Report 2003/2004

GMO Potato Project

The efficacy of potato lines expressing the *Bt-Cry1Ia1* (previously *Cry5*) gene against the potato tuber moth under field conditions, with preliminary environmental data.

Studies conducted in the Pretoria (Roodeplaat), Ceres (Koue Bokkeveld) and KwaZulu-Natal (Kokstad) regions

Permit number 17/3(4/03/068)

CONDUCTED BY

ARC-Roodeplaat

Vegetable and Ornamental Plant Institute

Private Bag X293, Pretoria, 0001

South Africa

ARC-LNR

and

Michigan State University

East Lansing, U.S.A.

Written and compiled by the Bt Potato Research Team

CONTENTS

EXECUTIVE SUMMARY

Three field trials were planted, one each at Roodeplaat (Gauteng), Ceres (Western Cape) and Kokstad (Kwazulu-Natal). Two transgenic potato lines (SpuntaG2 and SpuntaG3) containing the *Bt-Cry1Ia1* (old *Bt-Cry5*) gene and three non-GMO controls, Spunta, BP1 and Mnandi were evaluated against the potato tuber moth, *Phthorimaea operculella*. Results from all three field trials verified findings from the previous two years field work - absolute resistance against the tuber moth. Except for the Ceres trial, the total yield and size distribution of the two transgenes did not differ significantly from the Spunta control. In relation to the controls, the transgenes did not influence the incidence of the two most important virus diseases, PVY and PLRV. The percentage parasitism of the potato leafminer, *Liriomyza huidobrensis*, was approximately twice as high in Spunta and its two transgenes in comparison with that in a nearby insecticide sprayed potato field. Preliminary alternative host studies showed survival (>20%) on at least eight Solanaceae plants tested. Risk management for field trials with GMO-potatoes is discussed.

2. THE RESEARCH TEAM (2003/2004 SEASON)

The following persons were all directly or indirectly involved during the relevant season

South Africa

Dr. Graham Thompson.. Project manager and overall coordinator for South Africa.

Dr. Diedrich Visser ..... Supervisor for all field trials and Entomological aspects.

Dr. Cobus Coetzee ..... Coordinator for the Ceres trial.

Morgan Naidoo ..... Coordinator and data collection for the Kokstad trial. *PDA, KZN.*

Abroad (All linked to Michigan State University, U.S.A.)

Dr. Johan Brink..... International project coordinator.

Prof. Dave Douches ..... Overall project manager.

Prof. Walter Pett ..... Advisor on Entomological aspects.

Dr. Hector Quemada ..... General Biosafety advisor.

3. GENERAL BACKGROUND

The potato (*Solanum tuberosum* L.) is the most important vegetable crop in South Africa (Van Vuuren & Le Roux 2004). Between 55 000 and 60 000 hectares are planted annually in all nine provinces of which 16% is utilized for seed production (Potatoes South Africa 1999). Nearly 80% of all potato plantings are irrigated with an average yield of approximately 40 tons per hectare (Visser 2004). The total average yield (including non-irrigated farms), is approximately 30 tons per hectare. Seventy percent of all potatoes are planted with seed certified to be disease- and virus-free (Niederwieser 2003).

The potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), is a serious insect pest of potatoes in South Africa (Visser *et al.* 2003). It has also become increasingly important on tobacco (Van Vuuren *et al.* 1998) and tomato (Gilboa & Podoler 1994; D. Visser personal observation). Damage has also been reported on eggplant and other Solanaceous crops and weeds (Rahalkar *et al.* 1985; D. Visser, personnel observation). 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 (Visser & Schoeman 2004). 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 (Visser 2004). 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 (Visser & Schoeman 2004). Although no insecticide is registered against the potato tuber moth in South Africa under storage conditions (Nel *et al*. 2002), various control strategies are available for the small scale farmer (Visser 2004). However, the only control strategy that gave absolute control against the potato tuber moth (for nearly a year in storage) was the use of genetically modified potatoes (Visser & Schoeman 2003).

4. BACKGROUND TO THE RESEARCH

The current research (at ARC-Roodeplaat) with the *BtCry1Ia1 (formerly BtCry5)* gene started in 2001. This is the third year that field trials have been conducted with potatoes containing the *Bt-Cry1Ia1* gene in South Africa. During the first year one field trial was planted at ARC-Roodeplaat, the second year one each at Roodeplaat and Ceres (Western Cape) and this past season field trials were planted at Roodeplaat, Ceres and Kokstad (KwaZulu-Natal). During the first two years storage trials were also conducted at Roodeplaat. Currently (post the third year), three storage trials are in progress at Roodeplaat, Ceres and Kokstad. These storage trials fall under the current permit - all three trials are stored in secure, locked non-refrigerated environments (stores).

Seed for the Roodeplaat field trial, which is reported on in this report, originated mostly from mini-tubers (< 40 gram) planted in a greenhouse at Roodeplaat during the winter of 2003. Seed for the BP1 control plots was obtained from a commercial seed producer. Those were normal sized tubers (approximately 90 gram). Normal sized tubers were also used in the fourth repetition of the G3 treatment. This was due to the fact that insufficient mini-tubers of the G3 line were produced in the greenhouse during the previous winter.

The Ceres and Kokstad field trials were planted with tubers kept from the previous years field trial at Ceres. Only the Mnandi control seed for the Kokstad trial was obtained from a commercial seed producer. All the tubers for the Ceres and Kokstad trials were normal sized tubers. However, the Mnandi (Kokstad) seed tubers were physiologically in a much better state than the other seed tubers. The seed tubers of G2, G3 and Spunta had been kept for a much longer time period in a cool storage facility and were in an advanced stage of sprouting.

New tuber multiplications (mini-tubers) are currently in progress at Roodeplaat for the next years trials (2004/2005). We now report on the three field trials at Roodeplaat, Ceres and Kokstad conducted during 2003/2004. No storage trials were conduced during this period.

5. METHODS

a. Field trial layouts

The field trial layouts can be found in Fig. 1 (Roodeplaat), Fig. 2 (Ceres) and Fig. 3 (Kokstad). All three trials followed a randomized block design with four repetitions.

For the Roodeplaat trial two extra blocks were planted on either side of the trial. These plots were not part of the randomization and contained two other host plants of the tuber moth, namely tomato and eggplant. The aim was to monitor and compare the tuber moth activity in adjacent fields that contain other host plants of the tuber moth. The purpose was to determine whether the presence of GMO potatoes influence not only the activity of the tuber moth in adjacent related crops, but also the activity of all non-target arthropods in those fields.

For the Ceres and Kokstad trials, natural infestations of the potato tuber moth were allowed. For the Roodeplaat trial, more than 20 000 tuber moths were released (as pupae and/or moths), divided equally between all plots with every release (see Table 1 for release dates and numbers). The releases were done because of the known low tuber moth pressure at Roodeplaat.

b. Events in the field

The events (actions taken for the duration of the field trials) can be found in Table 1 (Roodeplaat), Table 2 (Ceres) and Table 3 (Kokstad).

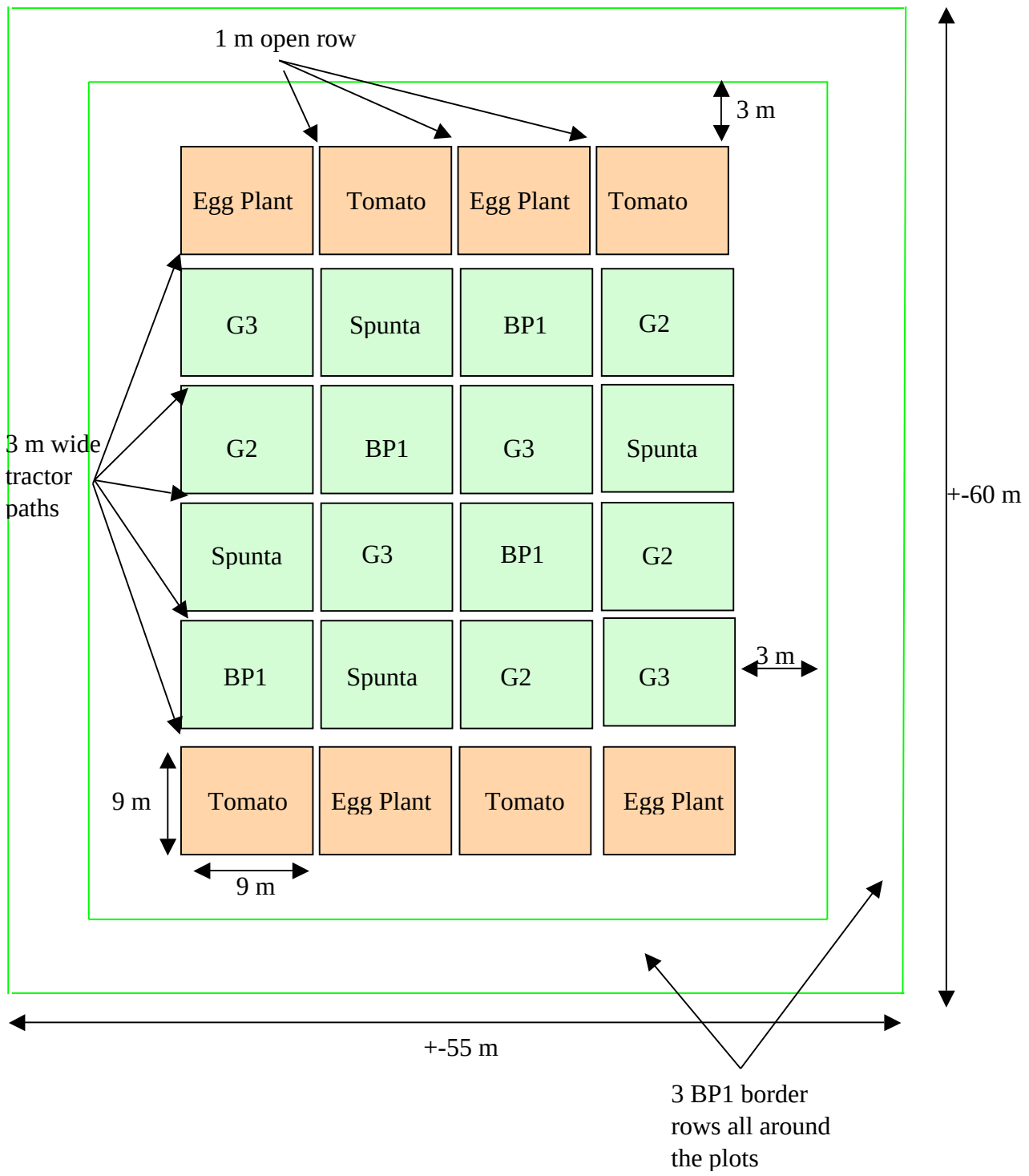


Figure 1. **ROODEPLAAT** field trial layout for the 2003/2004 season. The border/side rows were all BP1, non-GMO potatoes. Thirty tubers were planted per row with 9 rows per plot (in the potato plots). Paths between the blocks were 3m wide (not to scale in the fig).

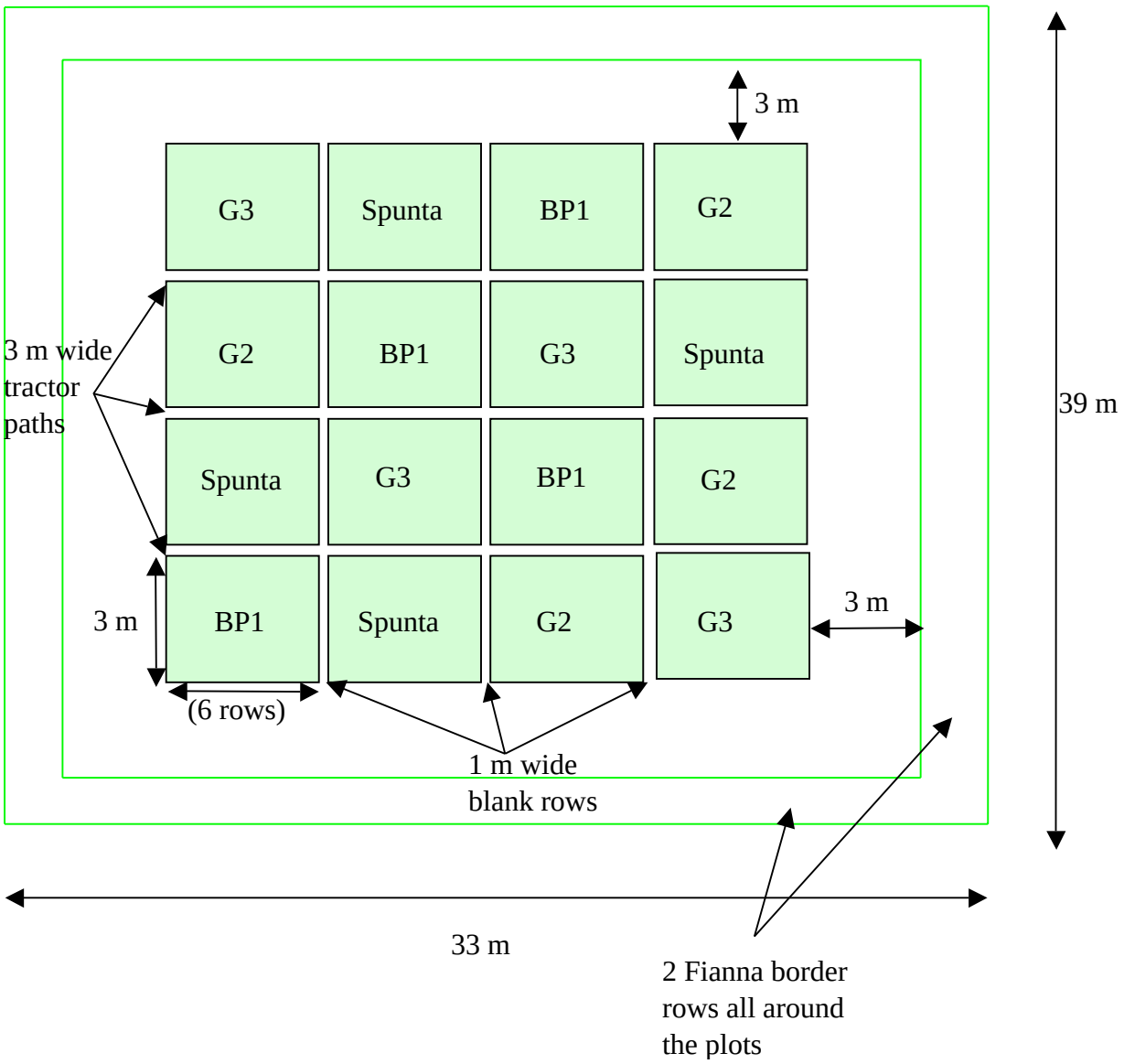


Figure 2. **CERES** field trial layout for the 2003/2004 season. The border or side rows were all of the Fianna cultivar, non-GMO potatoes. Ten tubers were planted per 3m row and 6 rows per plot (60 tubers per plot).

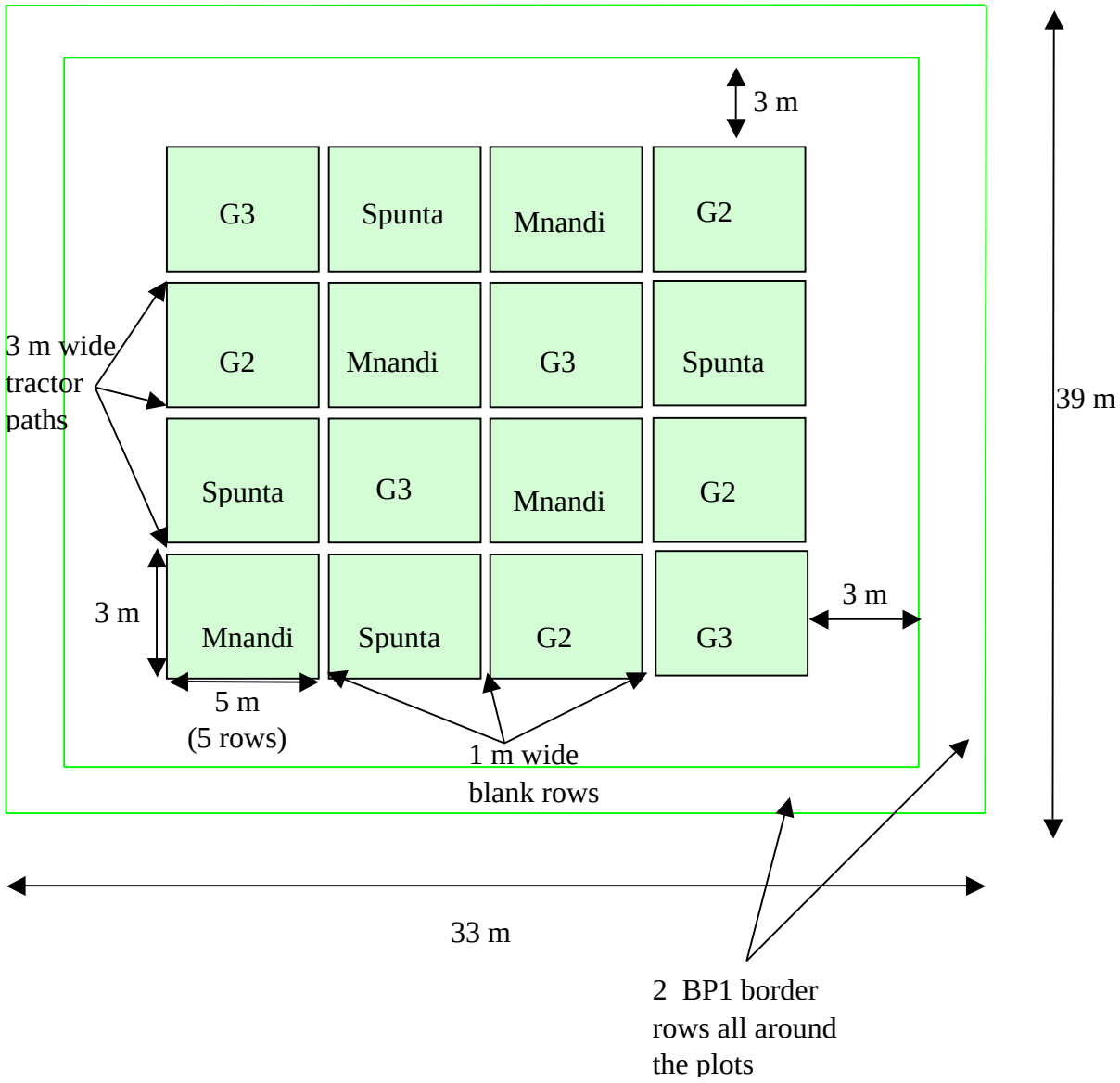


Figure 3. **KOKSTAD** field trial layout for the 2003/2004 season. The border/side rows were all of the cultivar BP1, non-GMO potatoes. Ten tubers were planted per 3m row with 5 rows per plot (50 tubers per plot).

Table 1. All actions taken for the duration of the Roodeplaat field trial 2003/2004.

Note: The field was visited nearly every day for collections of environmental data (non-target organisms). These visits are not stipulated here.

2003	**Actions**
12 May	Lay out irrigation system.
15 May	Irrigate field before cultivating.
19-21 May	Start cultivating.
4 June	Irrigate
18 Aug.	Spray Roundup @ 4l / ha in 200 l water.
25 Aug.	Complete cultivation of field.
26 Aug.	Apply pre-plant herbicide: Eptam @ 4l/Ha.
28 Aug.	Take seed tubers from cool storage and place at room temperature.
27 Aug.	Measure out trial.
28 Aug.	Make furrows and plant trial. Fertilize @ 1300kg / ha 3:2:1(25). Lay out the irrigation system.
29Aug.	Apply pre-emergence herbicide: Metagan Gold 1.2l /ha. Irrigate 9 mm
30 Aug.	Irrigate 8 mm
2 Sept.	Irrigate 5 mm
5 Sept.	Irrigate 14 mm
9 Sept.	Irrigate 2 mm
11 Sept.	Irrigate 10 mm
12 Sept.	BP1 ± 80% emergence = emergence date for BP1 all reps (normal tubers).
16 Sept.	Irrigate 13 mm
17 Sept.	Count emerged plants.
18 Sept.	Irrigate 11mm
19 Sept.	Count emerged plants.
22 Sept.	Irrigate 13 mm
23 Sept.	G3 Rep 4 ± 80% emergence = emergence date G3 Rep 4 (normal tubers were planted in Rep 4 because mini tubers were not enough).
25 Sept.	Count emerged plants. Irrigate 18 mm
26 Sept.	Measure out plots for applying fertilizer where tomatoes and eggplant will be planted.
29 Sept.	Count emerged plants. Irrigate 11mm
30 Sept.	Apply fertilizer and rotovate where tomatoes and eggplant will be planted.
1 Oct.	Make beds where tomatoes and eggplant will be planted.
2 Oct.	Plant tomato seedlings. Irrigate 7 mm
3 Oct.	Count emerged plants. Irrigate 5 mm
4 Oct.	Irrigate 5 mm
5 Oct.	Irrigate 7 mm
6 Oct.	Replant tomato plants that died. Irrigate 7 mm
7 Oct.	Top-dress with 100 kg LAN(28) and ridge plants that were ready.

8 Oct.	Count emerged plants. Irrigate ±7 mm
9 Oct.	Irrigate 9 mm
10 Oct.	Irrigate 8 mm
11 Oct.	Rain 18 mm
13 Oct.	Move pipes, clean and level paths. Plant eggplant seedlings and water with watering cans.
14 Oct.	Spray Mancozeb @ 2.5l / ha + Aqua Right 7 @100ml/100l H_2O + Complement @ 150 ml /100l water. Water eggplant seedlings with watering cans.
15 Oct.	Irrigate 18 mm
16 Oct.	Top-dress with 100 kg LAN(28) and ridge plants that were ready.
17 Oct.	Irrigate 7 mm
18-19 Oct.	Rain 26 mm
20-21 Oct.	Rain 32 mm
24 Oct.	Top-dress tomatoes. Install tensiometers. Irrigate 11mm
28Oct.	Replant tomato seedlings that did not establish because of the heat wave. Spray Mancozeb @ 3l / ha + Aqua Right 7 @100ml/100l H_2O + Complement @ 150 ml /100l water. Water tomato seedlings with watering cans.
29 Oct.	Irrigate 23 mm
31 Oct.	Irrigate 12 mm
1 Nov.	Rain 2 mm
2 Nov.	Irrigate 10 mm Rain 12 mm
3 Nov.	Rain 2 mm
4 Nov.	Rain 4 mm
7 Nov.	Irrigate 17 mm
10 Nov.	Irrigate 15 mm Rain 3 mm
12 Nov.	Spray Mancozeb @ 3l / ha + Aqua Right 7 @100ml/100l H_2O + Complement @ 150 ml /100l water. Released 10 000 tuber moths. Rain 6mm
14 Nov.	Irrigate 23 mm
18 Nov.	Irrigate 12 mm
20 Nov.	Spray Mancozeb @ 3l / ha + Aqua Right 7 @100ml/100l H_2O + Complement @ 150 ml /100l water.
21 Nov.	Irrigate 17 mm
22-23 Nov.	Rain 7 mm
26 Nov.	Irrigate 15 mm
26 Nov.	Rain 18 mm
27 Nov.	Rain 2 mm
2 Dec.	Irrigate 2 mm
3 Dec.	Spray Curzate Pro @ 3kg / ha + Score @ 350 ml / ha + Aqua Right 5 @ 100ml / 100l

	H_2O + Complement @ 150 ml /100l water. Released 10 000 tuber moths.
5 Dec.	Irrigate 17 mm
9 Dec.	Irrigate 19 mm
10 Dec.	Spray Curzate Pro @ 3kg / ha + Score @ 350 ml / ha + Aqua Right 5 @ 100ml / 100l H_2O + Complement @ 150 ml /100l water.
11 Dec.	Rain 6 mm
12 Dec.	Irrigate 16 mm
15 Dec.	Irrigate 17 mm
17 Dec.	Spray Curzate Pro @ 1.6kg / ha + Mancozeb @ 2.4 kg/ha + Score @ 350 ml / ha + Aqua Right 5 @ 100ml / 100l H_2O + Complement @ 150 ml /100l water.
18 Dec.	Irrigate 8 mm
19 Dec.	Irrigate 8 mm
22 Dec.	Irrigate 20 mm + Rain 37 mm
23 Dec.	Slash surrounding grass.
25 Dec.	Rain 44 mm
30 Dec.	Irrigate 15 mm
2 Jan. 2004	Irrigate 18 mm
3 Jan.	Rain 34 mm
7 Jan.	Spray Mancozeb @ 2.4 kg/ha + Score @ 350 ml / ha + Aqua Right 5 @ 100ml / 100l H_2O +Complement @ 150 ml /100l water.
6 Jan.	Irrigate 10 mm
7 Jan.	Plants dying off naturally. Foliage becoming yellow and no more collections or evaluations in field is possible.
2 Feb.	Spray Gramoxone herbicide to kill off all left-over foliage.
16 Feb.	Harvest starts.
23 Feb.	Sorting, weighing, evaluating and storing of harvested tubers starts.

Table 2. Actions taken for the duration of the Ceres field trial 2003/2004.

Action	**Date**
Planning of trial layout	29 September 2003
Inspection of farm by Dept. of Agriculture	9 October 2003
Counting and packing tubers for Ceres	3 November 2003
Planting of the trial	6 November 2003
Inspection of trial	9 December 2003
Potato tuber moth evaluation 1	7 January 2004
Potato tuber moth evaluation 2	22 January 2004
Potato tuber moth evaluation 3	4 February 2004
Inspection and meetings from MSU team & Virus sample	6 February 2004

collection	
Mechanical harvesting	23 March 2004
Sorting and grading of the trial	23 March 2004
Store 800 tubers at Ceres (non-refrigerated).	24 March 2004
Cool storage of the rest of tubers at Ceres	24 March 2004

Table 3. Actions taken for the duration of the Kokstad field trial 2003/2004.

Action	**Date**
Land history: The land has been left to grass, undisturbed for more than five years. *Land prep*. Rip, plough, disc and kongskilde.	
Planting date Target yield = 70 ton/ha with 100g MAP & 11g KCL/3m	6 Oct. 2003
Top dressing and ridging (270g KAN/3m)	19 Nov.
Spray for diseases • Bravo weekly: 21 Nov. to 12 Dec. • Amistar alternatively with Bravo weekly: 18 Dec. to 21 Jan 2004. • Mancozeb + Bravo: 26 Jan. • Mancozeb + Amistar: 2 Feb. • Amistar: 9 Feb. (no insecticides were applied)	
Irrigation every week from 16 Oct 2003. to 9 Jan 2004	
Hail damage	23 Dec.
Die back days G3 = 6 Feb and G2 13 Feb	
Slashing of leaves	18 Feb. 2004
Harvest, sorting and storing	8 Mar.

c. Leaf damage evaluations

Fields were inspected regularly for tuber moth infestations. This was done by scouting the control blocks while looking for any leaf mines present. When more than 15% of plants were attacked in the control blocks, a detailed scouting of the rest of the trial plots was followed. The following were recorded while scouting;

a) number of plants attacked per 10 plants (Roodeplaat) or the number of haulms attacked per 25 haulms (Ceres and Kokstad), randomly selected per plot
b) number of leaf mines found in plants or haulms from a)
c) number of live larvae from b)

d. Harvest: yields and tuber damage

Two weeks before harvest, all haulms were destroyed by either using the herbicide Gramaxone or by slashing or pulling the haulms. At harvest all tubers from each plot were kept separate in clearly marked containers. Weighing and sorting were done on sorting tables in the potato stores of Roodeplaat and Ceres and by hand in Kokstad. Tubers from all plots were weighed separately after sorting into large, medium, small and unmarketable. All tubers were stored in safe lock-up cooling facilities. All rotten tubers were first frozen and then buried in deep (>2m deep) refuge holes. The potatoes in the refuse holes were covered with a layer of soil after the dumping of the now destroyed tubers (the freezing process destroys all living plant material).

All sites will be monitored for volunteer potato plants for the next 12 months. Volunteers will be recorded and destroyed.

e. Gene flow

During the latter parts of the season, fields were inspected for any signs of true seeds. These seeds (in berries) are a possible source for new plants and thus potential transgenic potatoes. Harvested seed would be tested for the presence of the relevant Bt gene (*Cry1Ia1*).

f. Plant virus incidence

During the latter parts of the growing season, leaf samples were collected in all plots at the Roodeplaat and Ceres sites for the determination of virus incidence. Five leaf samples were collected in each plot, using five different plants. All samples were analyzed for four different viruses, totaling 1280 tests at each of the Roodeplaat and Ceres trials. The viruses that were included in these tests were potato virus Y (PVY), potato virus X (PVX), potato virus S (PVS) and potato leaf roll virus (PLRV).

g. Effect on Parasitism

The effect on parasitoids was tested by collecting potato leaves from all treatments (in the Roodeplaat trial) containing leaf mines of the potato leafminer, *Liriomyza huidobrensis*. Forty leaves containing leaf mines were collected randomly and placed in insect proof cages. These leaves were kept in these cages until the emergence of adult flies and parasitoids of the flies. The number of emerging flies and parasitoids were counted and the percentage parasitism calculated. This was the only time that any plant material (leaves) was taken from the field. All dried out leaves were destroyed by burning after these tests.

Parasitism of the potato tuber moth was not evaluated because no potato tuber moth leaf mines could be found in the GMO plots (all were killed by the GMO's, 100% mortality). No collection of tuber moth parasitoids were done in the non-GMO control plots because comparisons with the GMO plots are thus impossible.

h. Presence of other insects

The presence of other insects (pests and non-pests) in all plots in the Roodeplaat trial were observed and noted for the duration of the trial.

i. Non-target Arthropods

An effort was made to collect as many non-target arthropod species as possible in all plots in the Roodeplaat trial (including the tomatoes, eggplant, a sprayed potato field in the vicinity and follow fields adjacent). This was done using two kinds of traps, i.e. pitfall traps and sweep net catches. This was done approximately twice per week for four weeks in December. The aim was to find whether the arthropod diversity in the GMO plots differed from those in the non-GMO plots.

j. Storage trials

Three storage trials were initiated in 2004, one at each of the three locations, i.e. Roodeplaat, Ceres and Kokstad. Because these trials are still in progress, they will only be reported on in the next year's report (2005).

k. Laboratory bioassays – alternative hosts

Alternative host plants for the potato tuber moth were collected in the Pretoria region. Detached leaves were put in Petri-dishes containing wet filter paper. First instar potato tuber moth larvae, reared from the Roodeplaat insectaries, were transferred onto these leaves with a fine camel hair brush. The survival of these larvae were checked after 72 hours (3 days).

6. RESULTS AND DISCUSSION

a. Leaf damage evaluations

The results can be found in Fig. 4 (Roodeplaat), Fig. 5 (Ceres) and Fig. 6 (Kokstad).

Roodeplaat (Fig. 4)

The initial tuber moth infestations in November were low, but infestations increased during the December evaluations. Eggplant were the most severely attacked (leaf mines) while tomato plants were the least infested. Even the insecticide sprayed fields outside of the trial area showed severe infestations by the potato tuber moth, especially during November. Both the transgenic Spunta lines, G2 and G3, showed no signs of tuber moth infestation.

Ceres (Fig. 5)

The Ceres trial had good natural infestations on all three evaluation dates. Both the GMO-lines gave absolute control while heavy infestations were found in the two controls (BP1 and Spunta) cultivars.

Kokstad (Fig 6)

Only one evaluation date for Kokstad was possible. The plants died off earlier than expected, preventing further evaluations. This was as a result of the advanced physiological state of the planting material. During this evaluation in January, good infestation in the non-GMO controls was achieved. Both the two GMO lines, G2 and G3 showed no damage symptoms by the potato tuber moth.

b. Harvest: yields and tuber damage

Tuber damage

No damage by the potato tuber moth to tubers at harvest was observed for all three trials. This was not unexpected. Potato tuber moth larvae do not burrow into soil. The only way for them to reach tubers in soils is to move down cracks or to attack exposed tubers. Therefore, if cracks are limited or when tubers are not exposed, damage will be limited or non-existent. This is often the case, especially when irrigation is applied and where tubers are not exposed. We nearly always find this at the Roodeplaat site. The non-infestations of tubers at our trial sites were therefore not uncommon.

ROODEPLAAT Tuber Moth Efficacy Data

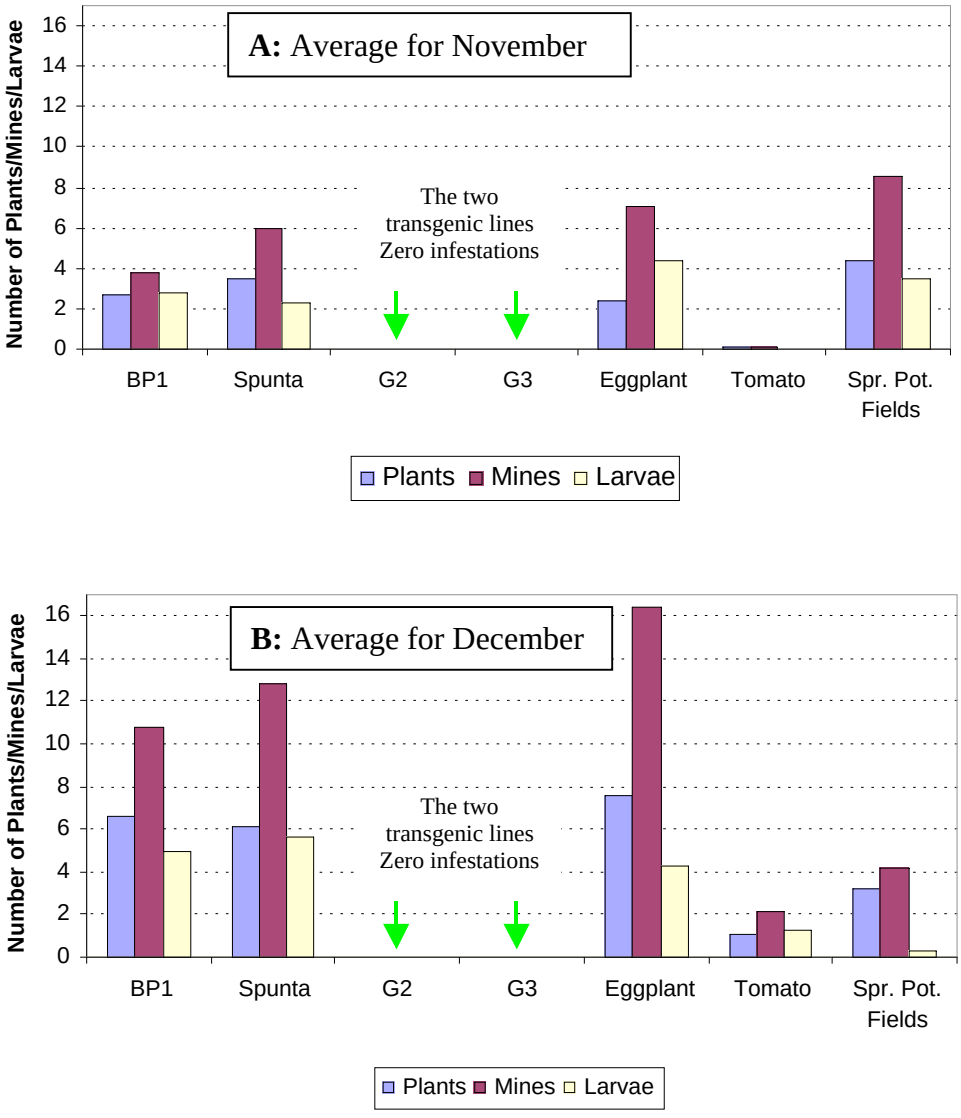


Figure 4. The level of potato tuber moth infestations at **ROODEPLAAT** (2003). The mean number of plants infested out of 10, the mean number of leaf mines found on those plants and the mean number of larvae found in those leaf mines. Four replicates used (as per trial layout, page 8). The mean data (three evaluations each month) for A: November (top) and B: December (bottom) is given.
Spr. Pot. Fields = sprayed potato field (mixed cultivars and lines) adjacent, +- 300m from the GMO field. All other treatments were part of the same field trial (see layout page 8).

CERES Tuber Moth Efficacy Data

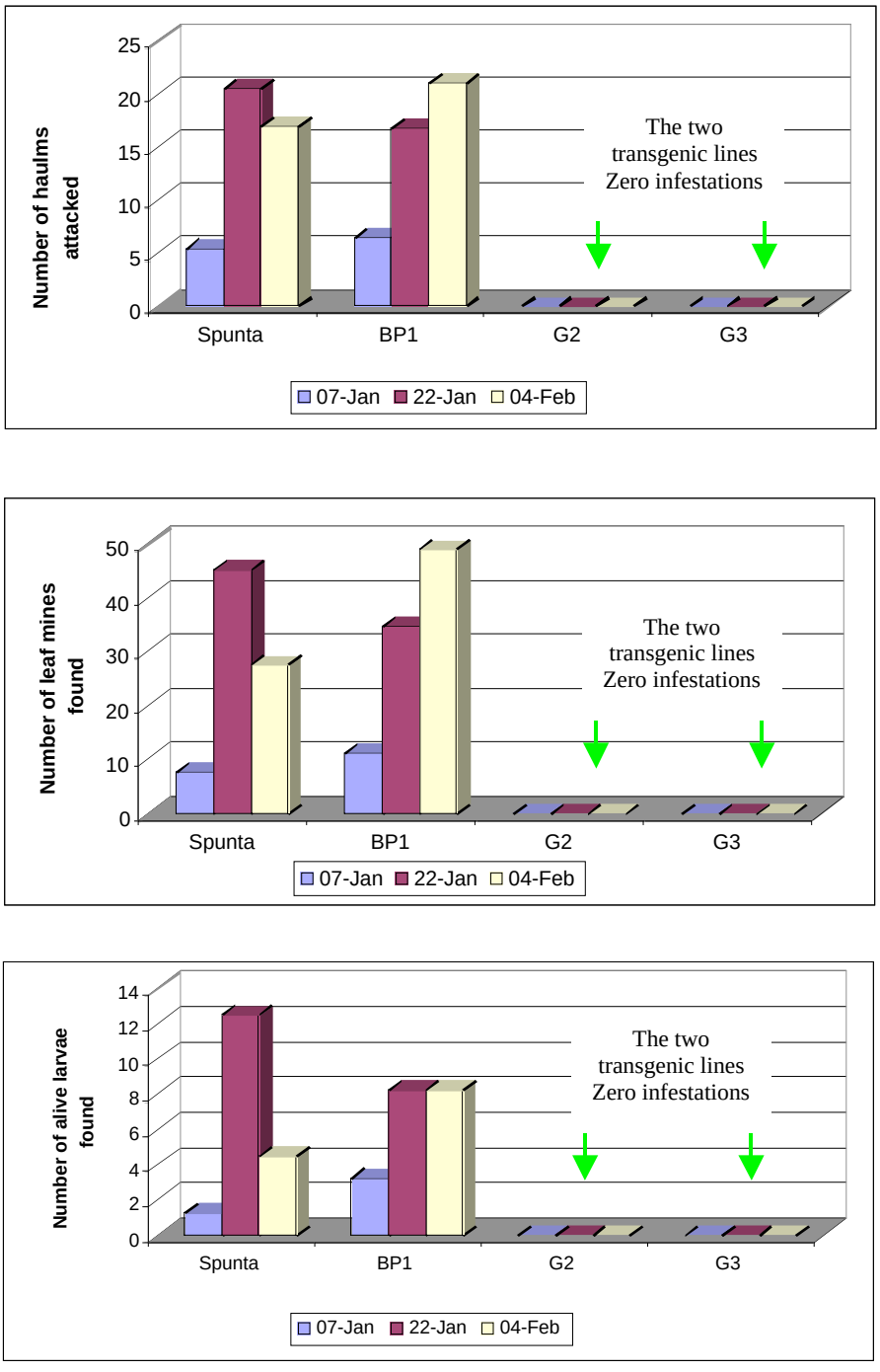


Figure 5. The level of potato tuber moth infestations at **CERES** (2003/2004). From top to bottom: The mean number of haulms infested out of 25 (top), the mean number of leaf mines found on those haulms (middle) and the mean number of larvae (bottom) found in those leaf mines.

KOKSTAD Tuber Moth Efficacy Data

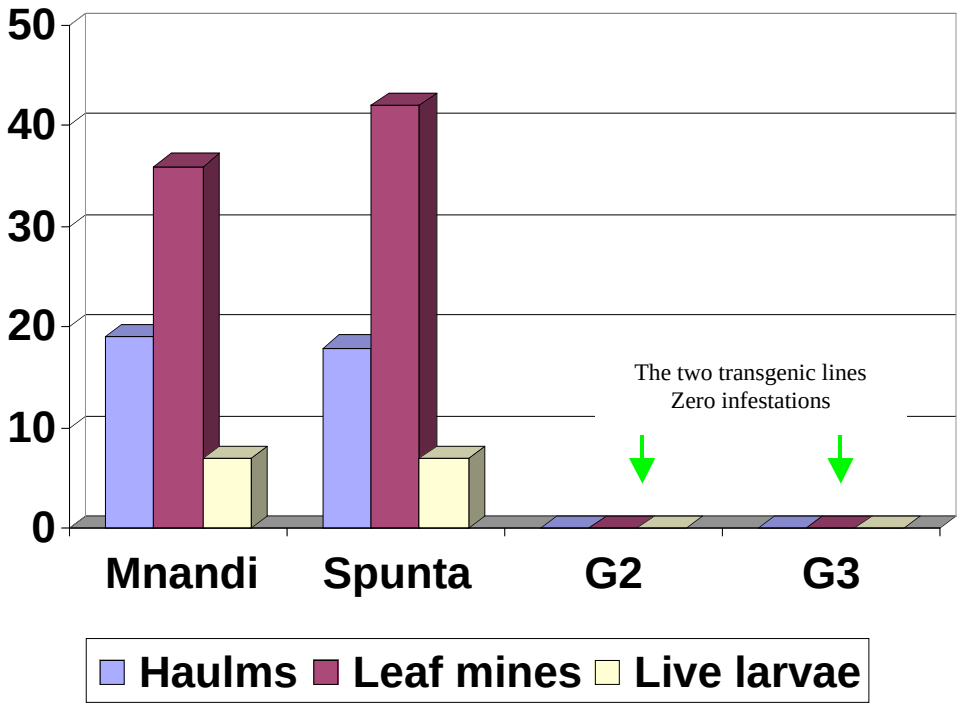


Figure 6. The level of potato tuber moth infestations at **KOKSTAD** (January 2004). The mean number of haulms infested out of 25, the mean number of leaf mines found on those haulms and the mean number of live larvae found in those leaf mines.

Yields

The yields obtained at each site from the two transgenic lines of Spunta were similar to those of the non-transformed Spunta. The yields obtained at each site were comparative to production levels normally obtained in those areas. However the yield of the Spunta lines could not be compared to the control varieties as the condition of the planting material had an influence on their growth and yield.

c. Gene flow

No true seeds were found on any of the potato plants in all treatments. That was observed at all three trial sites.

d. Plant virus incidence

The incidence of plant viruses in the Roodeplaat and Ceres trials were evaluated. The results can be found in Table 4. It is clear that the GM lines did not influence the incidence of the relevant viruses. This was similar to the results obtained in the previous year.

Table 4. The viruses found in treatments at the Roodeplaat and Ceres trials.

Line/Cultivar	Virus found at Roodeplaat 2004	Virus found at Ceres 2004	Virus found at Roodeplaat 2003
BP1	PVY, PLRV, PVS	Not taken	PVY, PVS, PVX
Spunta	PVY, PLRV	PVY, PLRV, PVS	PVY, PVS, PVX
G2	PVY, PLRV	PVY, PLRV	PVY, PVS, PVX
G3	PVY, PLRV	PVY, PLRV, PVS, PVX	PVY, PVS, PVX

PVY = Potato virus Y
PLRV = Potato leafroll virus
PVS = Potato virus S
PVX = Potato virus X

e. Effect on parasitism

Fig. 13 shows the percentage parasitism of the potato leafminer in the two GMO treatments, G2 and G3 as well as the Spunta control and in a sprayed potato field nearby. The percentage parasitism for the Spunta and the two Spunta GMO's were around 30%, while the sprayed field were lower at 16%. It is clear that the GMO potato lines had no adverse affect on parasitism of the potato leafminer. The insecticide-treated field, however, reduced the percentage parasitism by about a half.

ROODEPLAAT Influence on *L. huidobrensis* Parasitoids

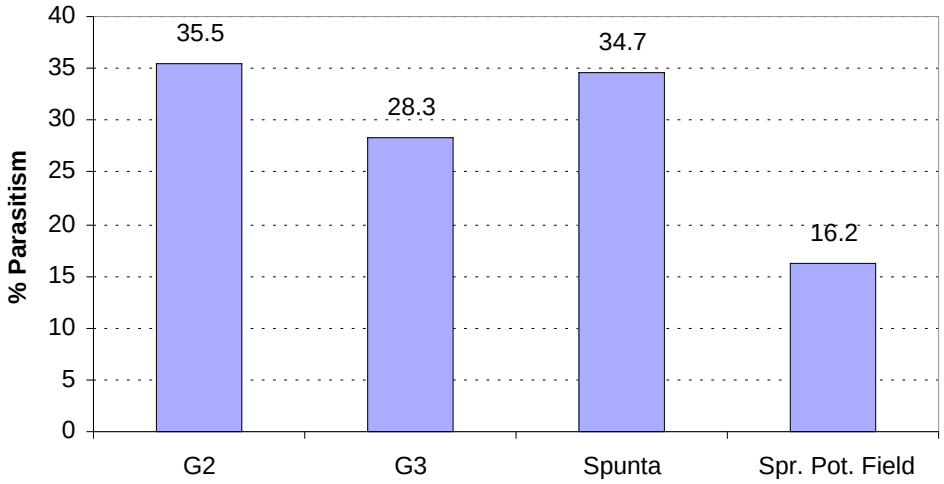
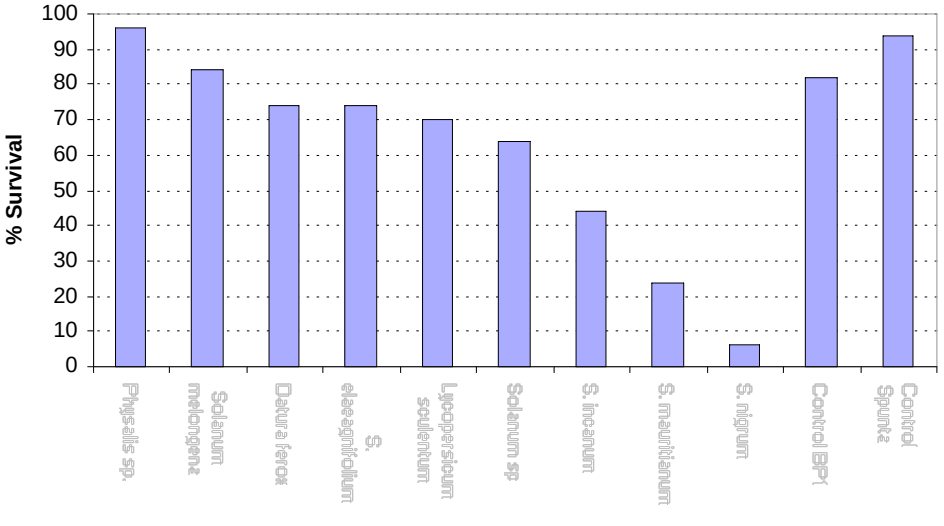


Figure. 13. The mean percentage parasitism (from 40 leaves) of the potato leafminer, *Liriomyza huidobrensis.* Spr. Pot. Fields = sprayed potato field (mixed cultivars and lines) adjacent (>100m) from the GMO field. Data is preliminary; only two repetitions were completed and only on the selected lines.

ROODEPLAAT Alternative hosts bioassays studies



Figu hed leaf bioassays. Data was taken at hour 72. Ten replicates with 50 larvae per replicate

f. Laboratory bioassays – Alternative hosts

Fig. 14 shows the survival of first instar tuber moth larvae on 11 different alternative hosts. The tuber moth could survive on all the alternative host plants tested. This shows that the introduction of GMO potatoes will not be responsible for the eradication of the tuber moth. There should thus be ample alternative host plants other than the potato on which the tuber moth can complete its life cycle.

g. Presence of other insects

The only other insect pest noted during the duration of the trial was the potato leafminer, *Liriomyza huidobrensis.* Effect on its parasitoids were noted above. However, there were no noticeable differences on the severity of attacks by this leafminer between any of the treatments. There were no other potato pests of note in the field during the duration of the trial.

h. Non-target arthropods

Tens of thousands of arthropods were caught in the pitfall traps and sweep net catches. The sorting, identifying and counting of these insects and spiders is a very laborious process that will take months to complete. Although this process is nearing its finishing point, at the time of writing this report it has not been completed. We will report on this data in our 2005 report.

7. Risk Management of the relevant GMO field trials

The risks involved with a field trial that incorporates genetically modified plants are higher than one without modified plants. The risks relating to a GMO field trial include the normal risks for any field trial, namely:

a) damage by animals

b) damage by hail, storms and frost

c) plant diseases

d) other pests destroying the plants

e) not high enough numbers of the tested insect

f) equipment malfunctioning

However, when working with GMO plants under field conditions, the following risks and precautions are added:

- theft under field growing conditions
- theft under storage conditions
- theft when moving potatoes between fields and storage
- accidental spillage
- the illegal movement of any plant material out of the trial environment
- escape of plants into the wild (e.g. cull potatoes)
- escape of genes through pollen transfer
- accidental mixing of lines/cultivars
- the proper movement of seed tubers between the field and storage
- proper notification that the trial contains potatoes not suitable for human consumption
- fencing off of the trial

The following can be reported for the three field trials.

All three fields were fenced off by wire fencing. The trial at Roodeplaat was incorporated in a 15 ha fenced (2 m high) experimental field while the Kokstad trial was secured by a new 10 feet security wire fencing. The Ceres trial had a normal 1,3 m wire fence.

Transportation of tubers from the field to storage was done in sealed bags. Bags were sealed with a wire sealer, the same type used on potato bags that is bought on the market. Only the designated responsible officers were allowed to open and handle the tubers.

Sign boards with warnings in three languages were fixed on fencing surrounding the fields and also next to the trial where applicable (Roodeplaat).

No plant material was allowed to be taken from the trial site during the season, except for experimental purposes. This was only done once when we removed 40 leaflets, taken from each treatment, for evaluation of parasitism of the potato leafminer (see above). These tests with the 40 leaflets were done in separate insect cages in a locked insectary room. After the tests, when the leaves were dried out, they were burned and destroyed.

Some tubers are left under the ground after harvest. It is impossible to remove all these cull potatoes during the harvest process – some always escape. However, cull potatoes will be managed by spraying them with herbicides once they emerged. The harvested fields will be inspected regularly for the emergence of cull potatoes.

Mixing of the different treatments when working with tubers is a high risk. Every step of the cultivation process (from preparing tubers, to planting, to growing, to harvest, to sorting, to storage) is vulnerable. To minimize the risk of mixing, extra precautions were taken. At planting time, each treatment was first planted before a next treatment was brought to the field. When crates were used to move the tubers to its relevant plots, it was ensured that these crates were not carried over the plots, but only moved along the paths. This ensured that no sprouts that broke off during the planting process could fall in a wrong plot. All plants that emerged in the paths later were removed and destroyed. When harvesting, it was ensured that no tubers from one plot get mixed with tubers from another plot. Paths were widened (from the previous field trial layout) and a one meter open row was inserted between treatments. While lifting, the tractor was also stopped between plots (in the open space/path) to ensure that all potatoes were first removed from the harvester chains (that might have got stuck). After putting the tubers in clearly marked bags (marked on the inside as well as on the outside), they were sealed until sorting. After sorting, each and every bag or crate into which the tubers were placed was clearly marked with the date, the treatment (line/cultivar), the tuber size and the repetition. Tubers were stored in locked cool storage facilities.

Destruction of left-over tubers

We are currently keeping all tubers harvested during the current season in cold storage. Once a decision has been made as to whether we need these tubers for any of the proposed plantings, the remainder will be destroyed. However the large quantity of tubers from these trials that may be destroyed makes the method of freezing and deep burial, as used previously, impractical. Thus it is recommended that this material be buried in a trench two meters deep and the site monitored for two years. According to Mr. Arno Visser (potato breeder and expert) no tuber will survive a burial of 2 meters.

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