat on the upper outside of the fine gauze material with three to four glass Petri dishes (100 mm diameter) as weights on top (Fig. 2b). The weights ensure contact of the tissue paper with the gauze material. The thin tissue paper will allow enough circulation of air and will not negatively

influence the moths. Moths lay their eggs on the underside through the gauze material onto the paper. The tissue paper is removed and replaced every morning. With this method, eggs can be removed without opening the container.

Moths mate during the first day after hatching and start laying eggs normally on their second day in the container. Eggs are only collected on days two to five. Moths lay most eggs on the third and fourth days. Because very little eggs are laid on the second and fifth days, these are handled as one batch. However, because the eggs from the second day will have developed further by day five, it is stored at 4 °C (to slow down its development temporarily) and taken out when collecting the fifth day's eggs. One batch of moths will thus provide three batches of eggs, namely those collected on the second and fifth days, those collected on the third day, and those collected on the fourth day. At day six some eggs that were laid inside the container (not on the tissue paper) at day two, may start hatching and contamination by active first instar larvae walking to other populations in the same room may occur. A batch of moths are therefore never kept for longer than five days after eclosion. After collecting eggs on day five, the container with the moths is placed in a deep freezer at -20 °C for three days. This kills all moths and any eggs that may be left inside the container. Moths do not have to feed and are never given any liquids. With this method it is important to make sure that all pupae eclose on the same day, otherwise some moths will only start laying eggs days later than others. See "pupation" section for methods to collect pupae of the same age.

The above mentioned method of placing tissue paper on the outside top of the gauze material results in eggs being deposited anywhere on the paper. However, when smaller pieces of paper containing eggs are needed for experimental purposes, e.g. for inoculating plants in the field or glasshouses or bioassays in the laboratory, filter paper is used. Any filter paper will do, but Watman® no. 2 with a 70 mm diameter was used in this study. The filter paper is firm enough to be cut into smaller pieces and these pieces can then easily be pinned to other substrates when inoculating. The filter paper is first roughened in straight lines with a scalpel (Fig. 2c). Tubers moths are known to prefer rough surfaces when laying eggs. When filter paper with roughened lines is supplied, they will only lay eggs on these lines. Six lines, approximately 10 mm apart, are made on each filter paper.

The filter paper must, however, be placed inside the containers, because moths ignore these lines when the filter paper is put on top of the gauze material. The filter paper is first folded in two and opened again halfway and put on the inside bottom of the container to form an inverted v-shape. Moths will use the undersides of this filter paper as shelters and as the substrate to lay their eggs on. After removal, the filter paper can be cut alongside the thin rough lines that will then supply six strips of egg containing filter paper. These strips can then be examined under a stereo microscope and smaller pieces can be cut off with the desired number of eggs. However, the whole process requires for the containers to be opened without the moths flying out. This is done by sedating the moths with $CO_2$ (Afrox® Class E; 1.8 kg/m$^3$; 6 kPa) for 30 seconds (20 ℓ/min). This will allow enough handling time of approximately half a minute before the moths recover. The collection and exchange of the filter paper, however, can easily be done in 10 seconds. If $CO_2$ is not available, moths can also be subdued by a cold treatment. Placing the container with moths in a deep freezer (-20 °C) for three to five minutes will sedate them long enough for the exchange of filter paper. In an effort to determine whether $CO_2$ and the cold treatment influenced the egg-laying ability of moths, batches of 50 moths were tested. From the second day after eclosion, moths were exposed to daily doses of $CO_2$ for 30 and 60 seconds and a cold treatment (-20 °C) of three and five minutes. The number of eggs laid after four days were compared to an untreated control. No differences were noticed between any of the $CO_2$ or cold treatments compared to the untreated control. It was therefore concluded that subduing tuber moths with either $CO_2$ or a cold treatment will not adversely influence their egg laying ability.

When eggs are collected for bioassays, there are two things to consider, the substrate and whether single, loose eggs are needed. Eggs are glued to substrates by moths and are difficult or sometimes impossible to remove without damaging them, e.g. when they are laid on a coarse substrate. This can be a limiting factor when loose eggs are needed for experiments. However this can be accomplished by allowing the moths to lay eggs on the fine gauze material without supplying paper on top. When the tuber moth lay her eggs through the gauze material, the nature of the material does not allow the eggs, that are deposited through the tiny holes to the other side, to be glued to the surface. Most of the eggs will be loose, lying on top, and will roll to one side when the container is tilted.

Others will be dislodged with the slightest bump of the container. When batches of eggs are used, they are, however, normally handled while still on the substrate. It is important to use a pH neutral substrate. A substrate like filter paper is usually not pH neutral and may influence the results when bioassays are performed with agents like insecticides.

*Handling of eggs and first instar larvae*

One piece of tissue paper with one day's harvest, as described above, is put into a plastic container with a tight fitting lid. This type of containers must have airtight fitting lids, otherwise the first instar larvae will escape. Eggs change colour with the development of the embryo inside. At first they are nearly white or cream coloured, then change to yellow, then to orange and hours before hatching to black. When black eggs are put in a container at 26 °C, most of the eggs will hatch the following day. They will be trapped inside the container (Fig. 2c) and can be used any time as required. The first instar larvae, however, will not survive for more than a day inside the container at room temperatures and it is recommended to use them the same day.

The food source for first instar larvae is punched potato tubers. This study used medium sized tubers, approximately 100 to 150 g. The punching of tubers is a critical step, because very little larvae will succeed in penetrating the tuber if not punched (Table 1). Punching is accomplished by rolling each potato over a spiked wooden bed (Fig. 2d). The spikes are the sharp ends of thin nails, 2 mm diameter, protruding 5 mm and spaced 10 mm apart. Holes must cover the whole surface of the potato. Although Etzel (1985) found that it was critical to place the larvae on tubers immediately after punching, this study did not find any differences for a tested 24 hour period (Table 1). However, this might be related to the type of potato cultivar that is used. Only the cultivar "BP1", the most widely obtainable potato cultivar in South Africa, was used in this study.

The first instar larvae are transferred to tubers by hand. The contents of one plastic container, approximately 1500 larvae, are shaken out over approximately 40 potatoes in a plastic crate (Fig. 2e). These crates (510 mm x 320 mm x 160 mm) are without any solid sides, allowing for air flow and thus preventing build-up of moisture around the tubers. It is also important for the bottom "floors" of the crates to be perforated to allow larvae to

escape at a later stage and to facilitate aeration. To further prevent the build up of humidity, potatoes must not be placed in layers, e.g. the 40 tubers in this study fit exactly in one crate. Care must be taken that a homogenous spread of first instar larvae is assured over all the potatoes. It is unavoidable that some larvae will not land on potatoes, but fall through the crate to the floor. When more than one crate of potatoes are used at once, it is recommended to put a second crate, with potatoes, beneath the current one to collect all larvae that may fall through. The positions of the crates are then exchanged before larvae are shaken out onto potatoes in the second crate. However, because the numbers of larvae are usually more than needed, the losses that occur are negligible. Care must be taken, however, to prevent larval contamination when more than one population is reared at the same time, e.g. the transfer of larvae of different populations must be done in separate rooms.

*Pupation*

After infestation, the potatoes are moved (still in the same crates) to one of the rearing rooms. The crates are packed on a metal frame (1,2 m x 0,6 m x 0,75 m) with three support racks (shelves), each capable of hosting three crates (Fig. 2f). The lowest shelf is 100 mm from the floor with a space of 300 mm between the other two shelves. After approximately 10 days the larvae inside the tubers are full-grown and will exit from the tubers in search for pupation locations. They always move downwards to the floor surface. When the larvae reach the floor, they crawl actively in all directions while searching for pupation locations. A few larvae pupate in or between the tubers when excess debris is available to construct a cocoon. The floor of the pupation room has a solid smooth cement surface and is always kept clean and swept when debris accumulates. There are four ways in which these active larvae can be contained and forced to pupate where the researcher wants them to; heated barriers as first described by Flanders (1945), sand barriers, sticky glue and tight sealing containers. For a medium size rearing program, a sand barrier was used in this study. This sand barrier is supplied on wax paper on the floor around the metal frame (Fig. 2g). The wax paper serves as a substrate onto which the sand cocoons are glued by the larvae, but because of the nature of the paper, are easily dislodged when shaken gently. The sand barrier should be at least 200 mm outside the vertical border of the frame to prevent larvae from flipping to the outer areas of the sand while moving down

from the crates. When the larvae reach the sand barrier, they immediately imbed themselves into the sand and start spinning a silken cocoon while impregnating sand particles into the cocoon. The larvae will not crawl underneath the wax paper and precautions in this regard are therefore not necessary. Most larvae will pupate in the first sand they encounter. However, a few individuals always crawl a little distance over the sand before they start spinning a cocoon, and may sometimes not be stopped by this method. To lesson the possibility of larvae walking over the sand barrier and thereby escaping, a layer of sand with a width of approximately five centimeters are applied around the frame, after which it is spread out with four fingers, parallel to the edges of the frame. The result is five narrow rows of sand with approximately one centimeter opening between them. The larvae that ignore the first sand rows usually pupate in the third or fourth rows. However, a few individuals always ignore all sand and pupate in crevices in other places in the room. These few larvae and the moths that appear from their cocoons are ignored.

At the end of the larval cycle, a large number of fourth instar larvae appear simultaneously from the infested tubers. However, some larvae develop slower than others and it takes a few days for all larvae to exit form the tubers. To collect larvae/pupae of the same age, it is therefore important to regularly remove cocoons that have already been formed in the sand. Cocoons are collected every second day for up to a week. However, it is critical not to disturb the cocoons while the fourth instar larvae have not yet pupated inside it. The fourth instar, after spinning a cocoon, takes up to two days to change into a pupa inside the cocoon. When the cocoons are disturbed before that, the larvae will exit from their cocoons and pupation will be postponed. To prevent this, the wax paper on all four sides of the frame, with the sand and cocoons on it, is slowly pulled outwards for 300 mm and left in that position for two days (Fig. 2g, white arrows). A new strip of wax paper is placed in the place of the previous one after which sand is again placed on it as described. After another two days, the first (now outer) sand barriers (with cocoons) are removed and shaken off into a plastic bucket. The inner sand barrier is then moved outward and the process repeated. The sand cocoons (which contain pupae), are taken to the handling room where the excess sand is removed by using a normal kitchen sieve (one to two millimeter holes). The pupae with cocoons can be stored at 6 °C for later usage.

After collection of cocoons on the sixth or seventh day, the used potato tubers are discarded.

When pupae without their cocoons are needed for experimental purposes, they are dipped in 5% NaOCl for one minute. The cocoons must be agitated or stirred lightly during this time. The NaOCl dissolves the silk and in the process free the pupae from the sand cocoon. The sand and most debris sink to the bottom while the pupae float on the water surface. The pupae are decanted into the same kitchen sieve as described. The pupae, still inside the sieve, are rinsed twice in clean water to get rid of the excess NaOCl. This is done by placing the sieve in a plastic bucket just larger than the sieve itself, while gently agitating the water by hand. It is not recommended to wash the pupae with running water due to possible injuries to the pupae. The pupae are then transferred onto a piece of double layered paper towel. The pupae are spread out during this process to shorten the drying process. The drying process takes only a few minutes when room temperature is above 20 °C. When dry, the pupae are counted and transferred to the oviposition container.

The described method for collecting pupae is for a medium size rearing program. When an average of 30 pupae per tuber is harvested, one metal frame with nine crates will yield approximately 10 000 pupae/moths per month. For larger rearing programs, where hundreds of thousands of moths are needed, the techniques of Finney *et al.* (1947) are recommended. However, when small laboratory studies are conducted, or where a few different populations are reared simultaneously, even the methods described above are too cumbersome and impractical.

When smaller populations are needed, slight modifications are made to some techniques used in the medium sized rearing program described above. The methods are altered to rear up to ten populations in one insectary. Only twenty tubers are used per population, which will supply about 500 moths per generation. Depending on the situation, this is usually enough for experimentation as well as continuing the rearing process. The tubers are handled in the same way as described for the medium sized rearing program, but two days after infestation, the tubers are placed in expanding plastic net bags (Netlon®

Produce Net). All 20 tubers are put into one net bag, and the bag is then put into an upright plastic bucket, 340 mm high and 330 mm diameter (Fig. 2h and i). It is critical for this bucket to have an airtight sealing lid, to prevent larvae from escaping. The lid of the bucket is cut open around the inside rim, and the rim is then used to keep a fine gauze material in place, which is fitted tightly over the opening (Fig. 2i). Air holes (approximately 20 mm in diameter) are supplied around the lower sides of the bucket, which is covered with gauze material which is glued to the sides. An electric fan is in constant use on the buckets while there are tubers inside. The air holes and fan remove the excess moisture that accumulates on the insides of the containers. When moisture accumulates on the insides, mortality of larvae increase and diseases appear in the population. One fan with a swivel head can be used to aerate up to five such containers. Before a bag of tubers is put in the container, a round piece of wax paper with sand on top is supplied on the inside bottom. A square piece of expanded metal, bent at the corners to form four 50 mm long legs, is then placed inside with the bag of potatoes on top (Fig. 2h). The expanded metals' only purpose is to prevent the tubers from touching the sand. When this happens, the larvae glue their cocoons to the tubers which are difficult to remove afterwards. One container forms a closed larval and pupation unit and is only opened to remove the cocoons. The wax paper with cocoons is removed every second day and replaced with new ones.

*Storing of stages*

Storage of certain stages of the tuber moth is often needed to synchronize their availability with experiments. To lengthen the life cycle of the tuber moth, eggs and pupae are stored at low temperatures. Mortality of eggs increases considerably over time at 6 °C, and are therefore kept at 10 °C. This is in accordance with Rahalkar *et al.* (1985) who found that the incubation period of eggs can be doubled when stored at 10-12 °C. They also found that adults could be stored at 10 °C for two days without affecting survival, fecundity and egg viability. This study tested freshly hatched first instar larvae and found that they can be stored at 6 °C for three to five days in an airtight plastic container without causing excessive mortalities. Pupae are stored for up to four weeks at 6 °C when needed. However, mortality always increases with time. In one instance, mortality of pupae that were stored for two months at 6 °C was as high as 90%. Variation in survival under cool

storage may be influenced by factors such as humidity and other volatiles in the cooling facility. When storing tuber moth and its stages at 6 °C it is recommended that eggs are not stored for longer than one week, first instar larvae not longer than five days and pupae not longer than one month.

*Precautions*

**Escape of insects**

Some authors have made extraordinary precautions to prevent the escape of tuber moth larvae (Etzel 1985) and adults (Rahalkar *et al.* 1985). However, for a small to medium size rearing program, this study has found that it was time consuming and not worthwhile to implement such precautions other than common insectary husbandry.

**Scales**

Scales and other body parts of Lepidoptera are known allergens and pose a serious health hazard for workers in rearing programs (Davis & Jenkins 1995). Care must therefore be taken when handling the adults. Before a container with dead or anesthetized moths is opened, it is placed directly under a large extraction fan in the handling room for a few seconds. Most of the scales get vacuumed through the gauze material into the fan and is thus removed from the containers and handling room. The containers with the moths are also put in closed insect cages fitted with air extraction pipes (Fig. 2a).

**Diseases and parasites**

Finney et al. (1947) described sanitation problems such as mites, unwanted parasitoids, red disease, black disease and protozoa. They went to extraordinary lengths to ensure that diseases does not break out, e.g. boiling the removable racks on which tubers were stored and pasteurizing tuber moth eggs at 47 °C for 20 minutes. However, this study has never had any serious sanitation problems in the rearing facilities for 12 years, despite the fact that no equipment was sterilize after usage. The only instance when a granulosis virus and a bacterium were observed infecting a few individuals was when population density and temperature/humidity were allowed to increase beyond the usual norms. This was when the containers in which the larvae (inside tubers) were incubated were not aerated well enough or when potatoes were stacked in more than one layer in crates. Findlay (1975) mentioned

that too high densities of tuber moth larvae may be correlated with disease outbreaks. The fact that larvae were never reared in such high densities as described by most researchers, plus the fact that all pupae were washed with 5% NaOCl before use, helped keeping diseases out.

Although some researchers discarded the sand in which larvae pupated after use (Etzel 1985), the sand supplied for pupation purposes in this study was always re-used. The sand is cleaned (with the pupae) when washed together (as sand cocoons) in 5% NaOCl and the chances of diseases spreading through this medium are thus limited. The virus that attacks the potato tuber moth larvae is a granuloses baculovirus. Infected larvae are easily identified by their milky white appearance, their lethargic manner of staying on the tubers and not actively searching for pupation locations like healthy ones. However, it was found that no extra precautions or even sterilizing of the insectary and equipment is needed after an outbreak. Only a thorough search and removal of any white larvae between tubers is necessary. An epizootic in the insectary is not easily established because there is no strong wind or water movement. This virus only breaks out when the temperature and/or humidity go too high (e.g. above 30 °C and 90% RH). If these two environmental factors are rigorously controlled, an outbreak will rarely happen.

Larvae infected with a bacterium sometimes appeared in the insectary populations. However, this bacterium has not been studied in detail, but Broodryk (1967) identified one with the same description as *Serratia marcescnes* Bizio. These infected larvae, when appearing from tubers, are usually smaller than healthy ones, are dark red in colour and when they succeed to pupate, the pupae are also dark red colour. The temperature and humidity preferences of the bacterium were not studied, but the same process than for the virus is followed to prevent outbreaks. Attempts to reinfest an experimental batch of larvae with suspensions of macerated red larvae failed, indicating that the bacterium might not be very virulent.

**Ants**

The only problem encountered regularly were ants that entered the rearing facility. They attack tuber moth larvae when they exit from the tubers to pupate. Originally glue was

used to cover a 10-mm wide band on the floor next to the walls. This method, however, require that the glue must be removed and reapplied regularly. The sticky nature of the substance was also very inconvenient when moving around in the insectary. It was later discovered that baby powder (Johnson's® baby powder, containing perfumed talk), applied liberally in a 50-mm band on the floor next to the walls, kept all ants out. The powder never came into contact with any of the tuber moth stages in the rearing program. However, in an effort to detect whether the powder, which may be suspended in the air due to human movement in the room, have negative effects on pupation, 10 fourth instar larvae were dipped in the powder for two minutes. The larvae were kept in the container with the powder, and sand was added after two minutes. All ten larvae succeeded in pupating in the sand/powder mixture and eclosed to form normal tuber moths a few days later. The conclusion was that the baby powder does not influence pupation or eclosion of the tuber moth, while ants do not succeed in crossing even a very thin layer of the powder.

## OVERVIEW OF THE LITERATURE ON THE BASIC BIOLOGY AND REARING TECHNIQUES FOR THE POTATO TUBER MOTH

### *Basic biology*

The basic biology and responses of the potato tuber moth to different environments have been studied in detail for the South African potato tuber moth (Broodryk 1967; Zimmerman 1967; Gouse 1969; Broodryk 1970; Broodryk 1971; Brits 1972; Brits 1976). Other comprehensive studies elsewhere include Finney *et al.* (1947), Cardona & Oatman (1975), Al-Ali *et al.* (1975), Fenemore (1977), Gomaa *et al.* (1978) and Foot (1979). More studies were observed as references in cited literature, but they were either in foreign languages or unobtainable. Very few of the cited studies were done under the same conditions and different or apposing conclusions are often observed from different researchers. Therefore, only a few important aspects regarding the biology of the potato tuber moth in rearing facilities are highlighted.

Developmental times and fecundity as described by different researchers can be found in Table 2. From this table the following can be summarized;

- Temperatures of 15 – 30 °C resulted in an egg hatch period of between three and 19 days. Larval periods inside tubers lasted between nine and 15 days for temperatures between 25 and 32 °C. However, at 11 °C it took 67 days for larvae to complete their development. Pupal periods lasted four to 18 days for temperatures between 11 and 32 °C. The total development time from egg to adult varied between 17 and 24 days for temperatures between 25 and 30 °C. Pupal weight varied between 6.6 and 11.6 mg. Male moths weighed less than females. Fecundity varied from 77 to 236 eggs per female. More eggs were laid when water or sugar solutions were supplied. Moths never lived for longer than 23 days and males seemed to live longer than females.

Other aspects relating to potato tuber moth biology include the following;

- Cardona & Oatman (1975) found that temperatures between 23.8 and 32.2 °C did not have a significant effect on reproductive capacity, sex ratio and survival, but found the lowest mortalities of immature stages at 26.7 °C.
- The development time of the tuber moth (egg to adult) can be as short as 14 days at 35 °C, but nearly half of all individuals die at this temperature (Cardona & Oatman 1975).
- Oviposition occurred over a wide temperature range (11 – 29 °C) and a wide relative humidity rage, demonstrating that tuber the moth is well adapted to varying climatic conditions (Broodryk 1971).
- The lower threshold of development for eggs is 9.5 °C for South African tuber moth populations, but thresholds as high as 13.8 °C have been reported for Egyptian tuber moth populations (Broodryk 1971). Relative humidity did not influence egg mortality and even eggs dipped into water shortly before hatch, survived (Broodryk 1971).
- At high temperatures, mating took place soon after emergence and female moths started to lay eggs one day after emergence (Cardona & Oatman 1975).
- No diapause is known for the potato tuber moth (Etzel 1985). The tuber moth and its stages do not overwinter, but some stages are able to utilize the daily portions of favorable temperatures that occur for its development (Broodryk 1971).
- Fenemore (1977) found that female moths lay more eggs than the number of eggs in their ovaries just after eclosion. This means that more eggs mature in the females'

ovaries during her adult life, which might explain the importance of food and water on fecundity.

- Fenemore (1977) found that virgin females lived significantly longer than mated females and laid a small number of non-viable eggs over an extended period. The life span of multi-mated females was also significantly shorter than that of females mated only once (Fenemore 1977). These factors may explain some of the variability regarding adult longevity and fecundity as reported in the literature.
- Al-Ali *et al.* (1975) and Cardona & Oatman (1975) found the sex ration of males and females to be in the region of 1:1 while Foot (1979) found that this ratio was not altered by temperature.
- Male moths emerged 1-2 days before females (Cardona & Oatman 1975).
- Fenemore (1977) found that females started laying eggs within hours after mating, most eggs were laid from day two to four and egg-laying was nearly completed after day seven.
- Cardona & Oatman (1975) found the total mortality figure of immature stages to be between 18% and 42% in a rearing facility.
- Mortality in the pupal stage was relatively low and never above 5% (Broodryk 1971).
- Rananavare *et al.* (1989) found an orange-eye mutant of the adults of the potato tuber moth.

***Rearing techniques***

From the literature it is clear that rearing techniques for the tuber moth were adapted to the extreme to fit the requirements of the researcher. Nearly every step of the process can be manipulated to suit the needs for the purpose the moth is reared for. Given below is an overview of some important or interesting aspects as described in the literature. Table 3 lists the rearing conditions of selected authors. From Table 3, the following can be summarized;

- The majority of researchers used temperatures of 26 – 27 °C. Most did not control humidity. Day/night exposures varied from 8:16 to 0:24. Where moths were subdued by anesthesia, either $CO_2$ or $CO_2$ /ether combinations were used. Most researchers supplied water or sugar solutions for moths. Egg substrates included filter paper,

muslin material, cotton material, tissue paper (handkerchief), surgical gauze and potatoes. Larvae were nearly always given potato tubers or potato tuber pieces as a food source. One rearer used an artificial diet. Most researchers supplied sand for pupation of the larvae.

Other important aspects relating to the rearing of insects and in particular the potato tuber moth include the following;

- Fisher & Leppla (1985) and Goodenough & Parnell (1985) described the design and engineering requirements for expensive and large insect rearing facilities.
- Meisner *et al.* (1974a) found that the potato tuber moth females laid nearly twice as many eggs when their larvae fed on potato tubers instead of potato foliage. He also could not find any other plant that could match the high numbers of eggs produced by females originating from larvae that were fed on potatoes. Gomaa *et al.* (1978) found that, in relation to any other food source, a larval diet of potato tubers resulted in better and faster development of all stages of the moth. They also found that pupae and larvae were heavier, moths emergence were higher and they laid more eggs and adults of both sexes lived longer when their larvae were reared on tubers.
- The tempo of growth of tuber moth larvae is dependent on factors such as crowding and food source (Finney *et al.* 1947). Larvae preferred mealy tubers to non-mealy tubers (Finney *et al.* 1947). Small egg-size tubers were also a better food source and larvae developed faster on them than on larger tubers due to the faster moisture loss in small tubers (Finney *et al.* 1947).
- Density of moths in cages did not influence their fecundity (Traynier 1983).
- Traynier (1983) found that fecundity of moths were higher when fed diluted honey, but not when fed pure honey.
- Singh & Charles (1977) could not find any differences in fecundity between females of equal weight fed water or 5% sugar or honey solutions.
- Martin & Finney (1946) discovered that sand for cocooning of larvae should be used in layers not higher than 10 mm. When this was done, the larvae pupated at the bottom of the sand and formed a mat of cocoons with little or no excess sand.

- The recommended number of punctures in potatoes must be at least one third of the maximum number of mature larvae that the potato can support (Finney *et al.* 1947). The ideal hole is at a right angle to the surface, 1.5 mm in diameter at the surface and 3 mm deep (Finney *et al.* 1947). Such punctures prevented the formation of heavy scar tissue in and around the hole.
- Broodryk (1971) found that overcrowding of larvae on potato tubers influenced the pupal weight of such larvae. However, pupal weight did not influence the number of eggs laid or the total life span of females (Fenemore 1977). The main negative effect of crowding was lower pupation rates (Broodryk 1971). He found that where five larvae were used per gram of potato, only 23% pupation was maintained while a pupation rate of 97% was maintained where one larva was used per five gram of potato.
- It is recommended that tubers be spaced loosely and not touching each other. This can be done just after larvae have been place on them and will prevent the build up of frass at later stages (Platner & Oatman 1968).
- The punching of tubers serve two purposes; it allows entry points for the neonate larvae and helps with moisture loss that helps larval development deeper inside the tubers (Finney *et al.* 1947).
- Some researchers did not use numbers (moths and pupae), but rather volumes (Etzel 1985).
- The egg-production stage can be eliminated by letting moths lay their eggs directly on tubers. However, special cages are needed for this and sanitation and contamination can become problematic. The tubers have to be taken out of the cage every day and replaced with new ones. Estimation for time of hatch for eggs will also have to be accurate, because holes have to be punched into the tubers just before egg hatch. This can become labour intensive, especially when tuber exchange is only possible through sleeves in cages. However, in small rearing programs, this should not be a problem. Meisner *et al.* (1974b) used this technique for his normal multiplication program. They used tubers inside cages as egg laying substrates, but also supplied squares of cheesecloth, hanging from the ceiling of the cage. Some moths laid their eggs on this cloth which were then collected and used for experimentation.
- Moths were kept immobile during handling by using $CO_2$ or a cold treatment. Although $CO_2$ may influence other insects negatively in relation to longevity and oviposition

(Hooper 1970), and nearly every other aspect (Nicolas & Sillans 1989), this study have never encountered any noticeable problems. The very short period of only 30 seconds used may be too short to influence the moths negatively regarding longevity and oviposition. Moths tended to get use to the gas when used repetitively over short periods (e.g. every few minutes) and usually need more gas than usual to put them out after a failed first attempt. Care must therefore be taken that all moths are lying on the bottom of the container before gassing is stopped.

- When dissolving cocoons, there are usually very few larvae (that have not pupated) present. They are easily picked out with forceps. However, according to Finney *et al.* (1947), these larvae can be separated by flotation in a saline solution having a specific gravity of 1.05.
- Brits (1980 & 1982) devised a method of estimating the age of pupae by the color of their eyes, where white pigmented eyes denote young pupae, red eyes older and black eyes the oldest pupae.
- Platner *et al.* (1969) described a technique by which tuber moth larvae can be recovered from tubers before the fourth instar.

## DISCUSSION

From the literature it is clear that nearly every aspect of the rearing procedure of the potato tuber moth can be adjusted according to the need of the researcher. Small adaptations or adjustments to the already known basic program can make the whole process much easier. During the past 60 years the basic rearing program has changed little, with punched tubers as food source for larvae forming the center of the program. However, some aspects of the rearing program have been changed and some interesting discoveries were made that allowed simplification of certain actions. The most important discovery was that moths generally laid eggs on rough surfaces (Finney *et al.* 1947). This allowed for moths to be contained in containers with smooth sides with the assurance that little or no eggs will be laid on the insides of the container when other rough substrates are provided. The moths' own ecological habits were thus exploited to force them to lay eggs where the researcher wanted them to. A second important discovery was the use of NaOCl to free pupae from cocoons (Bartlett & Martin 1945). This process helped in obtaining

clean pupae that could be counted and also helped in the disinfecting process. Broodryk (1971) made a third discovery. He found that moths postponed egg-laying when exposed to bright light. This discovery helped the researcher to obtain eggs of specific ages. Age-related experimentation with eggs were now simplified. Other miner changes in the techniques of the rearing program are mentioned in the literature, including this study. One important technique that has not been used by many researchers is artificial diets. The reason may be that the preparation procedures are extremely complicated and the diet of growing larvae has to be replenish at least twice during its development (Singh & Charles 1977). This, plus the fact that survival rates were lower than when they were reared on tubers (Griffith et al. 1979), made the use of artificial diets not worth the effort. However, such diets may prove to be valuable when controlled bioassays are performed where precise concentrations of chemicals have to be incorporated into the diet of larvae (Musmeci *et al.* 1999).

Quality control is an important aspect of any rearing program and is discussed in detail by Boller (1972) and Chambers (1977). Conlong (1991) compiled a list of quality control problems faced by South African insect rearers. Irrespective of the reason for which an insect population is reared for, it must be kept in mind that reared insects are kept under abnormal conditions. Bartlett (1985) lists 19 critical factors that is different between a wild and reared population. When rearing insects for augmentative releases in the wild, it is important to realize that the rearing process itself may alter the behaviour of individuals. These include sexual isolation from the wild strain, dispersal problems, search incapability, mating behaviour, and oviposition problems (Boller 1972). Chambers (1977) discusses other processes that contribute to genetic decay of an insectary population. These include the "founder" effect, inbreeding, genetic drift and selection. It is therefore important to study and investigate all these factors before an insectary colony is started or when insect releases are planned.

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**Table 1.** The number of pupae harvested 14 days after tubers were inoculated with first instar larvae (n = 30)

| Treatment of tubers | Repetitions | | | | Av. |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | |
| Not punched | 5 | 2 | 5 | 2 | 3.5 |
| Punched and inoculated immediately | 23 | 21 | 20 | 17 | 20.3 |
| Punched and inoculated 4 hours later | 20 | 17 | 22 | 16 | 18.8 |
| Punched and inoculated 24 hours later | 24 | 15 | 23 | 18 | 20 |

**Table 2.** Development times and parameters of the potato tuber moth as found/used by different authors.

| Authors (chronological) | Temp and RH | Eggs hatch period | Larval period | Pupal period | Egg to adult period | Mean weight of pupae (mg) | Fecundity eggs/female | Adult longevity | Misc. information |
|---|---|---|---|---|---|---|---|---|---|
| Hovey (1943) | 10 °C<br>15 °C<br>20 °C | all died<br>19 days<br>9 days | - | - | - | - | - | - | fully grown larvae will not pupate when put at 5 °C |
| Finney *et al.* (1947) | 26.7 °C<br>50% RH | 5 days | 10 days | 9 days | 24 days* | 10.0 | 80 - 200 | - | larvae pupate 4 days after exiting |
| Broodryk (1970) | 26.5 °C | 4 days | 12 days | 6-7 days | 22-23 days* | - | - | 13 days (+H) | Differentiated instars by head capsule widths |
| Broodryk (1971) | 11 °C<br>25 °C<br>32 °C | -<br>4 days<br>- | 67 days<br>12 days<br>9 days | 18 days<br>6 days<br>5 days | -<br>24 days*<br>- | 8.2 to 11.4 depending on competition | 0 - 232 (+H) | - | Found 39 °C the upper threshold for eggs and pupae |
| Cardona & Oatman (1975) | 26.7 °C<br>50% RH | 4 days | 10days | 8 days | 22 days* | - | 178 (-F) | 8 days (-F) | Found that 35 °C made males sterile |
| Singh & Charles (1977) 1st | 30 °C | 3 days | 12 days | 4-5 days | 20 days* | ♂ 9.1<br>♀ 11.4 | 169 (+W) | - | Tubers as substrate for larvae |
| Singh & Charles (1977) 2nd | 30 °C | 3 days | 13 days | 6 days | 22 days* | ♂ 7.7<br>♀ 8.3 | 3 – 170 (+W) | ♂ 11 days<br>♀ 10 days (+W) | Artificial diet for larvae |
| Fenemore (1977) | 25 °C | - | - | - | - | 11.6 | 46 - 236 (+S) | ♂ 23 days<br>♀ 14 days (+S) | multi-mated ♀♀ live shorter than single mated |
| Etzel (1985) | 22 °C<br>70% RH | - | - | - | - | 10.3 | 85 (-F) | - | - |
| Rahalkar *et al.* (1985) | 29 °C<br>65% RH | 3 to 4 days | 13 to 15 days | - | 17-22 days | ♂ 6.6<br>♀ 8.1 | 155 (+S) | - | - |
| This study (2003) | 26 °C | 4 days | 10 days | 9 days | 23 days | 10.0 | 77** (-F) | 8 – 10 days (-F) | - |

-F = no food or liquids supplied, +S = sugar supplied, +H = Honey supplied, +W = Water supplied. When RH is not shown, it was not indicated and most probably not controlled. When no information was given a "-" is inserted in the table

* not mentioned by author, but estimated using the stage times given; some figures are rounded of

** 0 - 97 when tested singly (the few moths that laid zero eggs were ignored)

**Table 3.** Rearing conditions for the potato tuber moth as used by various authors.

| Authors (chronological) | Temp & RH | Day: Night* | Anesthesia of moths | Food for moths | Egg substrate | Larval substrate | Cocooning substrate | Pupal freeing | larval yield | Misc. information |
|---|---|---|---|---|---|---|---|---|---|---|
| Finney *et al.* (1947) | 26.7 °C | - | $CO_2$/ether | - | muslin sheet | punched tubers | sand/ paraffin waxed plates | 2.5% NaOCl | - | wire barrier of 82 °C to contain larvae |
| Broodryk (1967) | 26.5 °C | 12:12 | - | - | filter paper | punched tubers | corrugated biscuit paper rolls | pulling paper apart | 0.2 per 1g tuber | larvae preferred red paper rolls |
| Platner & Oatman (1968) | 26.7 °C | - | $CO_2$ | water | muslin sheet | punched tubers | sand/hardboard | not freed | 75 per tuber | used emergence unit for moths |
| Wearne (1971) | 26 °C 45% | - | - | - | tissue paper | punched tubers | corrugated paper strips | pulling strips apart | - | - |
| Meisner *et al.* (1974b) | 26.5 °C | - | - | 5% sugar on cotton wads | potatoes and cheese cloth | potatoes with pin-holes | sawdust | - | - | tubers were replaced every other day in moths cage |
| Singh & Charles (1977) | 30 °C | 16:8 | - | 5% sucrose | surgical gauze | artificial diet | within or next to diet | by hand | 4 larvae per glass tube | - |
| Matthiessen *et al.* (1978) | 30-34 °C | - | - | - | tissue paper | - | sand | - | 0.2 per 1g tuber | - |
| Foot (1979) | 20-25 °C | 8:16 | - | 5% sugar solution | filter paper | punched tubers | double-layer tissue paper | pulling tissues apart | - | sugar solution on egg substrate |
| Powers & Oatman (1984) | 27 °C | 12:12 | - | - | muslin cloth | punched tubers | white sand | - | - | potato juice on egg substrate Cloth pieces with eggs pinned to tubers |
| Etzel (1985) | 22 °C 70% | 0:24 | $CO_2$/ether | nothing | muslin cloth | punched tubers | sand/wax paper | 1.3% NaOCl | 1 per 1 g tuber | wire barrier of 82 °C to contain larvae |
| Rahalkar *et al.* (1985) | 29 °C 65% | 12:12 | $CO_2$/ether | 10% sugar | cotton cloth | potato slices | sand/aluminum sheet | NA | 2.5 per 1g tuber | - |
| This study (2003) | 26 °C | 0:24 | $CO_2$ | nothing | tissue paper | punched tubers | white sand/wax paper | 5% NaOCl | 30-50 per tuber | - |

When no information was given a "-" is inserted in the table
*except during handling hours
NA: not applicable

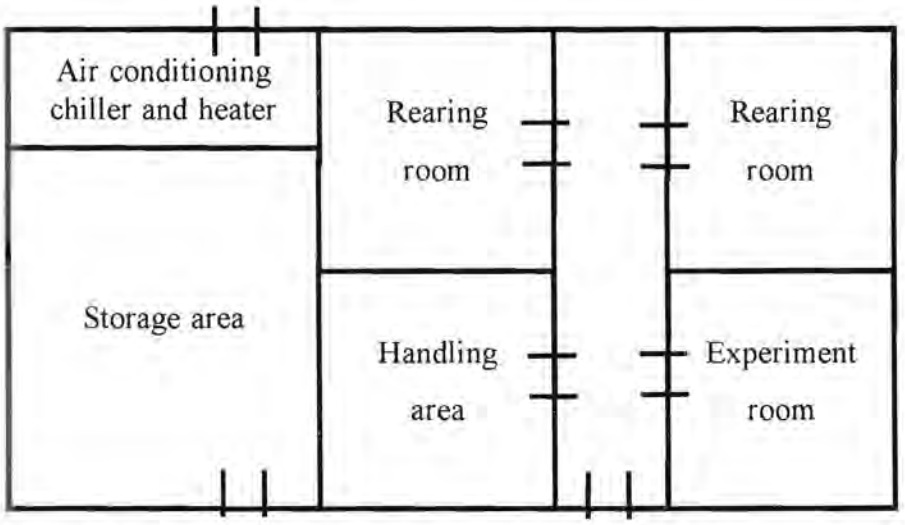


**Figure 1.** Plan of the potato tuber moth insectary at ARC-Roodeplaat

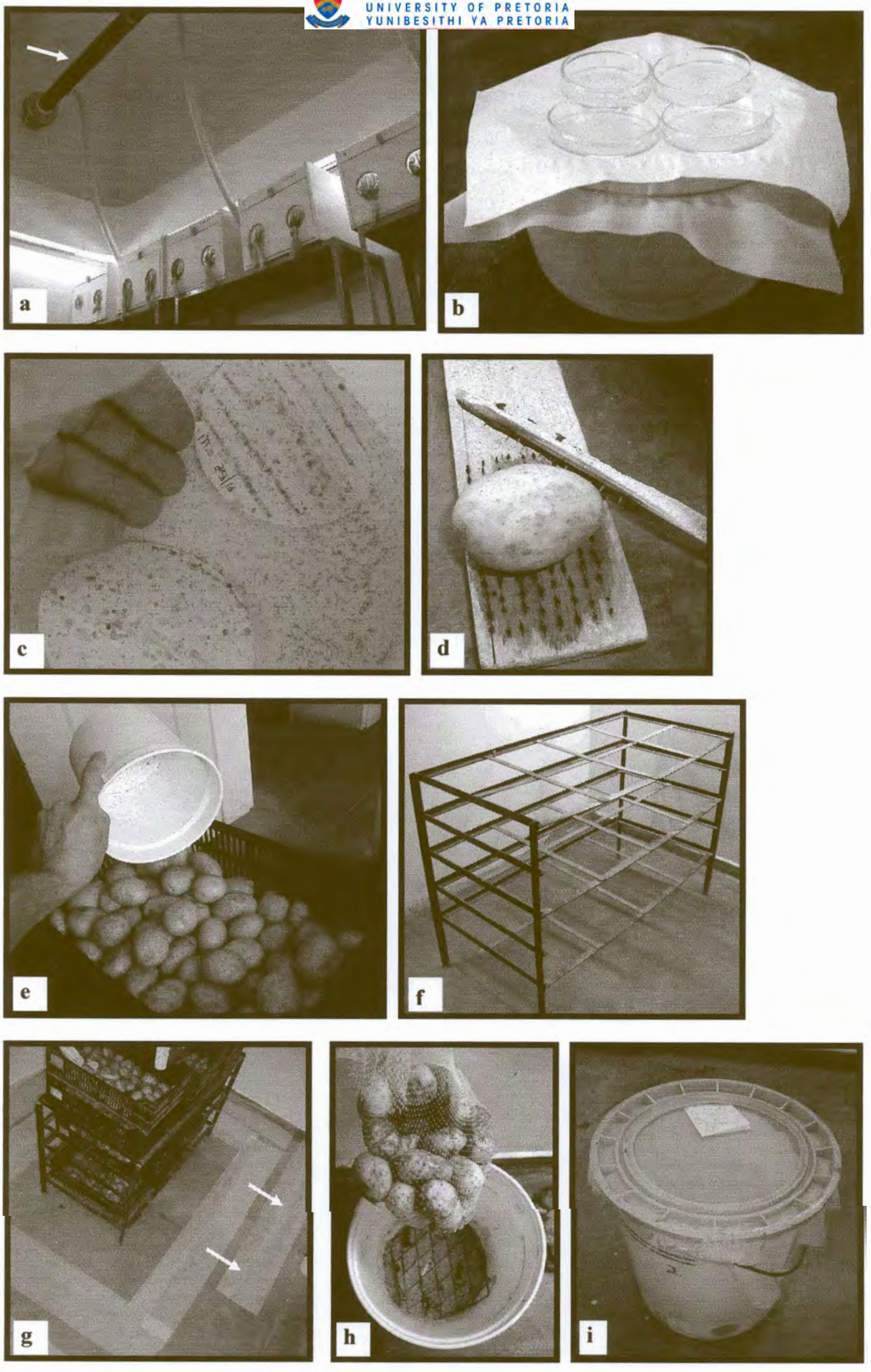


**Figure 2.** Aspects of the rearing program (see text).

# CHAPTER 3

## The potential of insecticidal powders to protect seed tubers against the potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), under laboratory conditions

### ABSTRACT

Three dusting powders; carbaryl, permethrin and gamma-BHC, a wettable powder (used as a dusting powder), *Bacillus thuringiensis* (*Bt*), and a domestic baby powder (as the control) were tested against the potato tuber moth on stored potatoes. Tubers were dusted by hand and artificial infestations were induced to simulate moth attacks in a store. A free choice experiment, where moths were allowed to infest any treatment, and a no choice experiment, where first instar larvae were put on treated tubers, were performed in an insectary. Moth attacks were induced immediately and 30 days after treatment and the larval attacks were induced 30 and 60 days after treatment. All the treatments protected the tubers from moth attacks immediately after treatment. However, only carbaryl, permethrin and *Bt* were effective against egg laying moths 30 days after treatment. All four insecticidal powders gave better than 70% control against first instar larvae 30 days after treatment. However, it was only carbaryl and *Bt* that protected the tubers 60 days after treatment. Gamma-BHC was the least effective of all the insecticidal powders in both the free choice and no choice experiments.

**Key words**: Potato tuber moth, *Phthorimaea operculella*, insecticidal powders, *Bacillus thuringiensis*, carbaryl, permethrin, gamma-BHC

## INTRODUCTION

The potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), is an important pest of potatoes in warm climates worldwide (Ferro & Boiteau 1993). In the protected environment of potato stores where tuber moths are present, however, the potato tuber moth is always a problem (University of California 1986). The larvae of the potato tuber moth create deep tunnels, three millimeters in diameter through tubers. The tunnels usually appear black, because they are filled with larval faeces and are often infected with fungi. Infested tubers are unmarketable. Ninety percent of tubers in stores may be destroyed (Lal 1987), while some studies reported a 100% loss after only two months of storage (Fuglie *et al.* 1991; Salah & Aalbu 1992).

Potato tubers are not only an important source of food, but also the main source of seed for the following season's crop. Potato cultivars can only be cultivated with seed tubers that have gone through a storage phase to break its dormancy. However, potatoes are also stored for various other reasons for three to nine moths after harvest (Dean 1994). In cool storage facilities, with temperatures below 10 °C, larvae of the potato tuber moth will stop feeding (Ferro & Boiteau 1993). Potato tubers can therefore be protected when they are stored in such facilities, but in developing countries, most potato farmers cannot afford the cost of storing potatoes in cold stores (Das 1995). There are, however, also times when seed potato tubers have to be left in open stores for lengthy periods (Dean 1994). During these times, the potato tuber moth populations that are present in the store may continue their development and infest potatoes in the rest of the store. Infestations are also possible from outside the stores; e.g. dumping sites (Daiber 1989).

Pesticides are often used on seed tubers to protect them from diseases (Dillard *et al.* 1993) and insects (Roux *et al.* 1992). The efficacy of these pesticides is mostly based on unconfirmed reports and usually no research has been done to evaluate them under controlled conditions. However, some insecticides have been evaluated for their effectiveness to protect tubers against the potato tuber moth (Morford 1964; Abdel-Salam *et al.* 1972; Hamilton 1983; Lal 1987; Raman *et al.* 1987; Das *et al.* 1992). Most of these tests were meant for the treatment of seed potatoes, because the use of insecticides on

consumer potatoes is highly undesirable (Ali 1993). Insecticides will not kill tuber moth larvae that have already entered tubers (Hamilton 1983). Abdel-Salam *et al.* (1972) found mercaptothion to be effective while Das *et al.* (1992) found deltamethrin to be effective, but *Bacillus thuringiensis* var. kurstaki (*Bt*) to be ineffective. Lal (1987) found *Bt* to be relatively effective (less than 15% damage) and carbaryl to be moderately effective (less than 35% damage), while Raman *et al.* (1987) found the dusting powder formulation of *Bt* to be very effective. Morford (1964) found that fogging with pyrethrum did not control tuber moth in a store, but succeeded in controlling tuber moth in a store by fogging with methyl bromide.

Twenty-three insecticides were registered in South Africa for tuber moth control in the field in 2002, but nothing has ever been registered to protect potatoes post harvest (Nel *et al.* 2002). This is partly due to the fact that registering insecticides is strictly controlled in South Africa, especially on edible crops. Because of long storage times, any insecticide that can potentially protect potatoes in a non-refrigerated store must have a long residue action. Chemical companies are therefore reluctant to test their chemicals on a potential edible crop because of the dangers of human poisoning. However, restricted registrations on edible crops are possible under certain circumstances, e.g. when colour-coded carriers are added to distinguish treated tubers.

Concern for the lack of research for the protection of seed potatoes against the potato tuber moth in stores has been expressed by Daiber (1988), and is still one aspect of potato production that has not been researched in South Africa. The potato farmers in South Africa, especially the western regions, have expressed their concerns about the lack of control measures to protect tubers when they are removed from cool storage. Post harvest treatment of tubers was therefore an important aspect of potato production that needed urgent attention to the benefit of the potato industry.

In an effort to find powdered formulated insecticides that can be used to protect seed tubers, the three most common powder insecticides on the South African market were evaluated. They represented three different groups of chemicals namely; pyrethroids, organochlorines and carbamates respectively. Liquid formulated insecticides were not

considered because of the potential of such treatments to spread diseases to healthy tubers (Dean 1994). Because the carriers of the mentioned insecticides were all in a powder form, and because powders (or inert dusts) are known to be irritants to insects (Romoser 1981), a non-insecticidal powder as well as a *Bt* powder (wettable powder formulation) were added as controls. The evaluations in this study was not meant to be a reflection of the conditions found in the vastly different store environments that can be found in different production areas, but only as an indication of the efficacy of these powders under controlled conditions for up to 60 days. The results can therefore be used to identify those insecticides that have potential as a future insecticidal powder for use against potato tuber moth on stored seed potatoes.

## METHODS

*Free choice experiment with tuber moths*

Four dusting powder formulated insecticides, a wettable powder insecticide, a domestic baby powder and a control were evaluated (Table 1). Medium sized tubers (100 to 150 g) of the cultivar BP1 was hand dusted through cotton stockings. A complete coverage of all tubers was ensured and the excess dust allowed to fall off before the storing process. For all the powders, except the *Bt*, a dosage of approximately one kilogram per ton of potatoes was used. The *Bt*, which was in an extremely fine powder formulation, was used at 300 g per ton of potato tubers. For these tests, the dusted tubers were removed from the container in which it was treated and placed in clean crates, i.e. no dust residues were allowed on the crates in which the potatoes were stored.

The experimental layout was a randomised block design with four replicates. Twenty crates, containing 25 potatoes each, were placed in an air-conditioned insectary with no windows. Because the distribution of potato tuber moth damage is aggregated when stored in piles (Roux *et al.* 1992), the tubers were placed in a single layer within crates. The crates were also not stacked, but put on the floor of the room. Two hundred potato tuber moths were released in the room on the first day of storage, 50 on each side against the wall, at a height of *ca* 2.5 m. The latter was to ensure that the moths would have an equal chance of reaching any crate on their way down to the floor. The moths had freedom of

choice to attack tubers. A temperature of 26 °C (±2 °C) was maintained with 24h darkness. Relative humidity was not controlled.

After 30 days, all tubers in all crates were examined for signs of tuber moth infestation. On the same day, all tubers were placed back in the same room and on their same places. Two hundred more tuber moths were released on the same day, in the same way as at day one. After another 30 days, a total of 60 days since the initial treatments, all tubers were evaluated again for signs of tuber moth infestations. There were thus two induced attacks, one on day one and one on day 30. It is important to note that although the final evaluation was done at day 60, the actual time the test lasted was just over 30 days. The final 30 days were just to give enough time for moths to lay eggs, for the eggs to hatch and the larvae that hatched from them enough time to induce damage. The powders were thus tested for immediate efficacy and then again efficacy 30 days after treatment. The schematic timeline can be found in Fig. 1.

*No choice experiment with tuber moth larvae*

A second experiment was conducted to investigate the possibility that the insecticides may have repelled the moths rather than killed them. This test tried to simulate the scenario of moths laying their eggs in the near proximity of the tubers, rather than directly on them. Ten tubers of each treatment, each in a separate crate, were used for these tests. A piece of filter paper containing approximately 20 potato tuber moth eggs (hatching age) was pinned to each potato tuber. This was done in such a way that the filter paper and the eggs they contained did not come into direct contact with the treated tubers. The pins with the filter paper and eggs were positioned in the middle of each tuber to ensure that the larvae that hatched from the eggs moved down and landed directly on the treated tubers. The first placing of eggs was 30 days after treatment and the second placing of eggs 60 days after treatment. After placing, the treatments were left for 12 days to show symptoms and were then evaluated. These tests thus evaluated the efficacy of the powders against first instar larvae at 30 and 60 days after treatment. The experiment had four replicates. The time line for this experiment is shown in Fig. 2.

The three dusting powders and the baby powder were obtained from a supermarket and the wettable powder, *Bt* (Ecoteck Bio), from the company *Ecogen* (experimental lot no. PC44286304). The *Bt* was used as a dusting powder because, at the time of study, a dusting formulated *Bt* was not obtainable and also not registered in South Africa (Nel *et al.* 1999). For the estimations of the cost of each treatment, all calculations (except the *Bt*) were based on the average price obtained from three suppliers. The *Bt* treatment was based on a price received from an agricultural chemical distributor for the *Bt* (Dipel). Dipel contains the same active ingredient as Ecoteck Bio.

For comparisons between treatments, 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

### Free choice experiment with tuber moths

All the powders gave excellent control immediately after treatment (Table 2). All the tubers in the controls were attacked when evaluated 30 days after releasing the first moths. No tubers in any of the insecticidal powder treatments were attacked, while only an average of three out of 25 tubers were attacked in the baby powder treated tubers.

Evaluations at 60 days still showed significant differences between all the treatments and the control (Table 2). In the carbaryl, permethrin and *Bt* treatments, less than four percent of tubers were attacked. These three treatments did not differ significantly from each other, but were significantly better than the baby powder and gamma-BHC treatments. However, the baby powder and gamma-BHC treatments showed significant less damage than the control.

### No choice experiment with tuber moth larvae

All the treatments showed significant less damage than the untreated control when larvae were put on tubers 30 days after treatment (Table 3). The carbaryl, permethrin and *Bt*

treatments resulted in a 100% control. Gamma-BHC and the baby powder were not as effective with 70% and 40% control respectively.

Evaluations of tests performed 60 days after treatment showed no control in all but the carbaryl and *Bt* treatments (Table 3). The *Bt* treatment gave 100% control while the carbaryl treatment resulted in 70% control.

## DISCUSSION

Three different stages of the potato production system are vulnerable to potato tuber moth attack, namely growing plants, tubers in the soil and tubers in storage. Although damage to potato plant foliage usually does not reduce yields, except when very young plants are infested (Bacon 1960), spraying programs are normally used to reduce attacks in the field. Tubers in the field can only be attacked when such spraying programs were not effective and when the larvae that survived, reach them under the soil. However, the situation in potato stores is different. Moths that originate from two separate sources may attack tubers under non-refrigerated storage conditions. Firstly, moths, eggs and larvae may arrive with the tubers from the field. Secondly, moths may fly in from the surrounding areas, e.g. other fields, crops or even dumping sites (Daiber, 1989). It is not always possible to identify and eliminate these sources of adult moths. It is therefore imperative that control measures be implemented to protect potato tubers when stored for lengthy periods in non-refrigerated store environments.

Results with the free choice experiment showed that potato tubers could be protected from potato tuber moths with all four insecticidal powders immediately after application. However, moths started infesting tubers in the gamma-BHC treatment 30 days after treatment. In the no choice experiment where larvae were allowed to attack treated tubers, it was gamma-BHC again that showed the first signs of breakdown 30 days after treatment. However, at 60 days after treatment, the permethrin treatment also did not protect the tubers from first instar larvae. Only the carbaryl and *Bt* treatments succeeded in protecting the treated tubers from first instar larvae 60 days after treatment.

It was shown that baby powder would prevent initial tuber moth infestations (only 12% damage), but the efficacy lowered to 34% damage when tuber moths attacked the tubers 30 days after treatment. Overall this is still acceptable under certain circumstances (e.g. the small-scale farmers) for such an irrelevant product. This unexpected result raises the possibility that other non-insecticidal powders may give the same result. Raman *et al.* (1987) experimented with three non-insecticidal powders in an effort to protect stored potato tubers. They were charcoal powder, wood ash and lime. None of them could protect tubers and resulted in 60% or more infestation levels in relation to the untreated control. It is possible that the strong smell of the baby powder used in this study may have acted as a repellent for moths looking for egg laying locations. The fact that non-insecticidal powders may prevent tuber moth attack, opens new options to the farmer. Some small-scale farmers in Africa use a powder formulated anti-sprouting agent when tubers are to be stored for lengthy periods (Fuglie *et al.* 1991). If this antisprouting agent show the same efficacy than the baby powder in this study, the application of other chemicals to protect such tubers against the tuber moth may be reduced or eliminated. Further work with more powders is thus warranted.

Because the *Bt* was used as a dusting powder rather than as a wettable powder (wettable powder applied dry), the excellent results were not expected. It was the only treatment that showed consistent control of both egg-laying moths and larvae for up to 60 days. Raman *et al.* (1987) found that *Bt* as a dusting powder formulation is more effective than its wettable powder formulation applied in a liquid form. Liquid formulated insecticides however, are not recommended on potatoes because of the potential of such treatments to spread diseases to healthy tubers (Dean 1994). The results clearly showed that the wettable powder formulation of *Bt* could effectively be used as a dusting powder to protect seed tubers against the potato tuber moth. The undesirability and potential dangers of applying large and uncontrolled quantities of insecticides to potato tubers destined for human consumption must be recognized (Raman *et al.* 1987). 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).

Small-scale farmers usually use low cost, unrefrigerated storage facilities to store seed tubers. These facilities vary according to the resources available to them. Many farmers simply stack their potatoes in heaps under a tree and cover them with a thick layer of potato haulms or foliage (Fuglie *et al.* 1991). Other farmers build temporary structures from wood, straw bails, and tree branches. Potatoes may be covered with a layer of paper, followed by a layer of loose straw from any plant residues, potato haulms, seaweed, reeds, branches or other handy materials (Fuglie *et al.* 1991). These structures will not prevent the tuber moth from reaching the tubers. The economic loss threshold for small-scale farmers cultivating consumer potatoes in Africa is between 20 and 30% (Fuglie *et al.* 1991). If potatoes in stores can be protected to show infestations of less than 20%, the farmer will experience very little economic loss. The findings of this study showed that this is possible with all treatments for at least 30 days. At 60 days, however, only carbaryl and *Bt* will protect tubers from first instar larvae that find their way to the stored tubers.

It is commonly accepted that infestation levels in potato stores are related to the population pressures from newly arrived or hatched potato tuber moths in the relative store. The damage experienced in any given store will thus be different depending on factors such as the infestation potential. In this study relatively high infestations were induced to ensure a 100% attack of the control tubers. The findings are therefore conservative and one may obtain better results when the relative treatments are administered in commercial potato stores where natural infestations are allowed.

The testing period of 60 days in this study was in accordance with Raman *et al.* (1987). They evaluated treated tubers for 120 days, but repeated the treatment after 60 days. This re-treatment of stored tubers after 60 days is a normal practice for small-scale farmers in South America (Raman *et al.* 1987). Farmers normally do this second treatment after they have sorted out any rotting or infected tubers. Because rotting of tubers can be caused by too many factors other than tuber moth, e.g. disease incidence of the stored tubers, store conditions etc., tubers were not kept for longer than 60 days in this study. At this stage, no rotting of tubers was evident.

Except for the toxic aspects of certain insecticides, dusting potato tubers have some other disadvantages as well. Helson (1949) name a few e.g. the objectionable white colour and unpleasant smell, the fact that some dusts may act as a medium for fungal growth under humid conditions and that high humidity may render most powders ineffective. When using the dust to protect seed tubers, however, the white colour is irrelevant. However, the different conditions under which tubers may be stored and the unknown effect which some powders may have on microorganisms, warrant further research.

Comparisons between treatment costs are problematic. Prices differ greatly from area to area and agent to agent. It must also be noted that prices differ dramatically when buying in bulk e.g. permethrin in 125 g quantities equals R184 per kg but only R32 per kg when buying in five kg quantities. Not all the powders are available in the same quantities, making price comparisons even more difficult. However, from Table 1 it is clear that *Bt* (R258/ton), is three to four times more expensive than that of any of the other powders. The high price of this treatment however, must be balanced against its efficacy compared to the other, cheaper powders as well as its safety aspect to humans and the environment.

It is concluded that the tested powders will protect stored tubers against attacks from the adult potato tuber moths for at least 30 days after treatment. Only *Bt* and carbaryl, however, will still provide excellent control of the larvae that find their way to the treated tubers after 60 days. The availability of commercial formulations of *Bt* should be considered as an important component in the development of integrated control of the potato tuber moth in storage, a view shared by Raman *et al.* (1987).

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**Table 1**. The powders, their common names, group, dosage and cost to treat one ton (1000 kg) as used for the evaluations against the potato tuber moth on potatoes.

| Trade name | Common name | Group | Dosage (ca.*) | Rand/ton |
|---|---|---|---|---|
| Johnson's baby powder | baby powder | perfumed talk | 1 kg/ton | R 58 (400g) |
| Coopex Dust DP | permethrin 5g/kg | pyrethroid | 1 kg/ton | R 32 (5kg) |
| Bexadust DP | gamma-BHC 6g/kg | organochlorine | 1 kg/ton | R 60 (500g) |
| Carbadust DP | carbaryl 50g/kg | carbamate | 1 kg/ton | R 76 (500g) |
| Ecoteck Bio WP | *Bacillus thuringiensis* *var. k.* 16 000 IU/mg | microbial toxin | 0.3 kg/ton | R 258 (500g) |

* approximate for medium sized tubers (100 to 150 g)

**Table 2**. The average number of tubers damaged by tuber moth larvae in the free choice experiment n = 25.

| Treatment | Moth attack induced on day one* | Moth attack induced on day 30** |
|---|---|---|
| Control | 25a | 25a |
| Baby Powder | 3b | 8.5b |
| carbaryl | 0c | 0.8c |
| permethrin | 0c | 0c |
| gamma-BHC | 0c | 6.8b |
| *Bt* | 0c | 0.3c |

Means followed by the same letter in each column is not significantly different at the 5% level ($P < 0.001$)
*evaluated on day 30,
** evaluated on day 60

**Table 3**. The average number of tubers damaged where first instar larvae were put on tubers, 30 and 60 days after treatment respectively. n = 10

| Treatments | Larval attack induced on day 30* | Larval attack induced on day 60** |
|---|---|---|
| Control | 10a | 10a |
| Baby Powder | 6b | 10a |
| carbaryl | 0d | 3b |
| permethrin | 0d | 10a |
| gamma-BHC | 3c | 10a |
| *Bt* | 0d | 0b |

Means followed by the same letter in each column is not significantly different at the 5% level ($P < 0.001$)
*evaluated on day 42, **evaluated on day 72

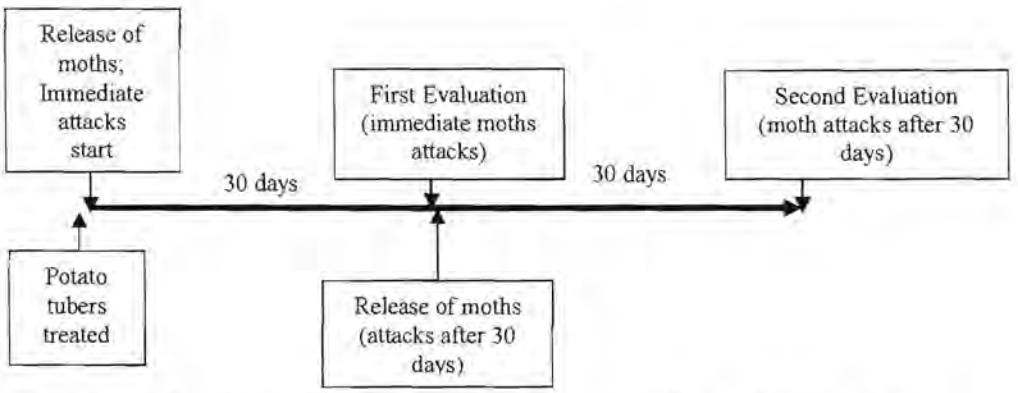


**Figure 1.** Time line of actions taken for the free choice, moth attack, experiment.

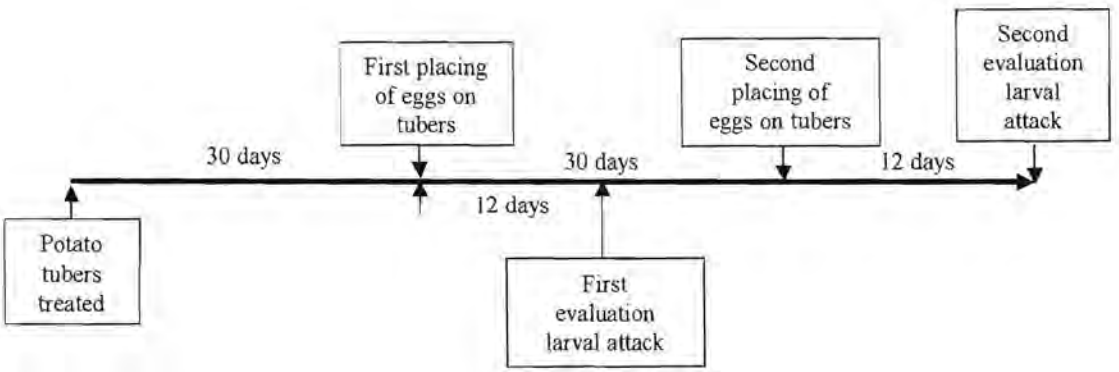


**Figure 2.** Time line of actions taken for the no-choice, larval attack, experiment.

# CHAPTER 4

## The efficacy of aluminium phosphide against the potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), under laboratory conditions

### ABSTRACT

Aluminium phosphide (Phostoxin®) was tested against all stages of the potato tuber moth at a dosage of four grams phosphine/$m^3$ (one pellet Phostoxin per 50 litre). Exposure time was 48 hours inside an airtight plastic container. The stages included; eggs, larvae inside tubers, first and fourth instar larvae outside tubers, pupae inside cocoons, pupae outside cocoons and adults. In all tests, aluminium phosphide was lethal to all stages of the potato tuber moth. It is concluded that aluminium phosphide can effectively be used to rid infested potatoes of any stage of the potato tuber moth. It can also be used in potato stores where potato tuber moths were known to be active and where possible eggs and/or pupae may be left unnoticed. Phytotoxicity of sprouted eyes occurred. However, eyes that where dormant at the time of exposure, developed normally a few weeks after exposure was completed. More work have to be done to find a safe, non-phytotoxic dosage and also to include the vast differences in real storage conditions under which such a product may be used.

**Key words**: Phostoxin, aluminium phosphide, potato tuber moth, *Phthorimaea operculella,* fumigation

## INTRODUCTION

The potato tuber moth *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) is a serious pest of potatoes, both in the field and storage world wide (Lal 1987; Roux & Baumgartner 1995; Visser & Steyn, 1999). The larvae tunnel into potato leaves and attack tubers under the soil when they are formed later in the season. These infected tubers find their way to the potato store where the life cycle of the moth continues (Kroschel & Koch 1994). Potato tuber moths prefer diffused light and are more active under such conditions (Trivedi *et al.* 1994). The inside of a potato store is therefore the ideal ecosystem for rapid increase of potato tuber moth numbers.

The larvae of the potato tuber moth tunnel into tubers, filling the tunnels with frass and webbing and allowing the entry of pathogens (Ferro & Boiteau 1993). The result is poor plant emergence followed by lowered yields. Moths originating from infested seed tubers can also result in the first infestation in the newly planted field (University of California 1986). Storage of potatoes at low temperatures will not necessarily kill tuber moth larvae inside tubers (Broodryk 1971). It is therefore important for farmers to plant clean seed, or seed that does not contain any live tuber moth larvae.

Commercial farmers only store seed potatoes. Table (consumer) potatoes are normally not a problem, because infested tubers are sorted out immediately after harvest and the non-infested ones sent to the market before damage becomes too severe (Gunn 1990). Commercial farmers rely on Co-operatives with large cool storage facilities, to store their seed tubers before the planting season. The temperature inside these facilities normally runs at 2 to 3 °C (University of California 1986) while the tuber moth larvae need temperatures above 10 °C to develop (Ferro & Boiteau 1993). Seed tubers are therefore protected from potato tuber moth attacks and damage in such facilities (Raman *et al.* 1987).

There are, however, times when seed potato tubers have to be left in open stores for lengthy periods. This can include the in-transit scenarios when seed tubers are send from Co-operatives to farmers, the two to four week period that seed tubers have to be taken out

of cool storage for sprouting or "reconditioning" purposes (Dean 1994), and the time tubers have to wait for the sorting process after harvest. At all of these stages the tubers are vulnerable to potato tuber moth attacks. It has happened that a seemingly clean batch of potatoes suddenly started to show infestation symptoms while no tuber moths were present (J. van Vuuren, personal communication). This is sometimes due to "latent" infestations – eggs or first instar larvae were present on or inside the tubers when the seed were bought, but not noted until the damage became more visual days later (Kroschel & Koch 1994).

The potato tuber moth is a nocturnal pest and is not often seen (Fenemore 1988). During the day it hides beneath leaves in fields, and between tubers in store environments (Annecke & Moran 1982). People handling seed tubers will therefore often not be aware of the presence of this inconspicuous pest. The situation therefore often arises when infestations of tuber moths are discovered too late. When moths have started to lay eggs and their larvae have already succeeded in entering the tubers, the situation is more severe. Because they are miners, and never leave the tuber except when they want to pupate, larvae inside tubers are relatively safe from adverse conditions on the outside. When a batch of tubers are infested, there is nothing that a farmer can do to rectify the situation. Of all the input costs, the single biggest investment of the season for the commercial potato farmer is the seed (Theron & Pieterse 1999). It was therefore important to find a remedy for those situations where a batch of seed potatoes was infested before planting.

In South Africa, twenty-three insecticides were registered for tuber moth control in the field in 2002 (Nel *et al.* 2002), but nothing was available to protect potatoes post harvest. There is also no known insecticide that can kill potato tuber moth larvae once they have entered tubers. Because of the lack of any insecticide that is registered to protect potato tubers, commercial farmers sometimes turn to illegal practices to protect their seed before planting. Powder formulated organophosphates are often applied excessively on bags containing seed tubers in an effort to kill off any tuber moth stages that may be present. These seed tuber bags are made of thinly knitted materials and the powdered insecticides are normally allowed to fall through to cover the tubers inside. These applications are normally aimed at the adults, because it will not kill larvae inside tubers.

It is not only the commercial farmer that experience problems with tuber moths infesting stored seed potatoes. The potato tuber moth is the most important pest of potatoes stored under traditional storage systems in Africa (Roux *et al.* 1992). These 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. Some farmers cultivate their own seed, which they store for a few months until planting (Kroschel & Koch 1994). Potatoes in such stores are open to attacks by the potato tuber moth originating from discarded tubers on dumping sites (Daiber 1989) or from infested tubers stored unknowingly with healthy tubers (Kroschel & Koch 1994). These farmers are known to sell their seed as soon as the first signs of infestations are noted. Seed bought from such sources will almost certainly be infested by various stage of the potato tuber moth. If left untreated, the contents of a potato store may be completely destroyed by potato tuber moth larvae (Fuglie *et al.* 1991; Ferro & Boiteau 1993). The small-scale farmer uses various natural substances to cover tubers (Raman *et al.* 1987). However, almost all of these control measures are preventative; none of these methods are able to kill off all stages of the potato tuber moth that find themselves in various places in a storage facility.

The potato tuber moth is known to lay its eggs on substrates near their host plant and does not require the presence of host-plant material for oviposition (Fenemore, 1978). In storage, larvae may crawl a considerable distance before pupating in crevices among building materials, in potato sacks, or at a similarly protected site (University of California 1986). 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 that get stored. The only logical way to kill pupae and eggs that may be found in various places in a store is by means of fumigation.

In an effort to address these problems that the potato tuber moth can cause in potato stores, the fumigant, aluminium phosphide, was evaluated for its efficacy against all stages of the potato tuber moth. In South Africa, aluminium phosphide is only registered against moths and beetles in tobacco stores (Nel *et al.* 2002). Nothing is registered to protect or clean infected potatoes or potato stores. These tests were aimed at a curative scenario where a store or a batch of potatoes was already infested. The only mention in the

literature of the use of aluminium phosphide on potatoes against potato tuber moth, is Andrew *et al.* (1992). However, no indication of its efficacy is given. This study was thus undertaken to establish whether aluminium phosphide can be used to treat infected tubers and stores to prevent further infestations of tubers as well as new fields planted with such seed. The aim of this evaluation was to find whether phosphide had any insecticidal activity against al the stages of the potato tuber moth. This study was not meant to find the efficacy of different dosages of phosphide under different conditions, but rather to serve as a starting point for further extensive storage trials for registration purposes.

## METHODS

Because no work has been done with aluminium phosphide on stored potatoes before, recommendations from the manufacturer, *Degesch SA* had to be used. The normal dosages for stored products ranged from one to five gram per $m^3$, exposed for three to eight days. Because potatoes in airtight environments give off moisture that can be detrimental relating to diseases and keeping quality (Dean 1994) it was decided to shorten the exposure time, but to use a high dosage. A dosage of one pellet per 50 liter was decided on (four grams phosphide per $m^3$), exposed for only two days.

All life stages of the potato tuber moth were exposed to aluminium phosphide in an airtight 50-liter plastic drum. To keep them separate, the different stages were first put in separate smaller containers before they were placed in the large 50-liter drum. To test the effect of aluminium phosphide against larvae inside tubers, they were first allowed to infect tubers. Forty first instar larvae, collected from an insectary rearing facility, were put on each tuber by means of a fine camel hair brush. These tubers were infested every day for nine consecutive days to ensure the presence of all instars in tested tubers. For each replicate, two infected tubers were put together in a plastic bucket. Eighty larvae were therefore tested per replicate. A piece of fine mesh material, held in place by the rim of the cut out lid, was used to close the open side of each bucket. This was done to prevent any surviving fourth instar larvae from escaping. A thin layer of white sand was supplied on the bottom inside of the buckets for pupation purposes of the larvae that survived. Because

seed tubers (sprouted) differ physiologically from fresh table potatoes (not sprouted), both sprouted and non-sprouted tubers were included in these tests.

Potato tuber moth eggs, laid on filter paper, were supplied by the potato insectary at ARC-Roodeplaat. The filter paper was cut into smaller pieces and the number of eggs on each piece counted. The pieces of filter paper with tuber moth eggs were put in small open plastic buckets. One bucket with the egg containing filter paper was considered a repetition. After treatment, the eggs were transferred to Petri dishes to check for hatching. The eggs were placed in the middle of the lower part of a Petri dish. A 10 mm wide layer of sticky glue was applied around the egg containing filter paper to prevent hatching larvae from escaping. The larvae did not attempt to cross the sticky glue and could therefore be kept alive for the duration of the test. However, because some first instar larvae started dying within the 48-hour testing period, the first instar larvae were evaluated after an exposure time of 24 hours. Fourth instar larvae were placed in similar buckets as described for tubers.

Pupae of the potato tuber moth are formed inside silken cocoons. Exposed pupae, with cocoons removed, and pupae in cocoons, were placed in small open plastic buckets before placing them in the bigger container. Freshly emerged moths were contained in similar small plastic buckets as describe for tubers.

Apart from the placement of the phosphide pellet on the inside bottom of the drum, both the treatment and the control were handled similarly. After the two day exposure time, the drums were opened and all contents taken out for aeration purposes. All the smaller containers inside the drums were also opened and aerated. The tubers containing the larvae were incubated for a further two weeks to allow any surviving larvae to complete their development before they exited from the tubers to pupate in the sand in the container. Evaluations of the larvae and the moths were done immediately, but were monitored for 5 more days to include any possible knockdown activity of aluminium phosphide. All eggs and pupae were monitored for four weeks to establish unsuccessful hatching or eclosion.

A separate treatment, without infesting the tubers, was included to study the effect of aluminium phosphide on the sprouting ability of the tubers. These tubers were kept for a further two months to monitor the possible emergence of dormant sprouts.

## RESULTS

### Efficacy

No larvae inside tubers survived the aluminium phosphide treatment (Tables 1 and 2). Both the fresh table potato tubers and older seed tubers in the controls produced healthy fourth instar larvae after nine days. The age of the larvae inside the tubers did not influence the efficiency of aluminium phosphide (always 100% control). No first, neither fourth instar larvae survived the treatment (Table 3) while all larvae in the controls showed a 100% survival rate. No pupae survived the treatment (Table 3) while all pupae in the controls stayed alive, developing to adult moths. No eggs survived the aluminium phosphide treatment (Table 4) while nearly all eggs of the four different ages in the controls survived. No moths survived the treatment (Table 3) while all moths in the controls showed a 100% survival rate.

### Phytotoxicity

The seed potatoes (sprouted) showed severe phytotoxicity, killing off all "eyes" that has already sprouted. However, the "eyes" that have not sprouted at the time of the treatments, sprouted normally and were not affected. The table potatoes sprouted normally during the two-month period following these tests.

## DISCUSSION

All stages of the potato tuber moth may be found in a potato store. Eggs may be laid on tubers or any place nearby while the first instar larvae that hatch from the eggs may find their way to tubers. Once inside the tuber, the larvae are relatively safe until the fourth instar larva have to exit the tuber again for pupation purposes. 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. This cocoon acts as a safe hiding place from natural enemies, but may also protect it from substances like insecticides. Moths hide during the day but fly

around after dark and are therefore relatively exposed. There are therefore a lot of different stages of different ages hiding in different places in a potato store. Insecticides may have a different effect on a stage, depending on where that stage find itself at the time of application. Different ages of the stages may also influence the effect of a chemical on that stage. The following scenarios were taken in to account: eggs of four different ages; 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) and moths.

All the mentioned stages and ages of stages were tested. This allowed for the evaluation of aluminium phosphide under the different circumstances that may be found inside a potato store at any particular time. However, it is important to note that the different store types and environments that exist could not be evaluated. These results only relate to controlled tests inside a 50 liter airtight container. The dosage was thus fixed and exact (no gas could have escaped), a situation that one will most probably never find in a real store environment. The results are therefore only an indication of the potential of the product to control the potato tuber moth and its stages in a post harvest scenario.

It was shown that the recommended dosage of one pellet Phostoxin per 50 liter would kill all stages of the potato tuber moth when administered in an airtight environment for 48 hours. However, most stores do not seal airtight. A typical situation will therefore be to cover a pile or stack of potatoes with a tarpaulin before fumigation begins. Spot treatments of different batches of potato inside a bigger store could therefore be an option. However, to rid an entire store of hiding pupae and moths, and possible eggs, the safer option will be to remove all potatoes from such a store and treat the whole store with higher dosages if the store can not be sealed to prevent gasses from escaping. More research is needed to find an optimum dosage under such conditions.

From the results it is clear that seed (sprouted) potatoes may harbor more than twice the number of larvae than table (not-sprouted) potatoes. This may be due to the fact that there are much more feeding niches and entry points on a tuber that has started to sprout. Seed

tubers may therefore be expected to show severe damage to eyes when infestations in a store are high. Fumigation by aluminium phosphide kills the larvae inside the tubers, while the damage done by the larvae still persists. It was shown that aluminium phosphide kill sprouted eyes, but that new eyes will develop later on the same tuber to compensate for the loss. However, the effect of aluminium phosphide on the vitality of seed tubers during the season (latent effect) is unknown. These two factors, damaged tubers by moth larvae and unknown effects of aluminium phosphide on the seed, therefore need investigation.

The data have showed that aluminium phosphide may kill the tuber moth and all its life stages. However, these tests have to be repeated with various dosages, different cultivars and tubers of different physiological ages to make final conclusions regarding phytotoxicity. The tested dosage of one pellet Phostoxin per 50 liter gave absolute control. It is therefore possible that lower dosages may still give good control while phytotoxicity may be reduced or eliminated. Another important factor not measured is residues that may stay behind in tubers after treatment. It is assumed that the gas entered the tubers to kill the larvae inside. It is unknown whether the toxic gas may get trapped inside the tuber tissue after exposure. Further studies regarding residues and penetration of the product into potatoes are thus needed before it can be regarded as safe for usage on potato tubers.

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**Table 1.** The number of larvae that survived inside table (not sprouted) potatoes after 48 hours exposure to aluminium phosphide in a sealed 50 liter container. (n = 80)

| Age of larvae (days) | App. instar | Untreated control | | | | | One pellet Phostoxin | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Repetitions | | | | Av. | Repetitions | | | | Av. |
| | | 1 | 2 | 3 | 4 | | 1 | 2 | 3 | 4 | |
| 1 | 1st | 11 | 17 | 15 | 16 | 15 | 0 | 0 | 0 | 0 | 0 |
| 2 | 1st | 28 | 33 | 36 | 18 | 29 | 0 | 0 | 0 | 0 | 0 |
| 3 | 2nd | 19 | 27 | 25 | 18 | 22 | 0 | 0 | 0 | 0 | 0 |
| 4 | 2nd | 14 | 22 | 16 | 25 | 19 | 0 | 0 | 0 | 0 | 0 |
| 5 | 3rd | 14 | 12 | 19 | 12 | 13 | 0 | 0 | 0 | 0 | 0 |
| 6 | 3rd | 36 | 39 | 34 | 29 | 35 | 0 | 0 | 0 | 0 | 0 |
| 7 | 4th | 16 | 19 | 20 | 12 | 17 | 0 | 0 | 0 | 0 | 0 |
| 8 | 4th | 29 | 27 | 24 | 22 | 26 | 0 | 0 | 0 | 0 | 0 |
| 9 | 4th | Larvae started to exit tubers. See next tables for tests on larvae outside tubers | | | | | | | | | |

App. = Approximate

**Table 2.** The number of larvae that survived inside seed (sprouted) potatoes after 48 hours exposure to aluminium phosphide in a sealed 50 liter container. (n = 80)

| Age of larvae (days) | App. instar | Untreated control | | | | | One pellet Phostoxin | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Repetitions | | | | Av. | Repetitions | | | | Av. |
| | | 1 | 2 | 3 | 4 | | 1 | 2 | 3 | 4 | |
| 1 | 1st | 68 | 59 | 80 | 60 | 69 | 0 | 0 | 0 | 0 | 0 |
| 2 | 1st | 76 | 69 | 79 | 67 | 73 | 0 | 0 | 0 | 0 | 0 |
| 3 | 2nd | 38 | 46 | 33 | 44 | 40 | 0 | 0 | 0 | 0 | 0 |
| 4 | 2nd | 78 | 76 | 71 | 76 | 75 | 0 | 0 | 0 | 0 | 0 |
| 5 | 3rd | 45 | 59 | 67 | 47 | 55 | 0 | 0 | 0 | 0 | 0 |
| 6 | 3rd | 69 | 56 | 48 | 73 | 62 | 0 | 0 | 0 | 0 | 0 |
| 7 | 4th | 68 | 79 | 59 | 76 | 69 | 0 | 0 | 0 | 0 | 0 |
| 8 | 4th | 51 | 35 | 59 | 52 | 49 | 0 | 0 | 0 | 0 | 0 |
| 9 | 4th | Larvae started to exit tubers. See next tables for tests on larvae outside tubers | | | | | | | | | |

App. = Approximate

**Table 3.** The number of larvae, pupae and moths that survived the aluminium phosphide treatment after 48 hours inside a sealed 50ℓ container.

| Stage | Untreated control | | One pellet Phostoxin | |
|---|---|---|---|---|
| | No. tested | No. survived | No. tested | No. survived |
| 1st Instar larvae outside tubers* | 10 | 10 | 10 | 0 |
| 4th Instar larvae outside tubers | 10 | 10** | 10 | 0 |
| Pupae in cocoons | 20 | 20 | 20 | 0 |
| Pupae not in cocoons | 20 | 20 | 20 | 0 |
| Moths | 10 | 10 | 10 | 0*** |

* Only tested for 24 hours.
** Pupated
*** Checked for five days for possible knockdown/survival effect – no survival.
Second and third instar larvae are nearly always found inside their food source (tubers or leaves) – t herefore they have only been tested inside tubers; see tables 1 and 2.

**Table 4.** The number of eggs of varying ages that survived the aluminium phosphide treatment after 48 hours inside a 50ℓ container.

| Age of eggs (days) | Control | | One pellet Phostoxin | |
|---|---|---|---|---|
| | Eggs tested | Eggs survived | Eggs tested | Eggs survived |
| <1 | 20 | 18 | 35 | 0 |
| 1 | 35 | 34 | 40 | 0 |
| 2 | 50 | 45 | 55 | 0 |
| 3 | 20 | 19 | 36 | 0 |
| 4 | 29 | 27 | 32 | 0 |
| 5 | Eggs started to hatch. See previous Table 3 for results on 1st instar larvae outside tubers. | | | |

# CHAPTER 5

## The potential of UV light-assisted insect electrocuter traps for controlling newly emerging potato tuber moth, *Phthorimaea operculella*, (Zeller) (Lepidoptera: Gelechiidae), in a non-refrigerated store environment

### ABSTRACT

An ultra violet (UV) light-assisted insect electrocuter trap was evaluated for its efficacy against newly emerging potato tuber moths in a non-refrigerated store environment. Two hundred potato tuber moths were released next to 300 potato tubers in 10 crates. One potato store was used with the control at one end and the trap treatment at the other end, with no line of sight between them. The UV light-assisted insect electrocuter trap was in constant operation directly above the 300 tubers in the treatment. One repetition lasted approximately two months, and was repeated eight times over a period of two years. After each repetition, the number of damaged tubers was counted in both the control and the trap treatment. It was shown that the trap treatment resulted in lower tuber infestations in only five of the eight months. It was also shown that very little tuber moths get killed during the first two days after releasing them, the time when infestations were most likely to occur. It is concluded that the use of an UV light-assisted insect electrocuter trap in a non-refrigerated store environment will not result in controlling tuber moth satisfactory.

**Key words:** *Phthorimaea operculella*, potato tuber moth, UV light, insect traps

## INTRODUCTION

The ability of insects to discriminate between different colours was discovered in 1913 by von Frisch, who pioneered the colour perception in insects with his work on bees (Burkhardt 1964). Over the past 30 years, much more has been learned about insect visual capabilities, especially their use of colour to find resources, including the ultraviolet spectrum (Land, 1997). Briscoe & Chittka (2001) reviewed the evolution of colour vision in insects. The responses of insects to visual stimuli have been used as a valuable tool in pest management programs (Prokopy & Owens 1983). These include the use of light traps for early detection, forecasting and estimating seasonal changes. Traps of various "human" colours (especially yellow) have been used to catch a variety of insects for monitoring and pest control. However, insects can see a much wider spectrum; 300 to 700 nanometer (nm), while the human eye can only perceive 400 to 700 nm (Shields 1989). Although the spectrum of insect vision is wider than that of the human eye, they can only see a limited number of colours (Romoser 1981). One of the colours to which the insect eye is most sensitive is ultraviolet (UV) light in the spectrum 350 to 400 nm range (Frost 1954; Romoser 1981; Briscoe & Chittka 2001). Lamps that emit light in this spectrum are also known as black light fluorescent lamps. Insect eyes are so sensitive to black light that it was shown that when black and white lights were placed only one meter apart, all insects that are attracted would nearly always prefer the black light (Frost 1954). It was also found that the "flickering effect" of these lamps was significantly more effective in attracting insects than the same lamps modified to emit a constant light source (Syms & Goodman 1987).

UV light-assisted insect electrocuter traps are also known as "Zappers®", "Bugwackers®", "Bug Masters®" (Frick & Tallamy 1996) and locally "Ultrablitz goggavangers®". Black lights are commonly used as integrated parts in these traps. UV light-assisted insect electrocuter traps are used to kill flying insects that are attracted to the black light by electrocuting them when they come into contact with an electrified iron grid below or next to the light. The most common use for these devices is in home gardens and camp sites where they are used to kill nuisance insects, e.g. mosquitoes, at night. However, Frick & Tallamy (1996) discussed a few unpublished studies that proved the

ineffectiveness of these devices, mainly because biting insects are more attracted to their prey (humans) than to the black light. Other non-biting insects however, are also attracted, and more than 3000 insects may be killed per day by these UV light-assisted insect electrocuter traps (Nasci *et al.* 1983). Frost (1954) found insects belonging to 35 families in black light traps while Frick & Tallamy (1996) found 104 families representing 12 orders. It is evident that black lights do not discriminate against any insect group.

No literature could be found on the use of UV light-assisted insect electrocuter traps in agriculture. However, potato farmers in South Africa commonly use these devices as a means to control the potato tuber moth. Farmers use them in their potato stores and some even use them in the field, mounted on central pivot irrigation structures, above the water sprayers. The farmers claim their electrocuter traps are effective in controlling various pests in fields and against the potato tuber moth in potato stores. However, these claims are untested because no scientific evaluations have ever been done to support their claims. It was therefore decided to investigate the potential of UV light-assisted insect electrocuter traps as a control method against the potato tuber moth in stores.

## METHODS

Two different size commercial electronic insect traps (Ultrablitz Goggavanger®), obtained from Magaliesberg Co-operative in Pretoria, were used in this study. The small device used an eight-watt lamp and the larger one two 20-watt lamps. The smaller one measured 320 mm x 60 mm x 260 mm and consisted of a 3000V/15mA iron grid below one F8T5BL 8W black light. The light was powered by a 220V, 50/60Hz 9W iron ballast. The larger insect trap measured 630 mm x 140 mm x 330 mm and consisted of a 3000V/15mA iron grid below two FL20SBL 20W black lights. These lights were powered by a 220V, 50/60Hz 1x40W ballast. Insects are attracted to these lights, fly into the grid and get electrocuted. These two insect traps were chosen because they were easily obtainable from co-operatives and commonly used by potato farmers.

### *Tests with the 8W insect trap*

Initial experiments were conducted with the smaller model to determine whether tuber moths were attracted to black lights. The small model made it possible to count the number

of moths killed, because they did not disintegrate when electrocuted by its grid. The moths that reached the trap got stuck to the grid from where they were collected and counted. One hundred moths were released in a closed insectary room (3 m x 2 m) with the insect trap in operation. The lights of the room were not switched on. The insect trap was examined once a day and dead moths removed and counted for one week.

*Tests with the 2 x 20W insect trap*

The bulk of infestations in stores are from larvae and pupae that originate from infested tubers in the relevant store (Kroschel & Koch 1994). It was therefore decided to test this device mainly against newly emerging potato tuber moths. The problem of possible variation in mated stages of female moths, and the influence it might have on their attractiveness to light, was thus eliminated. The experimental plan is illustrated in Figure 1. Both the untreated control and the insect trap treatment consisted of 300 tubers (30 tubers in 10 crates, placed in a single layer). All crates were placed on the floor and were not stacked. The insect trap was hung from the ceiling, approximately 300 mm above the crates. The insect trap was placed in such a way that the UV lights were visible to any insect in the vicinity. There was no obstruction between the lights and the tubers. Because the situation in very large stores would be different and obstructions would always occur, this arrangement was deliberately meant to favour the insect trap. These tests therefore slightly favoured the insect trap.

Two hundred tuber moths were released around the crates at the start of each experiment as illustrated in the experimental plan (Fig. 1). The moths were reared in the insectary at ARC-Roodeplaat, Pretoria. The pupae of the moths were kept separately in individual glass vials to prevent mating before releasing. Once the vials were opened, the moths could choose between seeking a mate, the tubers or the UV light. The treatments were then incubated for 30 to 60 days, with the insect trap in constant operation. This was to allow for mating, egg laying, egg hatching and larval development inside tubers until damage was visible. The tubers in both the treatments were then evaluated for tuber moth damage. A new experiment was conducted with uninfested tubers and newly hatched moths after evaluations were completed.

All insects that flew into the store at night and became attached to the shocking electrical grid were removed every morning by means of a small brush. To eliminate the effects of ants (attracted by the dead insects that fell to the ground) on the tuber moths that were released around the crates, all crates were raised from the ground by placing two 100 mm high plastic buckets underneath each crate. Glue was applied to the outsides, in a two-centimetre wide band around the buckets. Ants that tried to reach the crates or tubers got stuck in the glue.

The experiment was conducted in an old potato store that was only used for storing items other than potatoes. No potatoes that could have influenced the results were stored in the open. The insect trap treatment was placed approximately 50 meters away from the untreated control. A cold storage room (in the middle of the store) separated the two treatments from one another, eliminating the possible attraction of moths from the control treatment to the UV lights of the insect trap. This was better than in tests by Frost (1954), who used a distance of 30 meters between different light traps. The experiment was repeated eight times over a period of two years in the same store.

For comparisons between the control and the insect trap, a one-way ANOVA was used to test for differences between treatments and the control. Because of the large layout of the experiment, and because it would be impossible to get four stores to do repetitions, repetitions had to be done over time in the same store. Significant differences between treatments during specific moths could therefore not be calculated. The number of moths killed by the larger electrocuter could not be counted because they were disintegrated when electrocuted. The effectiveness of the trap was therefore measured in its ability to stop tuber moth infestation of potato tubers.

## RESULTS

### Tests with the 8W insect trap

Only 6% of released moths were caught during the first day (Fig. 2). This number increased steadily to 46% at day seven. Because moths mate and lay their eggs soon after emergence (University of California 1986), and because they don't live for much longer

than seven days (Debnath *et al.* 2000), it was decided not to extend the evaluations for longer than seven days.

**Tests with the 2x20W insect trap**

Tuber moths attacked the tubers in the crates in both treatments (Table 1, Fig. 3). The insect trap treatment showed lower levels of damage than the untreated control at five of the eight replicates. The difference, however, was noticeable only during two of these five occasions; (April '98 and June '98). A higher infestation level was experienced at the insect trap than at the untreated control on three occasions (January '98, February '99 and December '99). The average infestation over three years was 61% and 65% in the treatment and control respectively (Table 1).

## DISCUSSION

The results showed that the insect trap did not eliminate tuber moth damage. The damage to tubers varied considerably between replicates over time, but was never reduced below 20 percent. A minimum damage of 20 percent with an average of 61% is expected when using the electrocuter trap in the relevant store. Tuber moths are therefore not effectively controlled with unacceptable high damage to tubers the result.

A possible reason for the poor performance of the electrocuter trap is the moth's ability to find and lay their eggs on the tubers before they are attracted to the black light. Moths mate and lay eggs very soon after they emerge from their pupae (University of California 1986). If they don't get killed during the first two days after emergence, they most probably won't be prevented from mating and laying their eggs. Trap catches with the smaller electrocuter showed that only a few moths get killed during the first two days and that only 46% of released moths were caught after seven days. Although the smaller trap in this study used a less powerful lamp, Frost (1954) found that lamps with different intensities attract the same number of insects. Electrocuter traps with more powerful lamps will therefore also follow the same pattern and catch very little tuber moths in the first two days.

Various environmental factors (Morton *et al.* 1981) and also trap location (Harthstack *et al.* 1973) may influence the efficacy of black light traps. However, in closed stores most factors e.g. wind speed and rain, does not play any part and catches should not be influenced negatively. Morgan & Pickens (1968) found that more male houseflies were attracted to black light when temperatures were between 19 and 23 °C, but more females were attracted at temperatures of 28 to 32 °C. This study with tuber moths was repeated over eight months, representing warm and cold months and these influences are thus taken into account in the overall results. Hecht (1963) found that unfed houseflies were more attracted to light than fed ones. Adult tuber moths do not have to feed, and this factor was therefore not important in this study. One other factor that was not taken into account is the mated stage of females. Potato tuber moths that fly in from the outside may already have mated and may possibly only be interested in laying their eggs. Such moths may be less attracted to light. Stadelbacher & Pfrimmer (1972) found that approximately 45% of female moths (not tuber moths) caught in light traps have mated. Lopez *et al.* (1978) found that the reproductive condition of moths caught in black lights varies with the crop, the stage of the crop in which the traps are operated and the season. All these factors may thus influence the efficacy of electrocuter traps. This study was thus conservative, only testing newly emerging moths in a protected environment.

One of the reasons why farmers may believe that their electrocuter traps are effective may be the fact that most of the "kills" make a loud and distinctive noise. Frick & Tallamy (1996) stated that "the continuous snaps, crackles and pops originating from an active zapper seem to confirm their efficacy". The irony is that it is mostly non-destructive insects that get caught. Frost (1954) noted that very few mosquitoes, aphids and diamond back moths were caught in black lights. Frick & Tallamy (1996) states that even if the electrocuter traps do kill pests, "the resulting destruction of thousands of parasitoids, predators, aquatic insects and other members of the nocturnally active fauna would be difficult to justify". Their conclusion is thus that UV light-assisted electrocuter traps are not environmentally friendly.

Efficacy aside, other problems relating to the handling and installing of the traps may render them difficult to implement. Firstly, the larger commercial traps do not contain

shields to prevent humans from electrocution. Adaptations to the traps would therefore be necessary, or else their placement would be critical. Secondly, contamination of the grid by other insects would be a problem. The hundreds of insects that had to be removed from the grid every morning by means of a brush was labour intensive. If these traps were modified (e.g. covered by an outer wire mesh), or if they were located out of reach of humans, the cleaning problem would be even worse. By not cleaning them every morning, the efficacy of these traps would be affected considerably.

It is concluded that UV light-assisted insect electrocuter traps will not be effective in controlling potato tuber moths in potato stores. Because of the poor results obtained in the protected (favorable) environment of a potato store, it is unlikely that this device will be effective when used outdoors against the potato tuber moth in fields.

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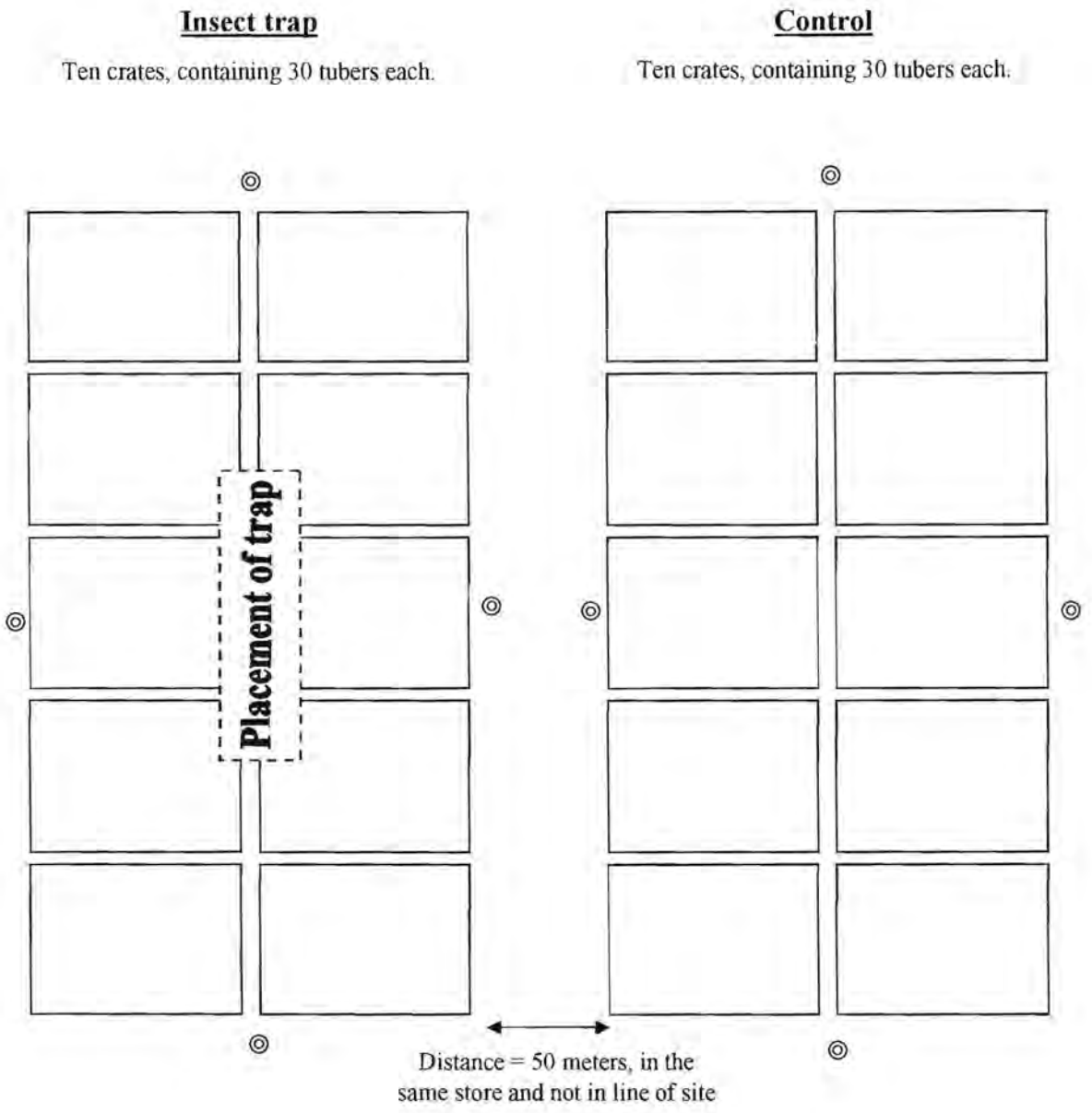
**Table 1**. The number of tubers attacked with and without the insect trap in operation above 10 crates with 300 tubers.

| Date | With insect trap | Untreated Control |
|---|---|---|
| Dec. '97 | 264 | 294 |
| Jan. '98 | 243 | 96 |
| Apr. '98 | 177 | 273 |
| Jun. '98 | 60 | 159 |
| Feb. '99 | 165 | 147 |
| Apr. '99 | 72 | 111 |
| May '99 | 216 | 225 |
| Dec. '99 | 255 | 243 |
| TOTAL | 1452 | 1548 |
| Average | 181.5a | 193.5a |
| Percentage | 61% | 65% |

The average number of tubers attacked per month over the two years was not significantly different (P = 0.676)




◎ = position of groups of 50 vials in which tuber moths were individually placed (200 moths per treatment).

**Figure 1**. The experimental plan of the store trial with the large UV light-assisted insect electrocuter trap.

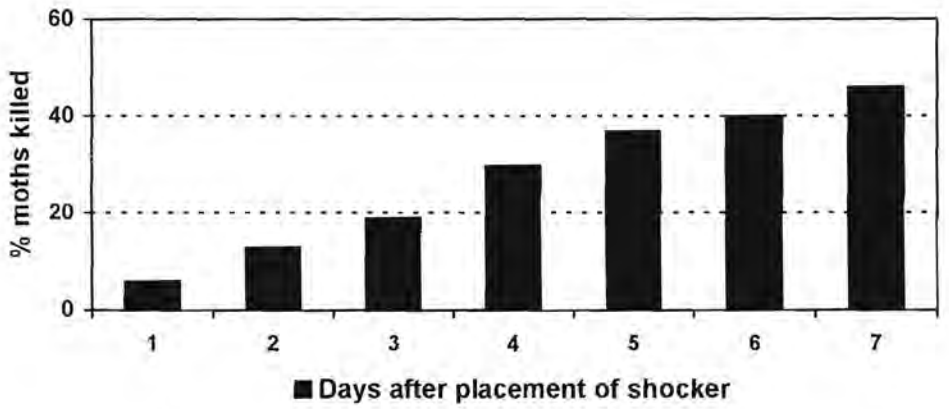



**Figure 2.** The cumulative increase in the percentage potato tuber moths killed over time with the small UV light-assisted insect electrocuter trap.

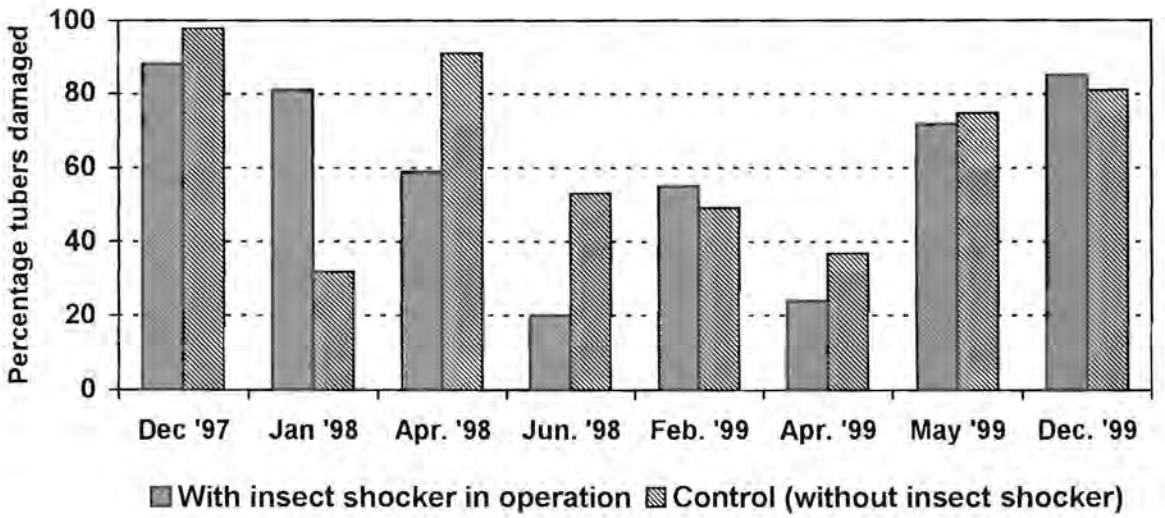



**Figure 3.** The percentage tubers attacked with and without the large UV light-assisted insect electrocuter trap in operation above 10 crates filled with 300 tubers.

# 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).

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**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.

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**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.

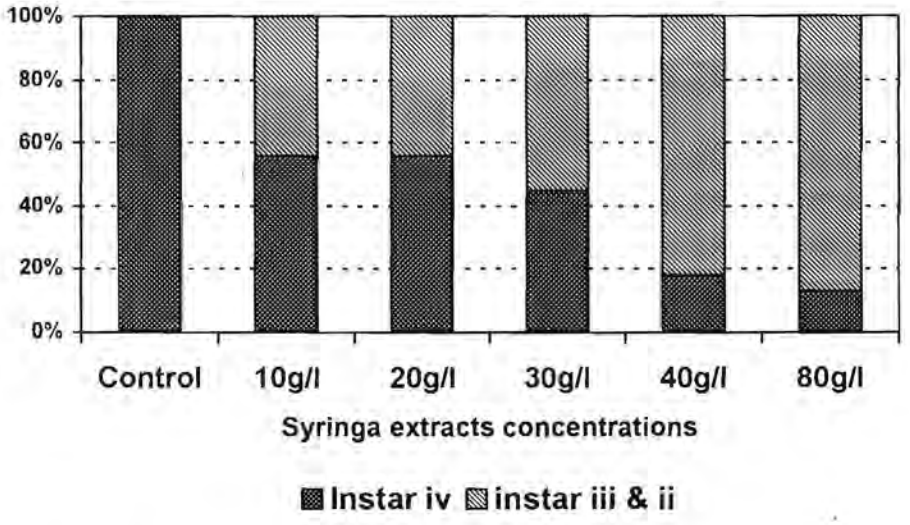


**Figure 1.** The percentage of surviving potato tuber moth instars after 12 days on syringa treated potato leaves (data recompiled from Table 1).

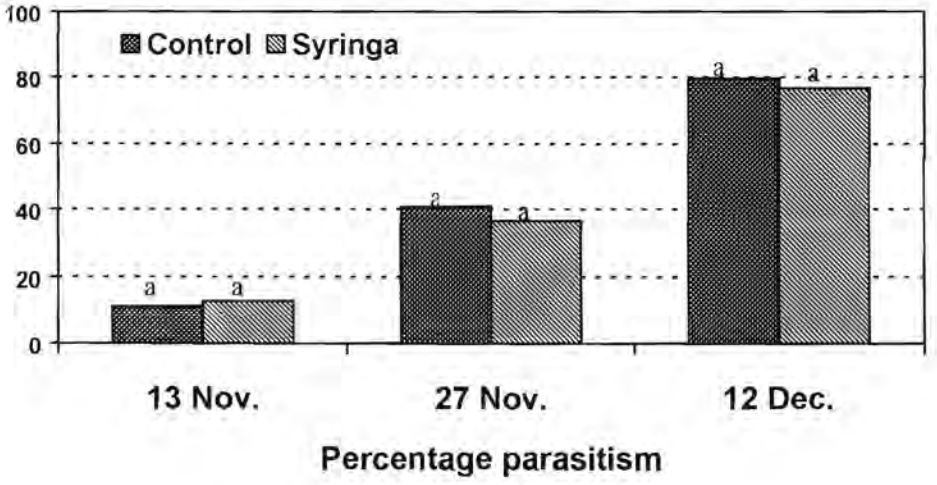


**Figure 2.** Mean percentage parasitism of potato tuber moth larvae in the syringa treated plots and controls. Means with the same letters were not significant at the 5% level for each monitor date.

# 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.

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**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.

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**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.

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