

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 $-2.4\text{ }^{\circ}\text{C}$ (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 (Kramblias 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

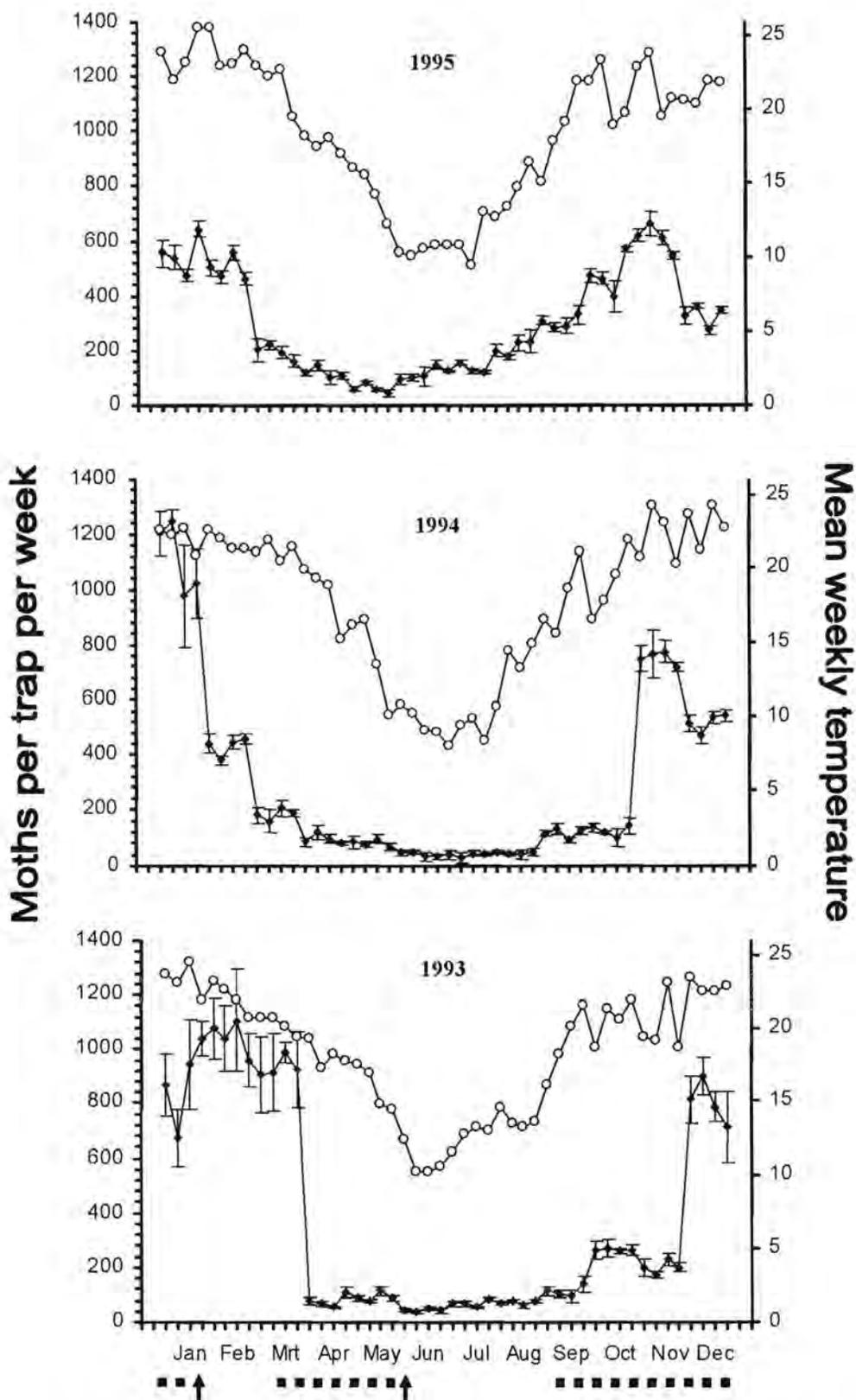


Fig. 1. The mean number of tuber moths (solid dots) caught per week with synthetic pheromones traps, from 1993 to 1995 on the farm Zeekoegat. The corresponding average temperatures (open dots) are given (centigrade). Bars represent standard errors (S.D.) of three traps. The dotted lines below the graphs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

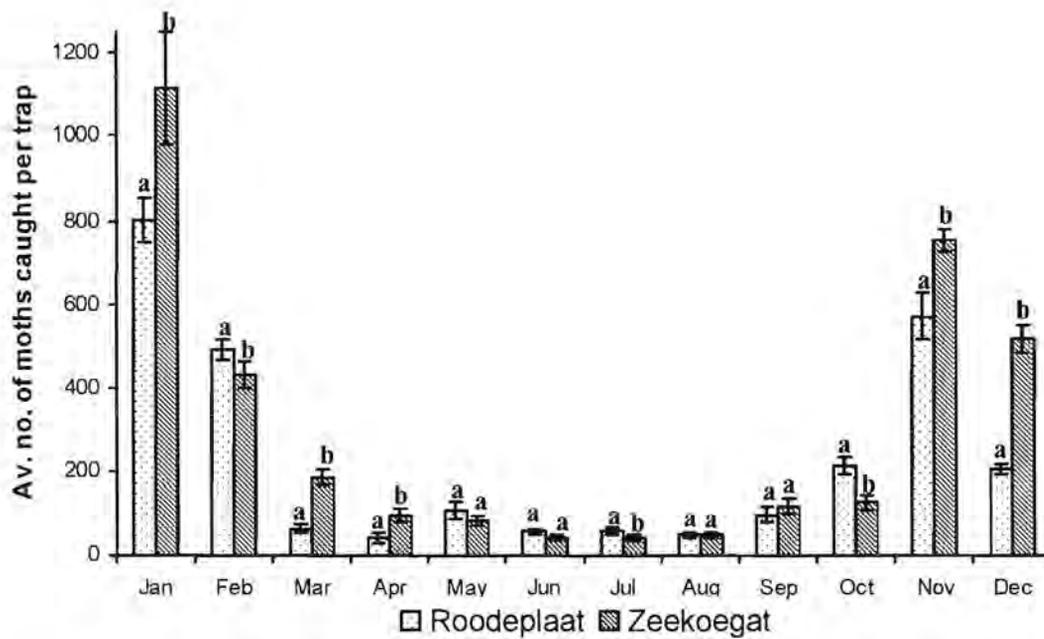


Fig. 2. The mean number of potato tuber moths caught per trap during 1994. The numbers are expressed as the weekly averages per trap for each month. Bars represent standard errors (S.D.) of three traps. Different letters (per month) indicate significant difference at the 5% level.

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 or 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 CO₂ (Afrox[®] Class E; 1.8 kg/m³; 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ or cold treatments compared to the untreated control. It was therefore concluded that subduing tuber moths with either CO₂ 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 from 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 marcescens* 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 range, 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 CO₂ or CO₂ /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 (Fenimore 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 placed 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 CO₂ or a cold treatment. Although CO₂ 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 minor 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 15 °C 20 °C	all died 19 days 9 days	-	-	-	-	-	-	fully grown larvae will not pupate when put at 5 °C
Finney <i>et al.</i> (1947)	26.7 °C 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 25 °C 32 °C	- 4 days -	67 days 12 days 9 days	18 days 6 days 5 days	- 24 days* -	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 50% RH	4 days	10days	8 days	22 days*	-	178 (-F)	8 days (-F)	Found that 35 °C made males sterile
Singh & Charles (1977) 1 st	30 °C	3 days	12 days	4-5 days	20 days*	♂ 9.1 ♀ 11.4	169 (+W)	-	Tubers as substrate for larvae
Singh & Charles (1977) 2 nd	30 °C	3 days	13 days	6 days	22 days*	♂ 7.7 ♀ 8.3	3 - 170 (+W)	♂ 11 days ♀ 10 days (+W)	Artificial diet for larvae
Fenemore (1977)	25 °C	-	-	-	-	11.6	46 - 236 (+S)	♂ 23 days ♀ 14 days (+S)	multi-mated ♀♀ live shorter than single mated
Etzel (1985)	22 °C 70% RH	-	-	-	-	10.3	85 (-F)	-	-
Rahalkar <i>et al.</i> (1985)	29 °C 65% RH	3 to 4 days	13 to 15 days	-	17-22 days	♂ 6.6 ♀ 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 <i>et al.</i> (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 <i>et al.</i> (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 <i>et al.</i> (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 <i>et al.</i> (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

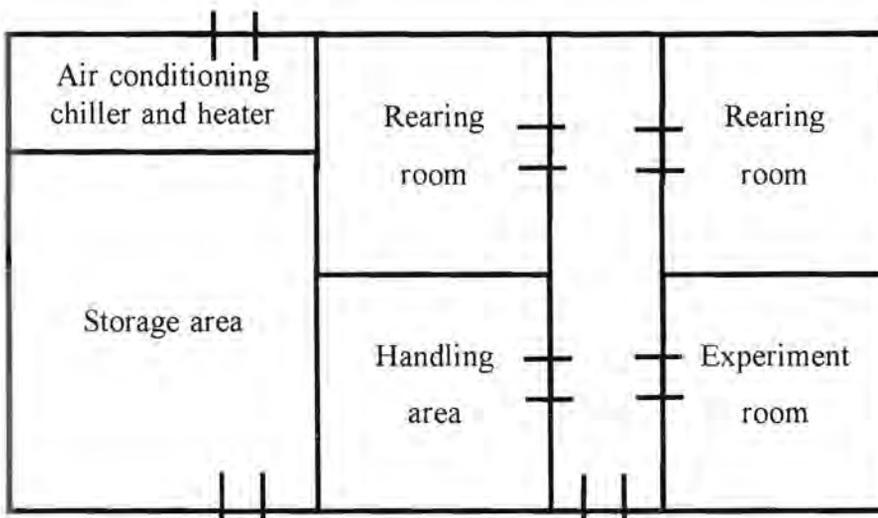


Figure 1. Plan of the potato tuber moth insectary at ARC-Roodeplaat

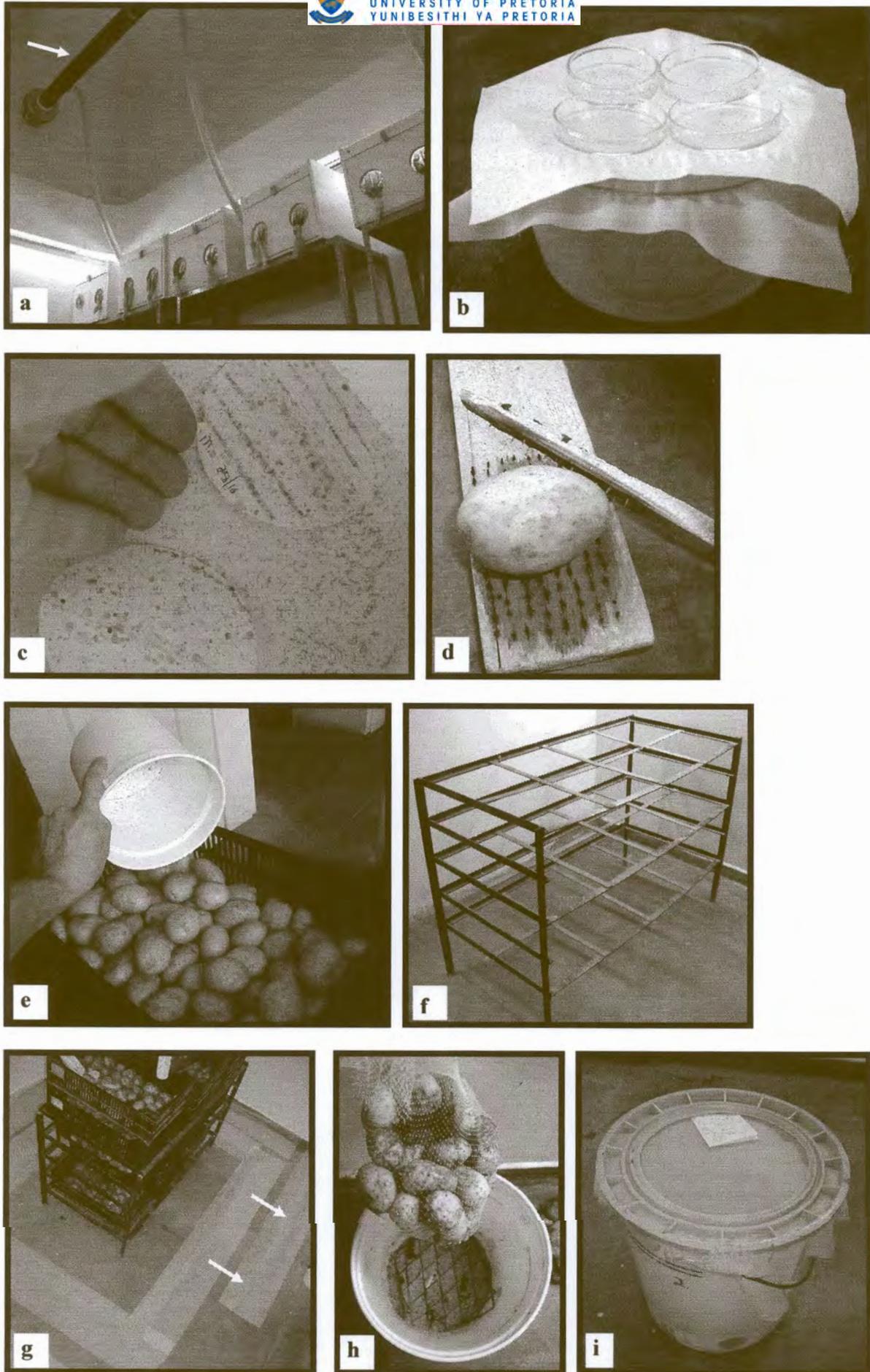


Figure 2. Aspects of the rearing program (see text).

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 months 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 (wetttable 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 wetttable 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 (wetable 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	<i>Bacillus thuringiensis</i> <i>var. k.</i> 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
<i>Bt</i>	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
<i>Bt</i>	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

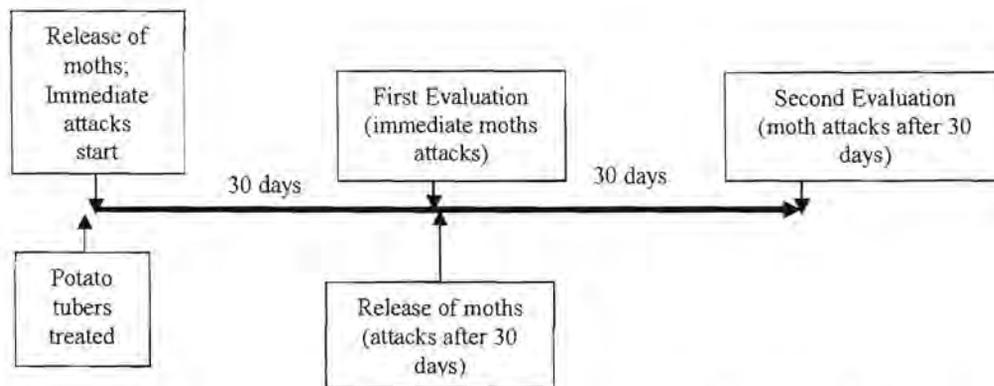


Figure 1. Time line of actions taken for the free choice, moth attack, experiment.

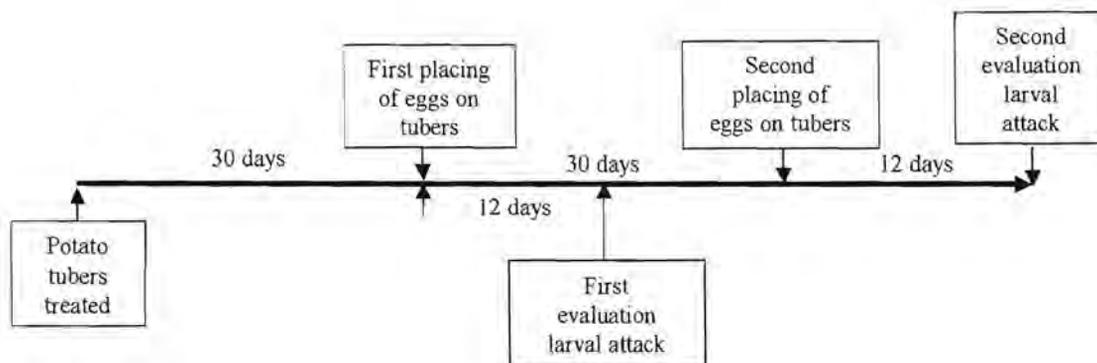


Figure 2. Time line of actions taken for the no-choice, larval attack, experiment.

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/m³ (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 were 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 sent 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 (Fenimore, 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 all 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 m³, 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 m³), 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	1 st	11	17	15	16	15	0	0	0	0	0	
2	1 st	28	33	36	18	29	0	0	0	0	0	
3	2 nd	19	27	25	18	22	0	0	0	0	0	
4	2 nd	14	22	16	25	19	0	0	0	0	0	
5	3 rd	14	12	19	12	13	0	0	0	0	0	
6	3 rd	36	39	34	29	35	0	0	0	0	0	
7	4 th	16	19	20	12	17	0	0	0	0	0	
8	4 th	29	27	24	22	26	0	0	0	0	0	
9	4 th	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	1 st	68	59	80	60	69	0	0	0	0	0	
2	1 st	76	69	79	67	73	0	0	0	0	0	
3	2 nd	38	46	33	44	40	0	0	0	0	0	
4	2 nd	78	76	71	76	75	0	0	0	0	0	
5	3 rd	45	59	67	47	55	0	0	0	0	0	
6	3 rd	69	56	48	73	62	0	0	0	0	0	
7	4 th	68	79	59	76	69	0	0	0	0	0	
8	4 th	51	35	59	52	49	0	0	0	0	0	
9	4 th	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
1 st Instar larvae outside tubers*	10	10	10	0
4 th 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) – therefore 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 1 st instar larvae outside tubers.			

CHAPTER 5

The potential of UV light-assisted insect electrocutor 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 electrocutor 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 electrocutor 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 electrocutor 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 electrocutor 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 electrocutor 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 electrocutor 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 electrocutor 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 electrocutor 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 electrocutor 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 electrocutor 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 electrocutor 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 electrocutor 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 electrocutor 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. Electrocutor 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 electrocutor 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 electrocutor 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 electrocutor 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 electrocutor 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 electrocutor 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%

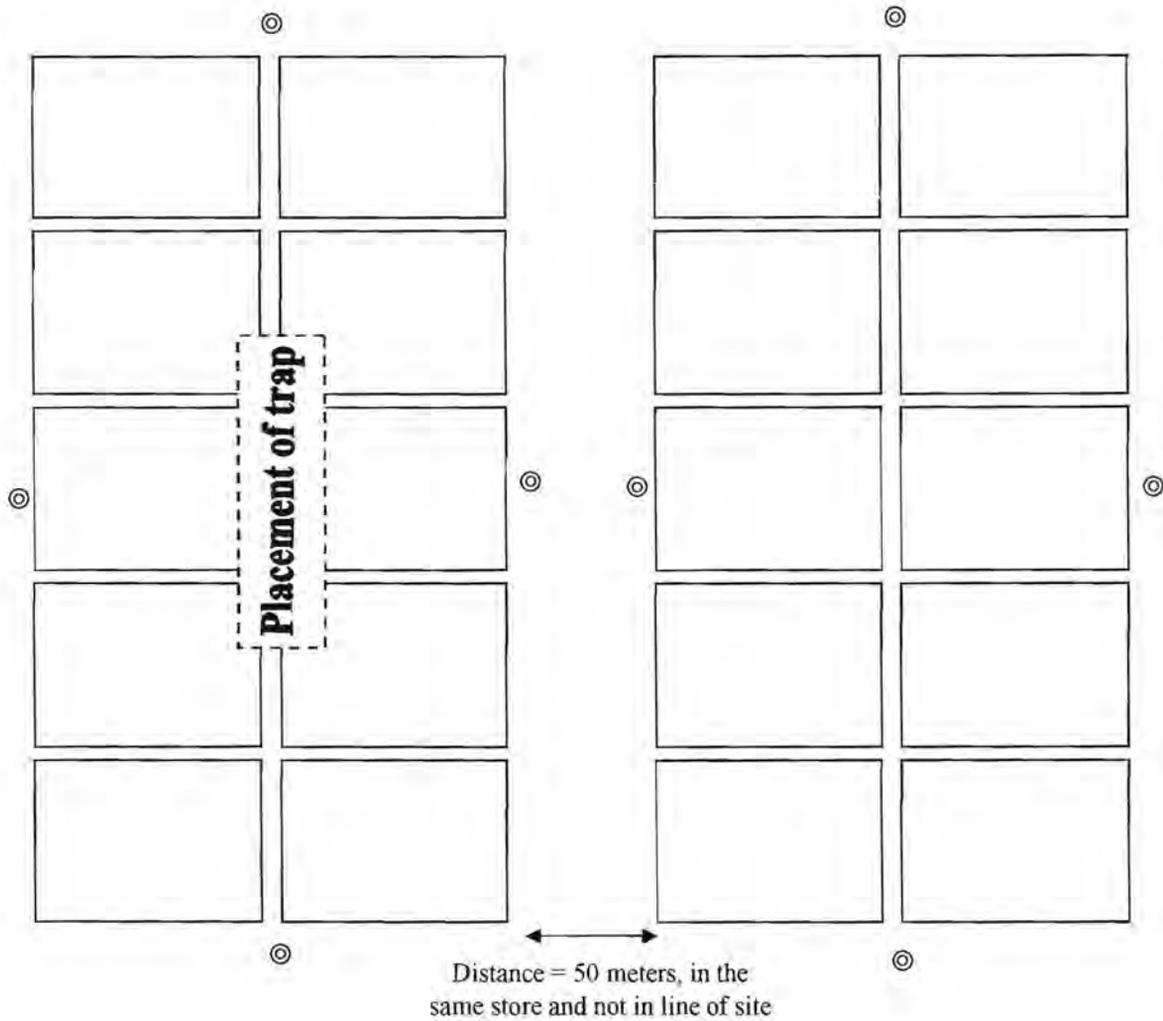
The average number of tubers attacked per month over the two years was not significantly different ($P = 0.676$)

Insect trap

Ten crates, containing 30 tubers each.

Control

Ten crates, containing 30 tubers each.



⊗ = 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 electrocutor trap.

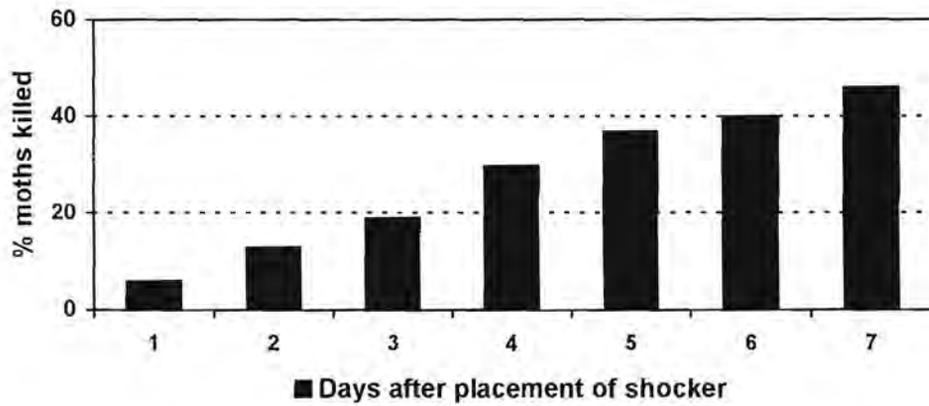


Figure 2. The cumulative increase in the percentage potato tuber moths killed over time with the small UV light-assisted insect electrocutor trap.

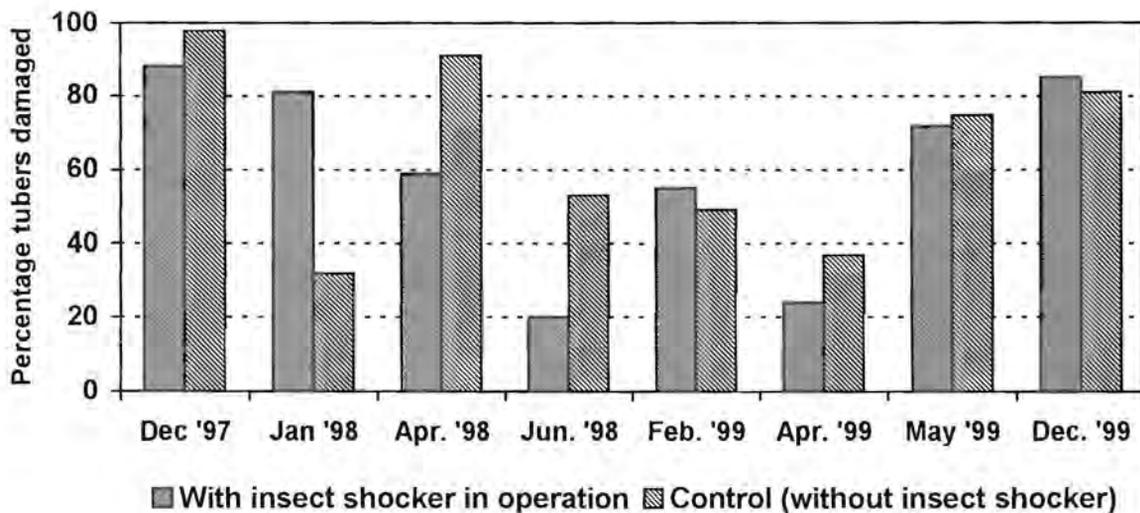


Figure 3. The percentage tubers attacked with and without the large UV light-assisted insect electrocutor trap in operation above 10 crates filled with 300 tubers.