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

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Introduction

The potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), is one of the most important insect pest of potatoes in South Africa. It attacks the foliage and tubers in the field, as well as tubers in non-refrigerated stores. Between 5 and 20% (depending on the production region) of tubers are discarded on the sorting tables after harvest, but in extreme cases this may be as high as 80%. Damage figures for stored potatoes in South Africa are not easily obtainable, but situations where infested batches of potato contaminated new clean batches in the same store are reported regularly. However, damage figures of potatoes in non-refrigerated stores in third world countries may reach 100% (Fuglie *et al.,* 1991; Ferro and Boiteau, 1993). The potato tuber moth is regarded as a serious post harvest pest problem for both the commercial and the small scale farmer.

The potato tuber moth attacks at least 40 plant species in the family Solanaceae (Foot, 1976). This extensive host range reduces the likelihood of a breeding program producing potato cultivars that are resistant to the tuber moth (Foot, 1976). Breeding for insect resistance in potato has been attempted since 1967 by the University of Minnesota (Flanders *et al.,* 1992) and since 1978 by the

International Potato Center in Lima, Peru (Raman and Palacios, 1982). Because resistance in already improved cultivars is very unlikely (Khalil *et al.*, 1987), researchers usually experiment with crosses between wild potato species and improved cultivars (Chavez *et al.*, 1988). Some of the wild potato species were shown to be resistant or tolerant to potato tuber moth attacks (Raman and Palacios, 1982; Malakar and Tingey, 1999). However, no commercial non-transgenic cultivar has ever been shown to express appreciable levels of resistance against the potato tuber moth (Lagnaoui *et al.*, 2001). This is disappointing in the light of the research that showed the huge potential of breeding for resistance against the potato tuber moth (Chavez *et al.*, 1988; Oritz *et al.*, 1990; Arnone *et al.*, 1998). The closest that certain commercial cultivars came to be labeled 'resistant' is where they were shown to be less preferred by the potato tuber moth than to other cultivars (Gyawali, 1989).

The common soil bacterium *Bacillus thuringiensis* (*Bt*) produces insecticidal crystal proteins that are harmless to mammals, including man (Raman *et al*., 1987). The proteins derived from *Bt* are called δ (delta)-endotoxins (Tabashnik, 1994), or insecticidal crystal proteins and sometimes protoxins (Ebora and Sticklen, 1994), while the genes that code for these proteins in transgenic plants are called *cry* genes (Ferre and van Rie, 2002). Some literature refers to these proteins as *cry* proteins (Honée and Visser, 1993). Five cry proteins (*Cry1* to *Cry5*) are known to have highly potent and specific insecticidal activity (Beuning *et al*., 2001). These *cry* proteins bind to specific receptors in the midgut after ingestion, causing the death of the insect larva (Gill *et al*., 1992). Plants that express these *cry* genes are therefore protected from those insects that are affected by these proteins.

Previous research on genetically engineered (GE) potatoes include: protein-rich genotypes (Gahukar, 2002), the production of edible vaccines against various animal diseases (Mason *et al*., 1999), resistance against plant viruses (Palucha *et al.*, 1998; Grieco *et al*., 1999), disease resistance (Lorito *et al*., 1999), resistance against the bollworm, *Helicoverpa armigera* (Chakrabarti *et al*. 2000) and resistance against the Colorado potato beetle (Haffani *et al*., 2000). The use of genetically engineered crops against the potato tuber moth always included the *cry1* or *cry1Ia1* (previously termed *Cry5*, Crickmore *et al*., 1998) genes. Van Rie *et al*. (1994) could not find control against the potato tuber moth using potato plants with the *Cry1B* gene, but noted that further research was needed to amplify the expression of the gene in the plant. Ebora *et al.* (1994) only found limited mortality (10%) in potatoes engineered with the *Cry1Ac* gene. The *Cry1Ab* gene gave 100% larval mortality in stored potatoes for up to seven months (Jansens *et al.*, 1995; Canedo *et al*., 1999). *Cry1Ac9* genes in modified tobacco plants were effective against the potato tuber moth (Beuning *et al*., 2001). Potatoes with the *Bt-cry1Ia1* gene showed 100% mortality against potato tuber moth larvae (Mohammed *et al*., 2000).

The objective of this study was to evaluate transgenic potato tubers containing the *Bt-cry1c* and *Bt-cry1Ia1* genes under laboratory and storage conditions against the South African strain of potato tuber moth. The main criterion was whether tubers were damaged or not, and not mortality of individuals feeding

on the transgenic tubers. The results are thus directly indicative of what the farmer who uses the GE potatoes can expect when potatoes are stored in the presence of potato tuber moths.

The importation, handling and experiments with the genetically modified cultivars in this study was authorized and strictly monitored by The Directorate, Genetic Resources of the National Department of Agriculture, the regulatory body of transgenics in South Africa. All the cultivars and lines used in this study have been issued with permits for experimental purposes only. Licenses for commercial use have not yet been issued at time of publishing this document.

Methods

Acquisition of the transgenic plants

The cultivars with the *Bt-cry1Ia1* gene (five *Spunta* modifications and one modified line) were transformed and supplied by Michigan State University, USA. They were received as test-tube plantlets and multiplied by the ARC-Roodeplaat, Pretoria (25°35'S, 28°21'E). The *Bt-cry1Ia1* gene is the property of Syngenta. The *Bt-cry1c* gene is owned by and was transferred into potatoes by *Vitality Biotechnologies, Israel* (Lochner, 2000). The four cultivars with this gene were obtained from *First Potato Dynamics* (Durbanville, South Africa). These modified cultivars were *Desiree* (two modifications), *O'Maya* (two modifications), *Shepody* and *Lady Rosetta.* The transgenic plants with the two different genes were not received and evaluated simultaneously and the results will therefore be handled separately. Two types of resistance may influence results with transgenic potatoes, namely antixenosis (non-preference) and antibiosis (affecting feeding) (Arnone *et al*., 1998). To test for both of these types of resistance, two experimental layouts (modified from Ortiz *et al.*, 1990) were followed. They were no-choice and free-choice experiments.

No choice experiments

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

Bt-cry1c

Two no-choice evaluations (with moths) were conducted with lines containing the *Bt-cry1c* gene; one week after harvest and 150 days after harvest. Medium sized tubers (100 to 150 g), 15 for the first test and 20 for the second test of each line and unmodified controls were placed in separate insect cages after which moths (30 for

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

Bt-cry1Ia1

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

Free choice experiments

Bt-cry1c

The free choice experiment was conducted in a closed air-conditioned insectary room, with no windows and a temperature of 20±2°C. This experiment was conducted at a lower temperature because the objective was also to extend the storage time and to limit the chances of rotting. Twenty medium-sized potatoes (100 to 150 g) of the above mentioned lines and unmodified controls were placed in crates. The crates were not stacked but were all placed on the floor of the room in a randomized block design with four replicates. Potato tuber moths were released in the room by placing a Petri dish in each crate containing pupae ready to hatch within 48 hours. Moths were released on two occasions, the first with 12 moths per crate and the second, 30 days later, with 25 moths per crate. The moths that emerged from the pupae had a free choice as to which tubers in which crates they wanted to lay their eggs on. The tubers were incubated for 30 days before an evaluation was performed. A second control was added before the second release. This was a BP1 control treatment and was meant to be an indicator treatment with no damage to start with in relation with the other controls, which already showed damage after the first evaluation. Fifteen randomly selected tubers from each

treatment (across replicates) were selected at the end of the second evaluation and transferred to separate containers with white sand. They were kept until the larvae inside exited and pupated in the sand. After pupation the pupae were counted and kept until moths emerged.

Bt-cry1Ia1

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

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

Results and Discussion

No choice experiments

When potato tuber moths had no choice but to lay their eggs in the same container as the tubers, or where larvae were put on tubers, no damage was recorded in any of the transgenic lines (Table 1, 3 and 5). The *Bt-cry1c* gene remained active for the tested 150 days after harvest and the *Bt-cry1Ia1* gene for the tested 200 days after harvest. All the unmodified controls were always damaged. Healthy progeny (moths) were collected from all the unmodified controls while the transgenic lines

did not give rise to any progeny.

Free choice experiments

All the transgenic lines were free of any tuber moth damage, except for the *Bt-cry1Ia1* transgenic Spunta-S4 line (Table 2, 4 and 5). However, only a mean number of 0.3 out of 10 tubers showed damage in this line during both of the two evaluation dates (Table 5).

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

Both the *Bt-cry1c* and the *Bt-cry1Ia1* genes were evaluated for their efficacy against the potato tuber moth under storage conditions. Lines with these two genes provided excellent control in environments where high numbers of potato tuber moths were present. Previous research results obtained with lines containing the *Bt-cry1Ia1* gene correspond with the results of this study. These works include Westedt *et al*., 1998; Li *et al*., 1999; Douches *et al*., 2000; Mohammed *et al*., 2000; Lagnaoui *et al*., 2001. This study showed that all the *Bt-cry1Ia1* transgenic lines, except Spunta-S4, which scored 97% in two of the five tests, would always control potato tuber moth. Even the 97% control observed with the Spunta-S4 is acceptable to label it as resistant. The resistance of lines with this gene lasted for the tested 200 days. Preliminary research conducted with potatoes containing the *Bt-cry1c* gene in South Africa was reported in the popular press (Lochner, 2001). The four cultivars with this gene always gave 100% control in all four tests. This absolute resistance lasted for the tested five months of storage. Most developed country markets rejects even slightly damaged potatoes and only a lethal antibiosis effect would therefore be acceptable (Arnone *et al*., 1998). The results with GM potatoes against the potato tuber moth comply with this prerequisite. However, potato production and markets in developing countries follows a different pattern. The economic loss threshold for small-scale farmers cultivating consumer potatoes in Africa is between 20 and 30% (Fuglie *et al.*, 1991). These farmers are also known to sell their seed potatoes as soon as the first signs of infestations are noted (Kroschel and Koch, 1994). The high resistance that the GM potatoes express will therefore add value to the crop, and will result in much longer storage times of potatoes in developing countries.

Genetically modified crops will most probably play a more important role

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

Geographic variability in the potato tuber moth has been documented (Briese, 1986). This variability in potato tuber moth populations was also indicated as the reason for varying results with resistance tests with wild potato species in Peru and Italy (Arnone *et al*., 1998). For a cultivar to be labeled as "resistant" against a certain pest, it therefore has to be evaluated against a wide range of different geographic populations. This study is the third country outside the USA to use a local potato tuber moth population in tests for the efficacy of the *Bt-cry1Ia1* gene in potatoes (the others being Egypt and Peru). The *Bt-cry1c* gene was also effective against the potato tuber moth in Israel (*personnel communication with* L. Olivier). It was therefore shown that the relevant *cry* genes are potent enough to control geographically removed populations of the potato tuber moth.

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

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

The use of tuber moth resistant potato cultivars will allow for the reduction or elimination of the use of toxic chemicals on an edible crop, a practice that is still common in some areas of the developing world. It will also possibly result in an increase of seed production of higher quality and will add much value to the table potato market. The high levels of resistance of potatoes containing the *Bt-cry1Ia1*

and *Bt-cry1c* genes will allow potato growers to lower the status of the potato tuber moth as a post harvest pest of stored potatoes. It is even possible that growers which use these resistant cultivars may remove the tuber moth from their list of problems to allow them to concentrate on other potential post harvest problems.

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

Lines	*Infested one week after harvest*		*150 days of storage*
	Number of tubers attacked (n=15)	*Number of healthy moths that appeared*	*Number of tubers attacked (n=20)*
Desiree (GE-1)	0	0	0
Desiree (GE-2)	0	0	0
Shepody (GE)	0	0	0
Lady Rosetta (GE)	0	0	0
O'Maya (GE-1)	0	0	0
O'Maya (GE-2)	0	0	0
Vanderplank	14	7	-
O'Maya	15	65	-
BP13	15	24	-
BP1	15	77	20*
Up-To-Date	15	72	-
Shepody	15	102	-
Desiree	15	24	-
Lady Rosetta	15	103	-

*fresh uninfested tubers were used; GE: Genetically engineered

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

Lines	*60 days of storage**	*90 days of storage*	*Number of moths from 15 tubers*
Desiree (GE-1)	0	0	0
Desiree (GE-2)	0	0	0
Shepody (GE)	0	0	0
Lady Rosetta (GE)	0	0	0
O'Maya (GE-1)	0	0	0
O'Maya (GE-2)	0	0	0
Vanderplank	7	18	9
O'Maya	6.8	20	17
BP13	4.8	19.8	16
Up-To-Date	8.3	20	18
Shepody	6.3	19	14
Desiree	7.8	19.3	21
Lady Rosetta	5.5	19	28
BP1	11.3	19.5	18
BP1(b)**	-	19	-

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

Table 3. The number of mini-tubers (10 to 20 g) from potato lines containing the *Bt-cry1Ia1* gene infested with potato tuber moth larvae and the number of healthy moths that appeared from them (no- choice moths, n=15)

Lines	*Number of tubers attacked*	*Number of healthy moths that appeared*
Spunta Control	12	15
BP1 Control*	15	440
Spunta-G2 (GE)	0	0
Spunta-G3 (GE)	0	0
L235-4.13 (GE)	0	0
Spunta-S1 (GE)	0	0
Spunta-S4 (GE)	0	0
Spunta-6a3 (GE)	0	0

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

Table 4. The mean number of mini-tubers (10 to 20g) from potato lines containing the *Bt-cry1Ia1* gene infested with potato tuber moth larvae and the mean number of healthy moths that appeared from them (free-choice moths, n=5)

Lines	*Number of tubers attacked*	*Number of healthy moths that appeared*
Spunta control	4	8.8
Spunta-G2 (GE)	0	0
Spunta-G3 (GE)	0	0
L235-4.13 (GE)	0	0
Spunta-S1 (GE)	0	0
Spunta-S4 (GE)	0	0
Spunta-6a3 (GE)	0	0

GE: Genetically engineered

Table 5. The mean number of tubers from potato lines containing the *Bt-cry1Ia1* gene infested with potato tuber moth larvae after 42, 72 and 200 days of storage in a diffused light store. The number of tubers that started to rot is also indicated (n = 10)

Lines	*Free choice (moths)*		*No choice (larvae)*	*Rotting*
	*42 days after harvest**	*72 days after harvest*	*200 days after harvest*	*200 days after harvest*
Spunta Control	5.5	8.5	10	NA
BP1 Control**	6.0	9.8	10	NA
Spunta-G2 (GE)	0	0	0	0
Spunta-G3 (GE)	0	0	0	0
L235-4.13 (GE)	0	0	0	0
Spunta-S1 (GE)	0	0	0	0
Spunta-S4 (GE)	0.3	0.3	0	0
Spunta-6a3 (GE)	0	0	0	0

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