STUDIES ON THE RESISTANCE OF WHEAT AND MAIZE TO FUNGAL PATHOGENESIS

by

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ABSTRACT

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17-Day-old seedlings of winter-wheat cultivar SST25 were inoculated with an avirulent race of wheat leaf rust, *Puccinia recondita* f.sp. *tritici*. After various time intervals the plants were reinoculated with a virulent wheat leaf rust race. No change in latent period or infection type was observed. However, the infection frequency was reduced by approximately 60 per cent.

The phytotoxic effects of three mycotoxins of *Fusarium* spp. (fumonisin B_1 (FB_1), moniliformin and T-2 toxin), and pathotoxin extracts of *Exserohilum turcicum* (HT-toxin) and *Stenocarpella macrospora* (SM-toxin) were studied using callus from the scutella of immature cobs of maize, *Zea mays*. The callus was grown on modified MS medium containing either 0, 0.1, 1.0, 10, or 100 mg (or ml) toxin per litre. For SM-toxin the concentrations used were 0, 0.01, 0.1, 1.0 or, 10 ml/l.

Mass increase of callus on medium containing FB_1, moniliformin, T-2 toxin, and HT-toxin decreased as the concentration of toxin increased, resulting in a significant reduction at the highest toxin level. SM-toxin caused a slight reduction in mass at 0.01 ml/l, but stimulated growth at 1.0 ml/l. At 10 ml/l a significantly lower callus mass increase was recorded.

Transmission electron microscopy studies of FB_1-treated callus showed an increased level of activity in the toxin-treated cells resulting in thicker cell walls, occurrence of starch grains and phenolic substances, when compared to the control. The mitochondria of callus cells were affected by SM-toxin, and starch was found in all toxin treatments.

When transferred to toxin-free medium after treatment with FB_1, a complete recovery of the callus occurred at all toxin levels but the highest, although regrowth occurred at this level. Callus treated with SM-toxin retained the same growth rate as during the toxin treatment, and it can be concluded that the toxin has a permanent effect on the growth rate of callus.

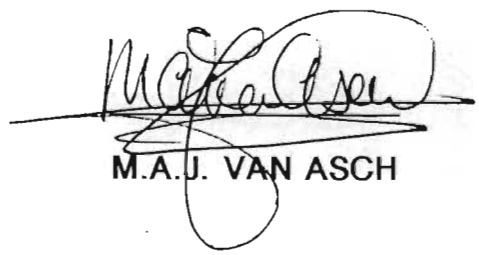
Maize seedling leaves, injected with a 10 g/l FB_1-solution at the stalk base, showed necrotic areas and chlorotic flecks. The toxin-treated plants were stunted and occasionally produced side shoots. *S. macrospora*-susceptible and -resistant seedlings, injected in a similar fashion with SM-toxin, gave a different response to the toxin. Susceptible plants were affected by the toxin, while no effects were observed in resistant plants.

PREFACE

The experimental work described in this thesis was carried out in the Department of Microbiology and Plant Pathology, University of Natal, Pietermaritzburg, under the supervision of Professor F.H.J. Rijkenberg.

All chapters have been prepared as for journal submission, and therefore some repetition was unavoidable.

I hereby declare that these studies represent original work by the author and have not been submitted in any form to another University. Where use was made of the work of others it has been duly acknowledged in the text.



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CONTENTS

CHAPTER 1

RESISTANCE INDUCED IN WHEAT, *TRITICUM AESTIVUM*, BY AN AVIRULENT RACE OF LEAF RUST, *PUCCINIA RECONDITA*, f.sp. *TRITICI*

INTRODUCTION

Since the work of Yarwood (1954), in which induced resistance by rust fungi was reported for the first time, it has become widely accepted that all pathogens, including rusts, are capable of inducing resistance. But, although the cereal - rust interaction is one of the most studied fields in plant pathology, relatively little work has been done on the induction of resistance by cereal rusts. Kochman & Brown (1975) used wheat leaf rust, *Puccinia recondita* Rob. ex Desm. f.sp. *tritici*, and wheat stem rust, *P. graminis* Pers. f.sp. *tritici*, both being non-host species, to induce resistance in oats, *Avena sativa* L., to the oat rusts, *P. coronata* Corda f.sp *avenae* and *P. graminis* f.sp. *avenae*. Results obtained by Johnson & Allen (1975) showed that resistance induced in wheat, *Triticum aestivum* L., by application of an avirulent race of *P. striiformis* West. can delay and reduce sporulation resulting from infection with a virulent race of the same rust. McRae & Brown (1983) found that resistance in wheat leaf segments to leaf and stem rust could be induced by previous inoculation with avirulent races of these fungi. A later study by Bahamish & Wood (1985) dealt with the induction of susceptibility to an avirulent race of *P. recondita* f.sp. *tritici* by a virulent race of the same rust in wheat.

The present research was conducted to determine the level of resistance to a virulent wheat leaf rust race, *P. recondita* f.sp. *tritici* race 3SA86, induced when wheat plants had been previously infected by an avirulent race (3SA126) of the same rust. Upon infection with the virulent race, the parameters used to assess possible induced resistance were latent period (LP), and infection frequency (IF), while the infection type (IT) was recorded to determine whether there was an effect on urediosorus size. Induced resistance was to be characterized by an increase

in LP, a decrease in IF, and a lower IT when challenged plants were subsequently infected with a virulent rust race.

MATERIALS AND METHODS

The spring-wheat cultivars Morocco and SST25, obtained from the Small Grain Centre, Bethlehem, South Africa, were selected for their specific reaction to the rust races used: 3SA86 and 3SA126. Morocco is susceptible to both rust races (reaction type 4), and SST25 gives a hypersensitive reaction (reaction type 0; 1$^{=}$) with rust race 3SA126 and a reaction type 4 infection with rust race 3SA86. This difference in resistance reaction is controlled by the *Lr24* gene. The rust races were bulked on Morocco plants.

Plants were grown in trays (26.5 x 18.5 x 6.5 cm). Each tray contained at least five Morocco and fifteen SST25 plants. One replication consisted of eight trays with plants consecutively inoculated with rust race 3SA126 and 3SA86, eight trays with plants inoculated with 3SA86 only, and, as control for infection with the first rust race, four trays with plants inoculated with 3SA126 only. Since the SST25 plants do not show any symptoms after inoculation with 3SA126, infection was recorded on the Morocco plants.

Secondary leaves of 17-day-old seedlings (growth stage 13 on the scale of Zadoks *et al.* (1974)) were inoculated on the upper leaf surface with a modified Andres and Wilcoxson inoculator (Crookes *et al.*, unpublished data). Leaves to be inoculated were affixed to a screen set at 20 cm from the inoculator orifice, horizontal speed was set at 9.0 and spray volume at 5.0. Per tray, approximately 2.5 mg rust spores was suspended in 0.8 ml of Soltrol® 170, which caused about 362 urediospores to be deposited per cm^2.

After inoculation, the plants were incubated for 12h in a mist chamber at 20 °C, where 100 per cent relative humidity and darkness ensured germination and penetration. Following incubation, the plants were transferred to a greenhouse

where the temperature ranged from 12 to 15 °C during the night and from 20 to 24 °C during the day.

For each replication, the viability of the spores applied was checked by spraying spores onto four water agar (2%) plates, of which two were incubated in a 20 °C incubator, while the remaining two were placed in the mist chamber with the inoculated trays. The percentage of germinated spores on the plates was microscopically determined.

The time intervals between the inoculation with the avirulent race (3SA126) and the virulent race (3SA86) were 1, 4, 7 and 10 days. The one and seven day interval were repeated twice, while the other tests were done once, since the results did not warrant a replication. The results obtained were analysed using the ANOVA statistical test.

The latent period (LP) was determined by counting daily the number of urediosori visible in a marked area on the leaves (using a 10 x pocket-lens) until the number of primary urediosori no longer increased. The time at which 50 per cent of the terminal number of urediosori had appeared, was estimated by interpolation. The LP was taken as the time period from the beginning of incubation to the time at which 50 per cent of the urediosori had appeared.

The infection frequency (IF) was measured using an aluminum sheet with a 2 x 0.5 cm window (Parlevliet & Kuiper, 1977). The metal sheet was randomly placed on the leaf over the inoculated area. The number of the urediosori within the window was divided by the number of rust spores applied per cm^2, corrected with a factor for germination percentage (as determined from water agar plates in the mist chamber), to give the IF. Infection types (IT) (Stakman *et al.*, 1962) were recorded 10 days post-inoculation.

To establish whether adult plants react similarly to seedlings, plants were grown to the flowering stage (stage 49-51 on the Zadoks *et al.* (1974) scale). At least three plants of Morocco and six plants of SST25 were inoculated on the adaxial surface

of the flag leaf, employing a 4-day-interval period, in a manner similar to that used for seedlings, and induced-resistance criteria were similarly assessed.

RESULTS

Latent Period. The LP of 50 per cent of the terminal number of primary urediosori for an infection with race 3SA86 was 210.6 $\pm$ 4.1 hours post-infection (hpi) on SST25 (Table 1). The average latent period of 3SA86 on leaves which had been inoculated previously with 3SA126 was 212.4 $\pm$ 6.9 hpi (Table 1). No statistical differences ($P < 0.05$) between the LP's at different time intervals were found for the single (3SA86 only) inoculation (Table 1). Statistical differences were found between the one-day-interval, and both the four-day and seven-day-interval of the double (both 3SA126 and 3SA86) inoculation (Table 1). The difference between the single and the double (both 3SA126 and 3SA86) inoculation was found to be 1.7 $\pm$ 3.1 hours on average (Table 1). However, the difference between the single and double inoculation was significantly different ($P < 0.05$) at four replications; once at an one-day-interval between inoculations, at both four-day-interval replications, and once at a seven day interval.
When the results are expressed as a percentage of the LP for 3SA86 only (Table 2), the low standard deviation indicates that no major differences existed between the time intervals.

Infection frequency. The average IF of inoculation with 3SA86 only was 20.5 $\pm$ 10.8 pustules per cm^2, for inoculation with 3SA126 only 22.7 $\pm$ 13.5 pustules/cm^2, and for double inoculation (3SA86 after inoculation with 3SA126) 11.7 $\pm$ 6.1 pustules/cm^2 (Table 3). No statistical differences ($P < 0.05$) of the IF between different time intervals were found for both inoculation with 3SA86 only, and the double inoculation. The IF of the inoculation with 3SA126 only showed significant differences ($P < 0.05$) between the one and the four day intervals (Table 3).

Compared with the inoculation with 3SA86 only, a decrease of the IF was found for

TABLE 1 Average LP values (in hours post-infection) at various time intervals between inoculation with an avirulent race (3SA126) and a virulent race (3SA86) of wheat leaf rust on leaves of winter-wheat cultivar SST 25 (see also APPENDIX 1.1)

TIME between consecutive inoculations	leaf	inoculation with both 3SA126 and 3SA86	inoculation with 3SA86 only	difference (hours)
1 day	second	202.4 a	203.1 a	- 0.7
1 day	second	205.0 a	208.4 a	- 3.4*
4 days	second	218.7 b	214.6 a	4.1*
4 days	flag	215.4 b	212.0 a	3.4*
7 days	second	213.3 b	211.8 a	1.5
7 days	second	221.2 b	215.2 a	6.0*
10 days	second	210.7 ab	209.4 a	1.3
average		212.4	210.6	1.7
standard deviation		6.9	4.1	3.0

Figures in a column followed by the same letter do not differ significantly ($P < 0.05$) in a LSD test

* difference significant in a LSD test ($P < 0.05$)

TABLE 2 Average LP values, expressed as a percentage of the LP for inoculation with 3SA86 only, at various time intervals between inoculation with an avirulent race (3SA126) and a virulent race (3SA86) of wheat leaf rust on leaves of winter-wheat cultivar SST 25

TIME between consecutive inoculations	leaf	inoculation with both 3SA126 and 3SA86	inoculation with 3SA86 only
1 day	second	99.0	100
1 day	second	98.4	100
4 days	second	101.9	100
4 days	flag	100.5	100
7 days	second	101.8	100
7 days	second	101.0	100
10 days	second	101.4	100
average		100.5	100
standard deviation		1.5	

the double inoculation at all time intervals, except for one replication of the one day interval, where the IF increased. All differences between the inoculation with 3SA86 only and the double inoculation were statistically significant ($P < 0.05$) at

TABLE 3 Average IF values (in pustules per cm^2) at various time intervals between inoculation with an avirulent wheat leaf rust race (3SA126) and a virulent wheat leaf rust race (3SA86) on leaves of winter-wheat cultivar SST 25 (see also APPENDIX 1.6)

TIME between consecutive inoculations	leaf	inoculation with		
		both 3SA126 and 3SA86	3SA86 only	3SA126 only on Morocco
1 day	second	2.5	2.3	0.5 a
1 day	second	15.6	17.9	14.0 a
4 days	second	16.5	26.6	33.9 b
4 days	flag	9.5	15.7	38.2 b
7 days	second	8.8	22.5	19.1 ab
7 days	second	20.7	37.6	15.9 ab
10 days	second	8.6	21.2	30.6 ab
average		11.7	20.5	21.7
standard deviation		6.1	10.8	13.2

Figures in a column followed by the same letter do not differ significantly ($P < 0.05$) in a LSD test

TABLE 4 Average IF, expressed as a percentage of the IF of an inoculation with 3SA86 only, at various time intervals between inoculation with an avirulent wheat leaf rust race (3SA126) and a virulent wheat leaf rust race (3SA86) on leaves of winter-wheat cultivar SST 25

TIME between consecutive inoculations	leaf	inoculation with	
		both 3SA126 and 3SA86	3SA86 only
1 day	second	108.7	100
1 day	second	82.2	100
4 days	second	62.3	100
4 days	flag	60.5	100
7 days	second	39.1	100
7 days	second	55.1	100
10 days	second	40.7	100
average		64.1	100
standard deviation		24.5	

each time interval.

The IF of the challenge inoculation (3SA126 only) was not correlated with the IF of the double inoculation (correlation coefficient (r); linear r = -0.38; multiplicative r

= -0.40). Significant differences between the IF of the challenge inoculum were not reflected in the IF of the double inoculation. This means that there is no evidence that the difference in IF between the single and the double inoculation is caused by the challenge inoculation.

The data of the average IF values, expressed as a percentage of the IF for 3SA86 only, indicate that the IF decreases as the time interval between inoculations increases (Table 4). However, this can not be supported statistically.

Infection type. No differences in the infection types of the different treatments were observed, nor was any zone of fungal inhibition noticed. Occasionally pustules were found on the edge of necrotic flecks caused by the inoculation with the avirulent race 3SA126.

DISCUSSION

Latent period. No major increase in LP could be demonstrated after a virulent race of wheat leaf rust was inoculated, at various time periods, after prior inoculation with an avirulent wheat leaf rust race. Significant increases in LP were found but were very small and may for all intents be disregarded as a calculation artefact.

Littlefield (1969) reported that pre-inoculation of flax, *Linum usitatissimum* L. with an avirulent race of *Melampsora lini* (Ehren.) Desm. caused a reduction in number, size and rate of development of virulent races of the fungus, and Johnson & Allen (1975) found that the onset of sporulation was delayed by seven days on seedlings which had been inoculated with a avirulent race of *P. striiformis* six days before inoculation with a virulent race. However, our data are in general agreement with those reported by both Cheung & Barber (1972), who used an avirulent race of *P. graminis* f.sp. *tritici* before inoculation with a virulent race of the same rust on wheat leaf pieces, and those of Kochman & Brown (1975), who used wheat rust, both *P. graminis* f.sp. *tritici* and *P. recondita* f.sp. *tritici*, as pre-inoculation for oat rust, both *P. coronata* f.sp. *avenae* and *P. graminis*

f.sp. *avenae*, on oats. These authors did not find any difference in the size or the rate of development, but only a reduction in the number of pustules per cm^2 leaf area.

The LP at the one-day-interval level was shorter than the LP of the control. Although this difference was not significant, it supports the findings of Bahamish & Wood (1985) that inoculation with an avirulent rust race, after previous inoculation with a virulent one, leads to the induction of susceptibility to the avirulent race, and to a lesser extent induction of resistance to the virulent rust race.

Infection frequency. In the present study a decrease in the IF was found at almost every time interval between the consecutive inoculations. A longer time span between successive inoculations seemed to further reduce the IF.

Cheung & Barber (1972) found a reduction of 80% in the number of pustules/cm^2 leaf area using two different races (inoculation with the avirulent prior to virulent) of stem rust, *P. graminis* f.sp. *tritici,* on wheat. In their research the time between the inoculations was three, or six days. Such a reduction was also noticed by Bahamish & Wood (1985), in conducting research on induced susceptibility in wheat to *P. recondita* f.sp. *tritici*, initially inoculated with a virulent race, followed by an avirulent race four days later. In the research of Kochman & Brown (1975) the challenge inoculation had no significant effect during the first two days after infection. The maximum effect was found four days after inoculation and the effect remained the same until the longest time interval (seven days) of their study. Johnson & Allen (1975) found a 70% reduction in total spore mass produced with a six day time interval between the successive inoculations. In the present study the IF was reduced by approximately 60%, with both a seven and a ten day interval between the inoculations.

The reduction in IF may result from killing or plugging of many stomata by the avirulent fungus (Johnston & Huffman, 1958). But since the number of stomata on the wheat-leaf surface is approximately 3000 per cm^2 (C.A. Crookes, unpublished data), and up to six appressoria can be found on one stoma (C.A. Crookes, unpublished data), it is unlikely that plugging of infection sites is the reason for the reduction in IF.

Diffusion of enzymes from germinating urediospores was suggested as the IF-reducing factor by Cheung & Barber (1972). They proposed that these enzymes, when produced by avirulent spores, may activate the synthesis of a resistance factor, which is also effective against virulent races of the pathogen. Even the presence of urediospore germination inhibitors, if capable of resisting the denaturing activity on the leaf surface for up to ten days, could be an explanation for the decrease in IF. However, this cannot explain the progressive decrease of IF with an increase of the time interval.

Both Johnson & Allen (1975) and McRae & Brown (1983) found that the induced resistance was systemic in the sense that it was expressed on the opposite leaf surface to that on which the inducer strain was inoculated. This indicated that a mechanism other than plugging or killing of stomata, and diffusion of enzymes from germinating urediospores played a role in the induction of resistance. However the mechanism involved was not discussed in these articles.

Kochman & Brown (1975) postulated that a toxic substance, such as a phytoalexin, produced as a reaction to pre-inoculation with an alien rust species or an avirulent race of the same rust species, might prevent development of the fungus beyond the appressorial stage. Another possible explanation for the decreased IF is that the penetrating avirulent propagule confers a resistance effect on surrounding mesophyll cells, rendering cells in the immediate vicinity resistant to subsequent infection. This would reduce the number of potential infection sites for the subsequently applied virulent race. The present authors believe that this resistance-inducing substance is far more localized in wheat than e.g. "the signal" in cucumber described by Dean & Kuc (1986).
Work by C.A. Crookes (unpublished data) indicated that *P. recondita* f.sp. *tritici* in a resistant wheat cultivar developed to the substomatal vesicle (ssv) or haustorium mother cell (hmc) stage before the development stopped. It may therefore be postulated that, after a certain number of propagules have reached the ssv/hmc stage, the resistance mechanism is activated to such an extent that development beyond the appressorium/infection peg stage, of subsequent infecting spores, is not possible.

On the basis of the work by Kuc (1983) and others, pre-inoculation with an avirulent race renders the plant more resistant to subsequent challenge by a virulent race. It is noteworthy that, in the experimental system used in the present study, such increased resistance, is only manifested in a lower number of pustules per cm^2 leaf area, and not by an increase in latent period or a change in infection type. It will be interesting to establish if this lack of response is encountered more widely in monocotyledonous taxa.

LITERATURE

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CHAPTER 2

EFFECTS OF MYCOTOXIN FUMONISIN B_1 ON GROWTH AND DEVELOPMENT OF MAIZE CALLUS

INTRODUCTION

Fusarium moniliforme Sheldon occurs world-wide on a great variety of plant hosts and is one of the most prevalent fungi associated with maize, *Zea mays* L., in most tropical and subtropical maize-producing areas of the world (Smith & Moss, 1985). Over the last decade an increase in the incidence of *F. moniliforme* in stored maize has been recorded in the U.S.A. (Richardson, 1986, cited by Holley *et al.*, 1989). This fungus is associated with human and animal dietary staples and has been suspected of being involved in diseases, e.g. human oesophageal cancer (Marasas *et al.*, 1981 & 1988a), since its original description in the previous century. *F. moniliforme* cultures have also been proven toxic to a wide variety of experimental animals (Marasas *et al.*, 1984). Although several mycotoxins are found in cultures of this fungus, only recently a new group of mycotoxins, the fumonisins, were isolated (Gelderblom *et al.*, 1988). Sydenham *et al.* (1990) reported the natural occurrence of fumonisin mycotoxins in mouldy maize, collected from an area of the Transkei, Southern Africa. It has been found that the major compound of this group of toxins, fumonisin B_1 (FB_1), can induce the symptoms of equine leukoencephalomalacia (LEM) (Marasas *et al.*, 1988b). This disease caused the deaths of hundreds of horses in the United States as recently as 1978-79 (Buck *et al.*, 1979, cited by Marasas *et al.*, 1984). Although FB_1 exhibits toxic effects in rats (Gelderblom *et al.*, 1988), nothing is known about the phytotoxicity of this metabolite.

The effect of FB_1 on callus cells was established by using different levels of the toxin in the culture medium of maize callus. Regrowth tests on toxin-free culture medium after six weeks of growth on FB_1-containing medium were done to measure the regrowing capacity of the treated callus. Transmission electron

microscopy (TEM) studies were conducted on the callus after six weeks of growth on the toxic culture medium, in order to study the damage at cellular level. Seedling tests were performed to observe the effect of the toxin on plants.

MATERIALS AND METHODS

Callus initiation. Maize callus was initiated from the scutella of immature embryos (Green & Phillips, 1975). From previous research (Hughes, 1984) it was known that the maize inbred line B14 was responsive to tissue culture, and its growing capacity over longer periods was excellent. However its shoot-forming capacity was poor (Hughes, 1984), and it has been established that this genotype is not suitable for regeneration (Van Asch, unpublished data). Since no better genotypes were available at the start of this research and regeneration was only a secondary objective, tests were performed with this inbred line.

The culture medium, as described by Green & Phillips (1975), contained the inorganic components of Murashige & Skoog (1962) medium, 7.7 mg L-glycine, 1.98 g L-asparagine, 1.3 mg niacin, 0.25 mg thiamine-HCl, 0.25 mg pyridoxine-HCl, 0.25 mg Ca-pantothenate, 20 g sucrose, 8 g agar, and 2.0 mg 2,4-D (all quantities given per litre medium). The pH was adjusted to 6.0 with 0.1 N NaOH before autoclaving at 115 °C (or 0.75 kgf/cm^2) for 15 minutes.

The cultures were grown in incubators at 26 °C with a 16 hour photoperiod. The callus was maintained by transferring small pieces of approximately 20 mg to fresh medium every 4-6 weeks.

Callus mass increase tests. For testing the effect of the mycotoxin on the mass increase of the callus, pre-weighed pieces of B14-maize callus (average approximately 0.14 gram), were placed on 6.5 ml of culture medium in flat-bottomed test tubes (100 mm x diam. 24 mm). Before autoclaving, either 0, 0.1, 1.0, 10.0 or 100.0 mg fumonisin B_1 (supplied by Prof. W.F.O. Marasas, Medical Research Council, Tygerberg, South Africa) per litre was added to the culture medium. FB_1 is heat stable (W.F.O. Marasas, pers. comm.). Per treatment, 49 pieces of callus were used and each treatment was repeated three times. The callus was incubated for

six weeks in an incubator at 26 °C with a 16-hour photoperiod. After this time, the callus pieces were weighed to determine the mass increase. After weighing, the pieces of callus were placed onto culture medium without FB_1 to observe regrowth. This experiment was replicated three times.

To establish the growth rate of callus during the toxin treatment, ten pieces of callus were randomly taken, at weekly intervals, weighed under sterile conditions, and returned to the culture medium. This proved to be a very laborious method, and the results obtained from the first replication did not warrant a continuation of this approach, therefore this determination was made once only.

Regrowth. After seven weeks of growth on FB_1-free medium, ten pieces of callus were selected randomly from each toxin level and weighed, to determine whether differences in regrowth rate existed. The regrowth rate was calculated by dividing the final callus mass, i.e. the mass at the end of the regrowth period, by the initial mass, i.e. the mass at the beginning of the regrowth experiment. The callus mass increase per day was also calculated.

Pieces of callus were photographed every two weeks to visualize differences in regrowth.

Transmission electron microscopy. Callus pieces, taken randomly from each treatment after the six weeks of exposure to toxin-containing medium, were fixed in a 3% glutaraldehyde in 0.05 M sodium cacodylate buffer (pH 6.8-7.2), washed twice in the same buffer, and post-fixed for 2 h in 2% buffered OsO_4. After washing in 0.05 M sodium cacodylate buffer (pH 6.8-7.2) twice, the material was dehydrated in an ethanol series and embedded in Spurr's epoxy medium (Spurr, 1969) under high vacuum. The specimen blocks were sectioned and the sections were stained for 10 minutes with 2% uranyl acetate, washed twice with double-distilled water, post-stained in lead citrate (Reynolds, 1963) for 10 min, and washed again in double-distilled water. Two or three blocks per treatment were sectioned for examination. The sections were viewed with a Jeol® 100 CX transmission electron microscope at 80 kV.

All measurements of cell components were taken from 10 randomly selected TEM contact-prints.

Seedling tests. The effect of the toxin was tested on ten 21-day-old maize seedlings of both inbred line I137TN WP87/17->19 (I137TN) and F2834T x B383Y 07/53x54 (F2834T) (supplied by Dr. H.O. Gevers, Summer Grain Sub Centre, Pietermaritzburg, South Africa). The plants were injected at the base of the stalk with 0.1 ml of either a 0.1 g/l or a 10 g/l FB_1 solution. Two sets of control plants were used; one set injected with deionised water at the stalk base, while the second set was left untreated. This experiment was replicated twice.

All seedling tests were performed under greenhouse conditions, and the plants were allowed to grow for four weeks after treatment. To establish the effect of the toxin, all plants were carefully examined for necrotic spots or lesions. The height of the plants, from stalk base to the tip of the longest leaf, was measured. Leaf pieces from the site of injection were prepared for transmission electron microscopy as described previously. After these assessments, the above-ground parts of the plants were dried in an oven at 100 °C for seven days, and the dry mass of the combined sample was recorded.

RESULTS

Callus mass increase tests. The data showed only a statistical mass increase difference ($P < 0.05$) between both the control and the 0.1 mg/l FB_1 concentration and the 100 mg FB_1 per litre treatment (Table 1). To eliminate differences between

TABLE 1 Average mass increase (g) of maize, *Zea mays*, callus grown for six weeks on culture medium (Green & Phillips, 1975) containing different amounts of fumonisin B_1 (see also APPENDIX 2.1)

concentration (mg/l)	replication 1	2	3	average
0 (control)	0.300 a	0.704 a	0.428 a	0.443 a
0.1	0.203 b	0.696 a	0.416 a	0.402 a
1.0	0.058 c	0.382 b	0.275 b	0.242 b
10	0.062 c	0.317 b	0.164 c	0.177 b
100	-0.027 d	0.078 c	0.055 d	0.035 c

Figures in a column followed by the same letter do not differ significantly ($P < 0.05$) in a LSD test

the replications, statistical analysis was done on the relative mass increase of the control. The mass increase of the callus differed significantly ($P < 0.05$) between the treatments with a low toxin concentration (0 and 0.1 mg FB_1/l), the intermediate concentrations (1.0 and 10 mg FB_1/l), and the highest concentration of 100 mg FB_1/l (Table 1 and Fig. 1).

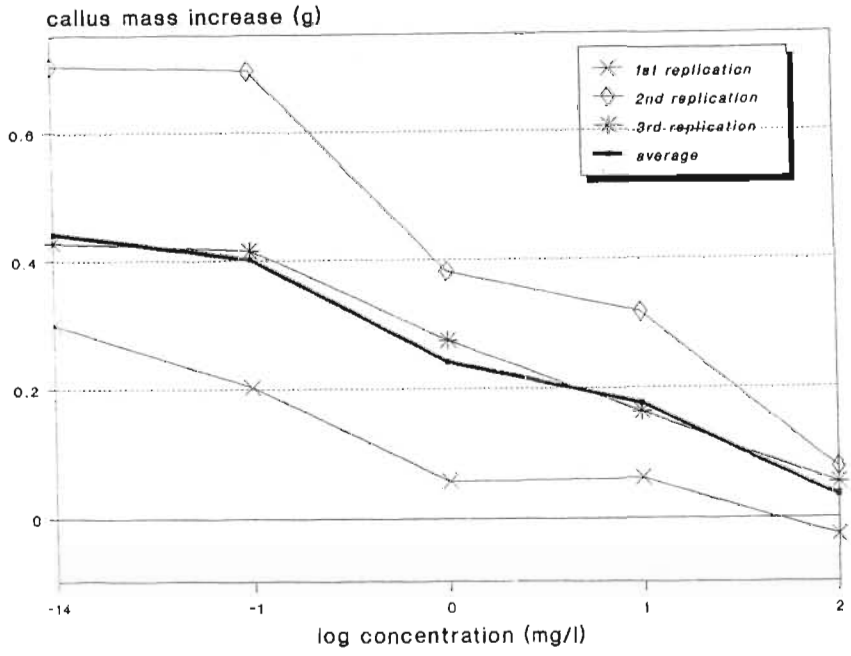


FIGURE 1 Average mass increase (g) of maize, *Zea mays*, callus grown for six weeks on culture medium (Green & Phillips, 1975) containing different amounts of fumonisin B_1 (see Table 1)

The trend of the mass increase of callus during the six weeks of the experiment was logarithmic for the control and the 0.1 mg/l concentration, while the curve of toxin-treated callus at all other concentrations of FB_1 showed a more linear trend (Fig. 2, see also APPENDIX 2.5).

After six weeks, the callus of the control treatment and that grown at the lowest FB_1 concentration of 0.1 mg/l looked very healthy and had increased considerably in size (Table 1, and column 1/row A and B resp. in Plate 1). The calli treated with the intermediate toxin levels (1.0 and 10 mg FB_1/l) were poorly developed, but had grown visibly (Table 1, and column 1/row C and D resp. in Plate 1). However, the callus treated with the highest toxin level (100 mg FB_1/l) had a brown colour and

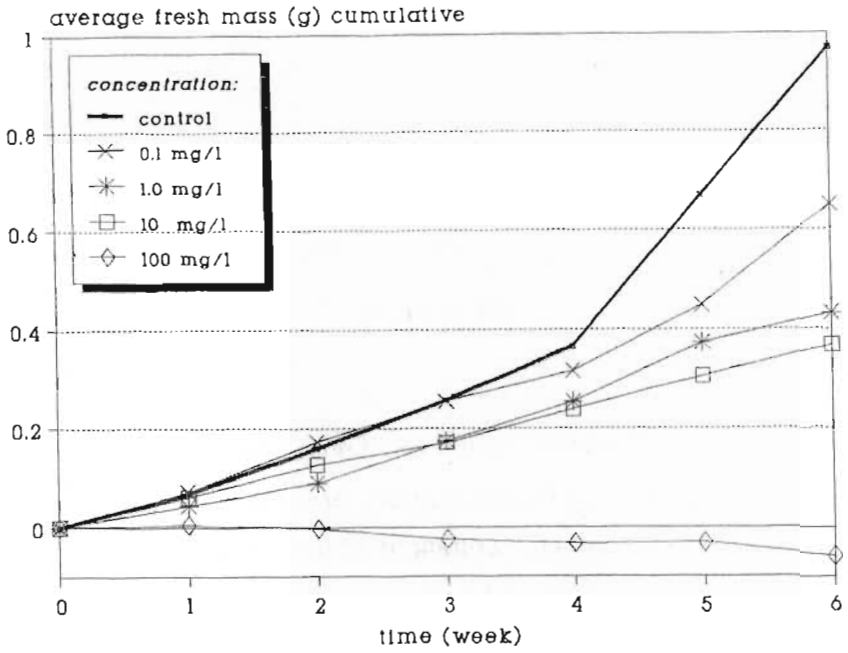


FIGURE 2 Cumulative average fresh mass increase (g) of maize, *Zea mays*, callus grown on culture medium (Green & Phillips, 1975) containing different amounts of fumonisin B_1 toxin over a six week interval

had not grown visibly (Table 1, and column 1/row E in Plate 1).

Regrowth. After the callus was transferred to fresh medium without FB_1, regrowth of calli from all FB_1 concentration levels occurred, although there was a visible difference between the size increase of callus derived from the highest toxin level (100 mg FB_1/l) and that from all other levels (Plate 1: column 4/row A to E). The growth rate at the intermediate levels (1.0 and 10 mg/l) was significantly ($P < 0.05$) greater than that of the control and the 100 mg FB_1/l concentration (week 6-14 in Table 2). However, no significant differences ($P < 0.05$) in growth rate over the duration of the callus mass increase and the callus regrowth experiments (week 0-14) were found between the control and any treatment, except the 100 mg per litre (Table 2, and columns 2 and 3/ row A to E in Plate 1). Although regrowth did occur at the highest toxin level, the callus remained significantly ($P < 0.05$) smaller at the end of the experiment.

PLATE 1

Regrowth over a six-week period (columns) of maize, *Zea mays*, callus pieces growing on culture medium (Green & Phillips, 1975) after treatment with different concentrations of fumonisin B_1 (FB_1) (rows) for six weeks (tube-width is 24 mm)

A. Control, no FB_1 added to the culture medium
B. 0.1 mg/l FB_1 in the culture medium
C. 1.0 mg/l FB_1 in the culture medium
D. 10 mg/l FB_1 in the culture medium
E. 100 mg/l FB_1 in the culture medium

1 Week 0; start of the experiment, the ending of the callus mass increase test
2 Week 2; two weeks after week 0
3 Week 4; two weeks after week 2
4 Week 6; two weeks after week 4

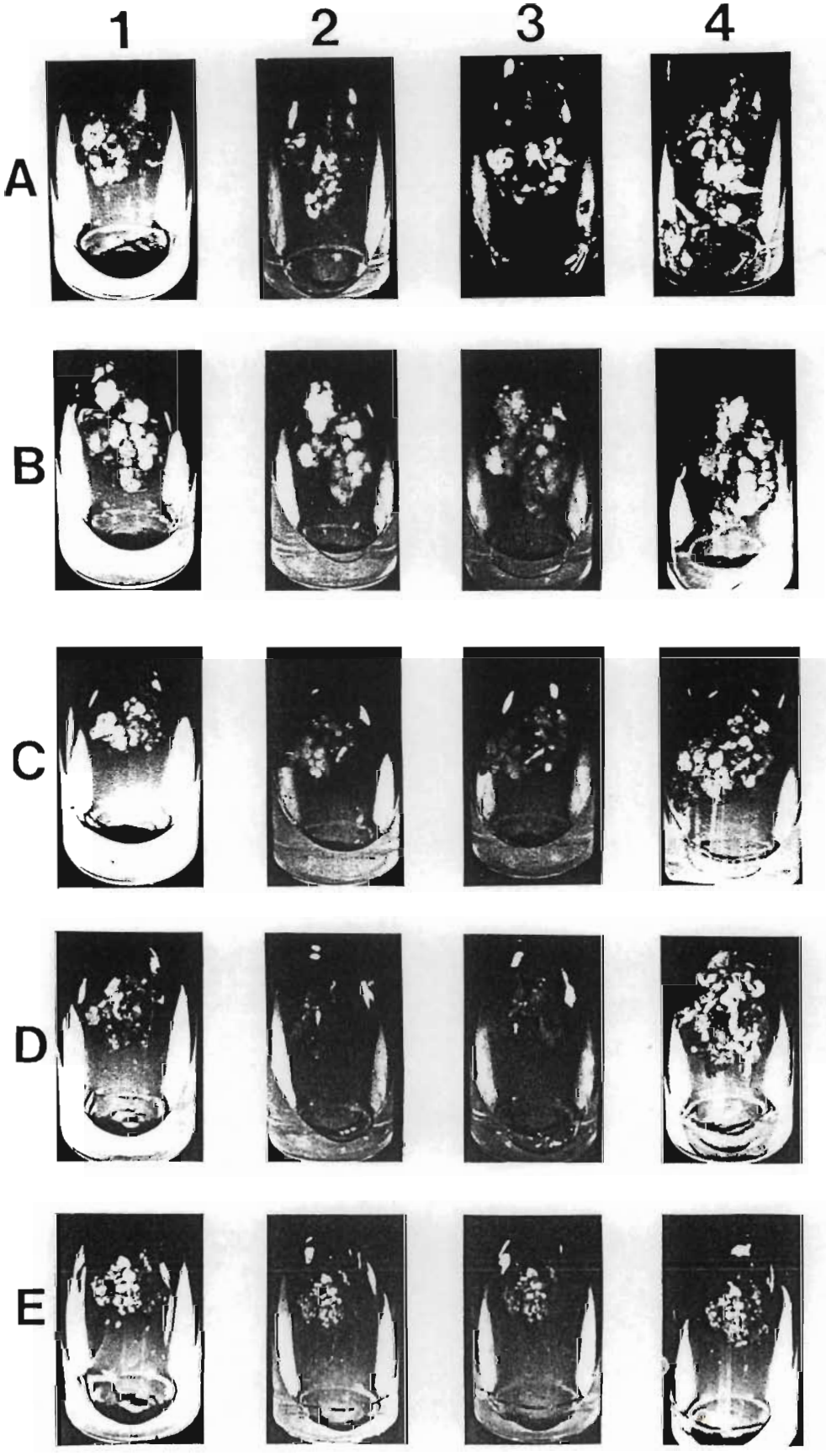

1
2
3
4
A
B
C
D
E

TABLE 2 Growth rate of maize, *Zea mays*, callus growing on culture medium, 1975) during (week 0-6) and after (week 6-14) treatment with different concentrations of FB_1 toxin (see also APPENDIX 2.6)

concentration (mg/l)	growth rate week 0- 6	week 6-14	week 0-14
0 (control)	4.93 a	1.97 a	9.66 a
0.1	3.79 b	2.22 ab	7.98 a
1.0	3.50 bc	2.80 bc	9.89 a
10	2.66 c	3.02 c	7.84 a
100	1.41 d	2.12 a	3.00 b

Figures in a column followed by the same letter do not differ significantly in a LSD test ($P < 0.05$)

The increase in mass per day over the regrowth period (week 6-14) showed significant differences between all treatments and the 100 mg/l (Table 3). If the mass increase per day is taken over both the toxin and toxin-free experiment (week 0-14 in Table 3), significant ($P < 0.05$) slower mass increase occurred at the 10 mg/l FB_1 treatment when compared with the lower toxin concentrations and the control, while the increase in mass at the 100 mg/l level was again significantly ($P < 0.05$) less than that at the 10 mg FB_1 per litre (Table 3).

TABLE 3 Average mass increase per day (g) of maize, *Zea mays*, callus (n = 10) growing on culture medium, during (week 0-6) and after (week 6-14) treatment with FB_1 toxin (See also APPENDIX 2.6)

concentration (mg/l)	mass increase (g per day) week 0- 6	week 6-14	week 0-14
0 (control)	0.014 a	0.011 a	0.012 a
0.1	0.011 b	0.010 a	0.010 ab
1.0	0.008 c	0.013 a	0.011 a
10	0.005 c	0.010 a	0.008 b
100	0.001 d	0.004 b	0.003 c

Figures in a column followed by the same letter do not differ significantly in a LSD test ($P < 0.05$)

Transmission electron microscopy. The TEM study of the increasing concentrations of FB_1 toxin compared to the control revealed that callus cells responded to the presence of the toxin by an increase of cell activity, the formation of phenolics, lipid bodies and starch grains, and an increase in cell wall thickness.

The structure of the cytoplasm became more granular with the increase in toxin concentration.

The cells of the control show large vacuoles (Plate 2, Fig. 1), mitochondria (Plate 2, Fig. 2) and nuclei with nucleoli and complete nuclear envelopes (Plate 2, Fig. 2). Proplastids are present but small (approx. 0.6μm) (Plate 2, Fig. 3), and occasionally chloroplasts were observed. The average thickness of the cell wall is 0.11 $\pm$ 0.02 μm (Table 4).

No differences between the 0.1 mg/l concentration (Plate 3, Figs. 2 and 3)) and the control were observed. The cell wall thickness was 0.18 $\pm$ 0.02 μm, but this change was not significant ($P < 0.05$) (Table 4). The chloroplasts in Plate 3 (Fig. 1) are a result of the developmental stage of the callus, and have also been observed in the control.

TABLE 4 Average cell wall thickness (in um) of maize, *Zea mays*, callus grown for six weeks on culture medium containing different amounts of fumonisin B_1 (see also APPENDIX 2.8)

concentration (mg/l)	cell wall (μm)
0 (control)	0.11 a
0.1	0.18 ab
1.0	0.23 bc
10	0.31 c
100	0.52 d

Figures followed by the same letter do not differ significantly in a LSD test ($P < 0.05$)

At the 1.0 mg/l level, lipid bodies in the cytoplasm (Plate 4, Figs. 1 and 2) and phenolic substances in the vacuoles (Plate 4, Fig. 3) were apparent when compared with the control. The phenolic products were not observed in any of the other treatments. The proplastids are enlarged (to about 2.5 μm) (Plate 4, Fig. 2). In the proplastids some stromal lamellae are visible, this might indicate a transformation of the proplastids into chloroplasts (Plate 4, Fig. 2). These structures can also be observed in the proplastids of the control (Plate 7, Fig. 1). The cytoplasm structure of the cell in Fig. 2 (Plate 4) is clumping and this might indicate that the cell is dead. Cell wall thickness was increased to 0.23 $\pm$ 0.03 μm (Table 4), which is a significant ($P < 0.05$) increase compared to the control.

Lipid bodies were still present at the 10 mg FB_1 per litre concentration (Plate 5), but were not as numerous as in the 1.0 mg/l treatment (Plate 5, Fig. 2). Large numbers of starch grains were observed in proplastids (amyloplasts), which had

enlarged to about 3.0 μm (Plate 5, Figs. 2 and 3). The cytoplasm of the cell in Figure 1 (Plate 5) is very granular and this might indicate cell death. The cell wall thickness measured was 0.31 ± 0.05 μm (Table 4), which is a significant ($P < 0.05$) increase compared to control and 0.1 mg/l treatment.

The highest FB_1 concentration (100 mg/l) showed an abundance of rough endoplasmic reticulum (ER) (Plate 6, Fig. 1) and membrane structures (Plate 6, Fig. 2), a break-down of the cytoplasm (Plate 6, Fig. 3), enlarged amyloplasts (approx. 3.0 μm) with many starch grains (Plate 6, Figs. 1, 2 and 3), and splitting of the nuclear membrane (Plate 6, Fig. 3). The structure of the cytoplasm in Plate 6 (Figs. 2 and 3) indicates that the cells are dead. The cell wall thickness was about 0.52 ± 0.06 μm (Table 4), which is a significant increase ($P < 0.05$) compared to all other treatments. This difference in cell wall thickness between the control and the 100 mg/l FB_1-concentration is illustrated in Plate 7 (Figs. 1 and 2 resp.). No differences were noticed in the structure of the mitochondria, nor the nucleus.

Seedling tests. No significant difference ($P < 0.05$) between the two lines, I137TN and F2834T, was found in either the height or the dry mass of the above-ground parts of the plants, in any of the test or replications (see APPENDIX 2.10). All the data of the two lines have therefore been pooled into one group.

In the water-injected control plants, lesions surrounded by a small necrotic area appeared at the site of injection (Plate 8, Fig. 4). No other effects were noticed. The seedlings injected with 10 g FB_1/l solution often showed an extended necrotic area around the site of injection and chlorotic flecking in other parts of the leaf (Plate 8, Fig. 4). The lower concentration of 0.1 g FB_1/l did not show such a distinct reaction.

No significant ($P < 0.05$) height differences were found between the untreated (55.8 ± 7.8 cm) and the water-injected control plants (53.7 ± 8.8 cm) (Table 5). The toxin-treated plants were significantly ($P < 0.05$) shorter (39.4 ± 10.6 cm for the 0.1 g/l solution and 36.0 ± 7.6 cm for the 10 g/l solution) than both controls (Table 5), but only a slight concentration effect was observed (not statistically significant). Toxin-injected plants had roughly the same number of leaves as the control plants, and the stunting seemed to be due to a failure of the stalk to

TABLE 5 Average length (cm) of maize, *Zea mays*, seedlings, four weeks after injection with 0.1 ml of a fumonisin B, solution at the base of the stalk at 21 days (see also APPENDIX 2.9)

treatment		replication 1	2	average
FB1	0.1 g/l	39.1 a	40.1 a	39.4 a
	10 g/l	34.4 a	38.6 a	36.0 a
control (water)		52.9 b	55.3 b	53.7 b
control (untreated)		54.0 b	59.0 b	55.8 b

Figures in a column followed by the same letter do not differ significantly in a LSD test ($P < 0.05$)

TABLE 6 Average dry mass (g) of the above-ground parts of maize, *Zea mays*, seedlings, four weeks after injection with 0.1 ml of a fumonisin B, solution at the base of the stalk at 21 days (see also APPENDIX 2.9)

treatment		replication 1	2	average
FB1	0.1 g/l	0.24 a	0.41 a	0.30 a
	10 g/l	0.27 a	0.34 a	0.30 a
control (water)		0.41 b	0.59 b	0.47 b
control (untreated)		0.37 b	0.63 b	0.47 b

Figures in a column followed by the same letter do not differ significantly in a LSD test ($P < 0.05$)

elongate (Plate 8, Figs. 1 and 3). Side shoots were formed at the base of the stalk of treated plants (Plate 8, Fig. 2). This occurred at both the 0.1 and the 10 gram per litre treatments with approximately a fifth of the plants.

Significant mass differences ($P < 0.05$) were found between the controls and toxin-treated plants at all concentrations of the toxin (Table 6), but no significant ($P < 0.05$) differences were recorded between the two control or the two toxin-injected treatments.

No differences were found in the ultrastructure of the leaves between toxin-injected and control seedlings. There was a slight indication that the toxin has an

PLATE 2

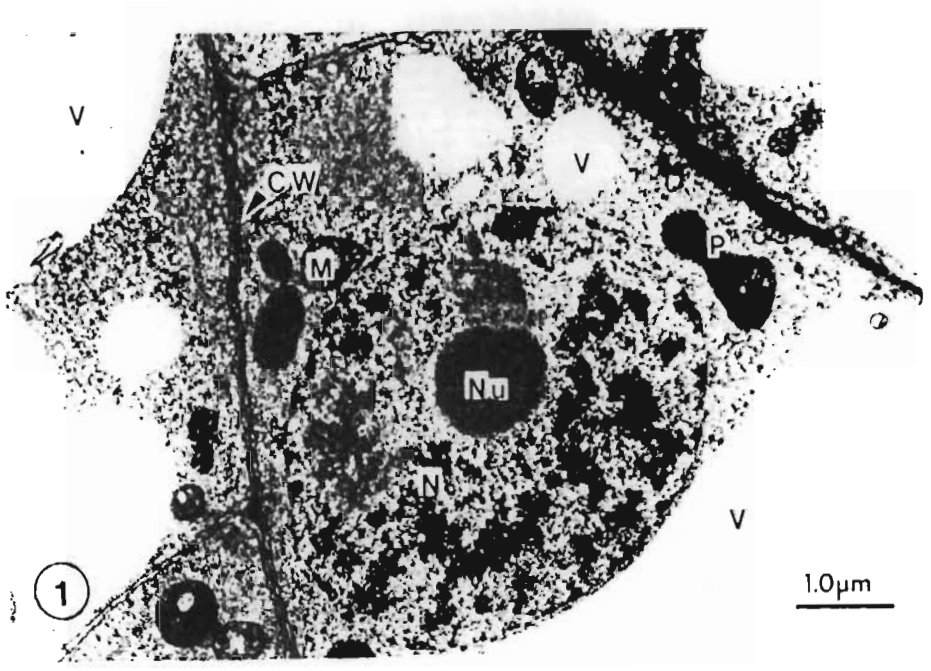
Transmission electron micrographs of callus cells of maize, *Zea mays*, grown for six weeks on culture medium (Green & Phillips, 1975) without fumonisin B_1 (control)

FIGURE 1 Cells showing a smooth cytoplasm, a nucleus with nucleolus, and large vacuoles

FIGURE 2 Cell with a nucleus with nucleolus and intact nuclear membrane, and mitochondria

FIGURE 3 Cells with nuclei and proplastids

CW = Cell Wall
Go = Golgi system
M = Mitochondrion
N = Nucleus
NE = Nuclear Envelope
Nu = Nucleolus
P = Proplastid
V = Vacuole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CW
V
M
P
Nu
N
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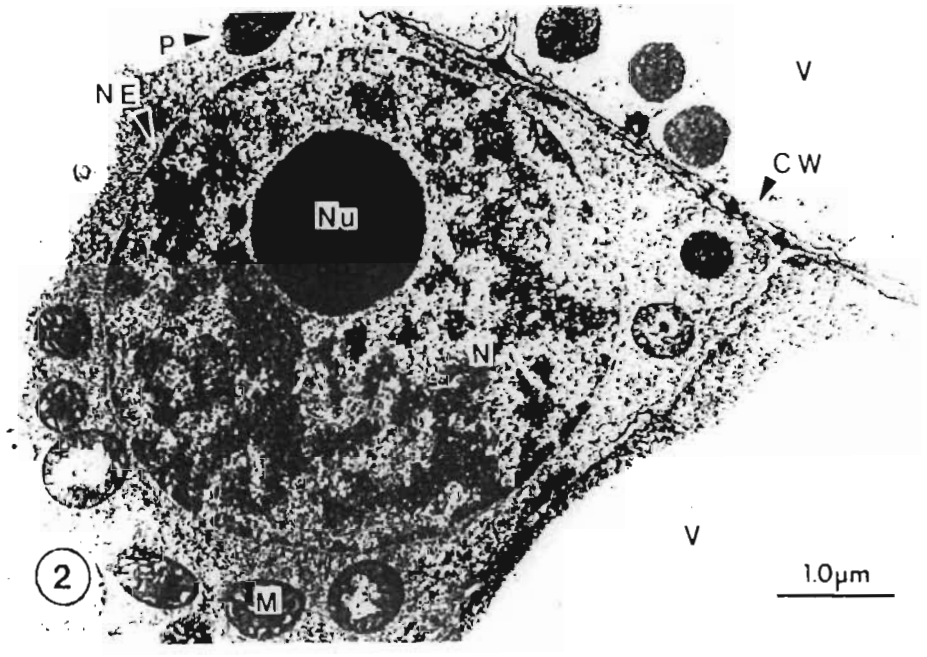
1
1.0μm


P
NE
V
CW
Nu
N
V
2
M
1.0μm

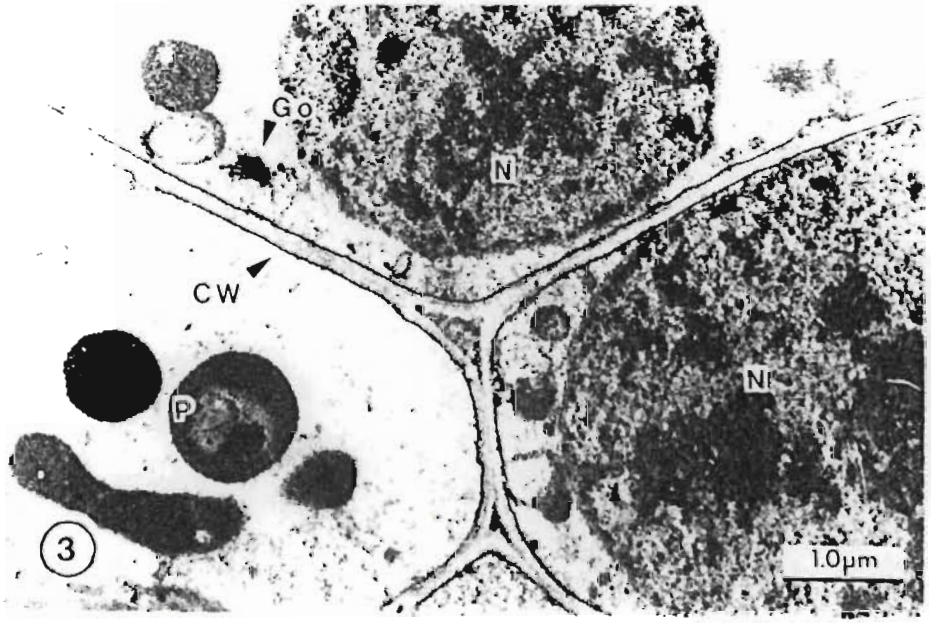

Go
N
CW
N
P
3
1.0μm

PLATE 3

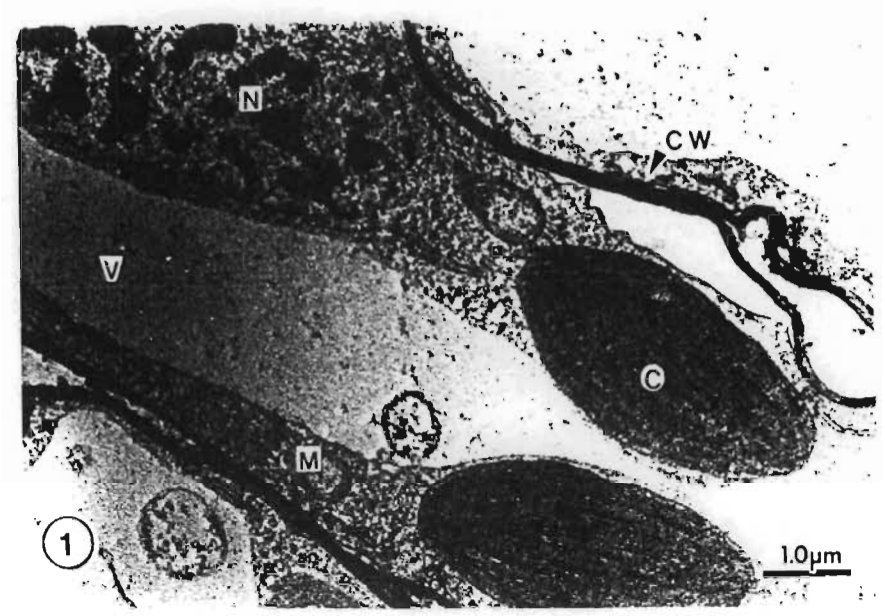
Transmission electron micrographs of callus cells of maize, *Zea mays*, grown for six weeks on culture medium (Green & Phillips, 1975) containing 0.1 mg/l fumonisin B_1. Please note that no difference in appearance with the control cells exists

FIGURE 1 Cell showing two chloroplasts and large vacuoles

FIGURE 2 Cell showing a nucleus, mitochondria and proplastids

FIGURE 3 Cells showing nuclei and large vacuoles

C = Chloroplast
CW = Cell Wall
M = Mitochondrion
N = Nucleus
P = Proplastid
V = Vacuole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CW
V
C
M
1
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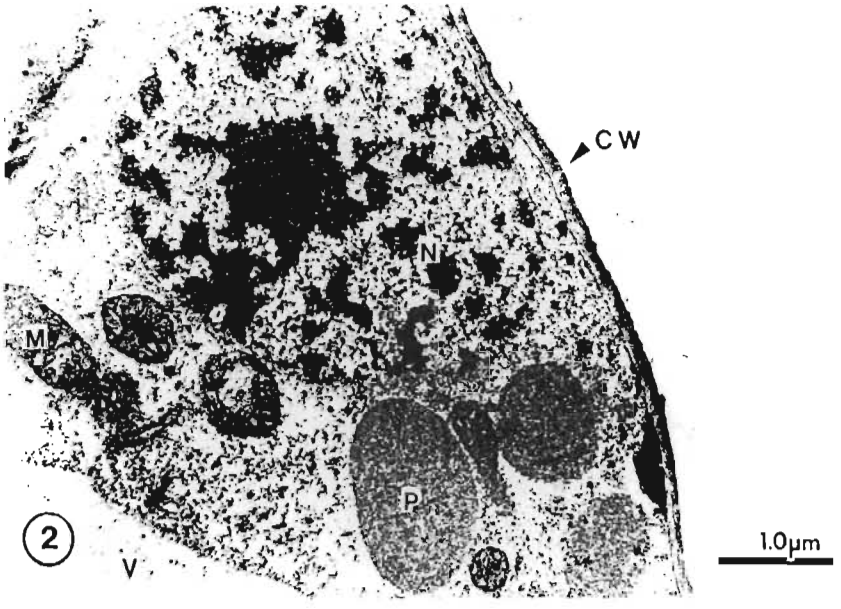
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M
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2
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1.0μm

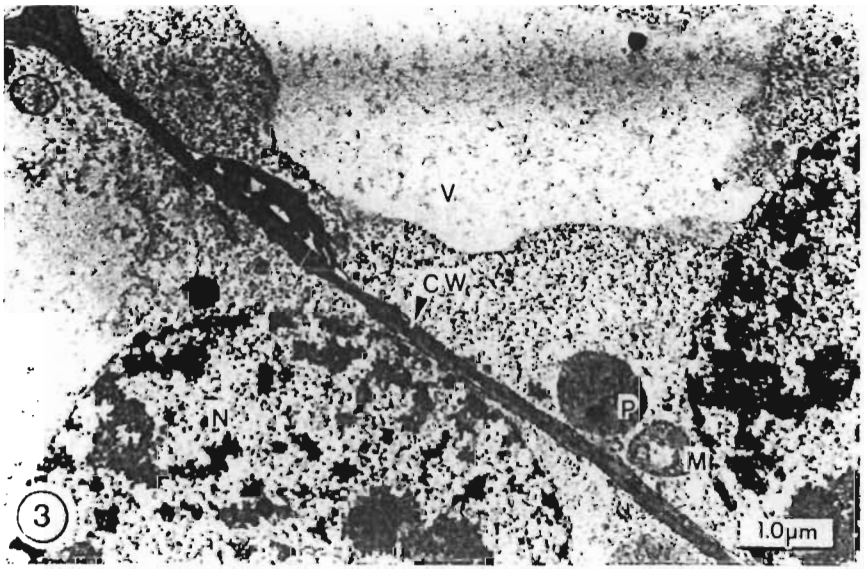

V
CW
P
N
M
3
1.0μm

PLATE 4

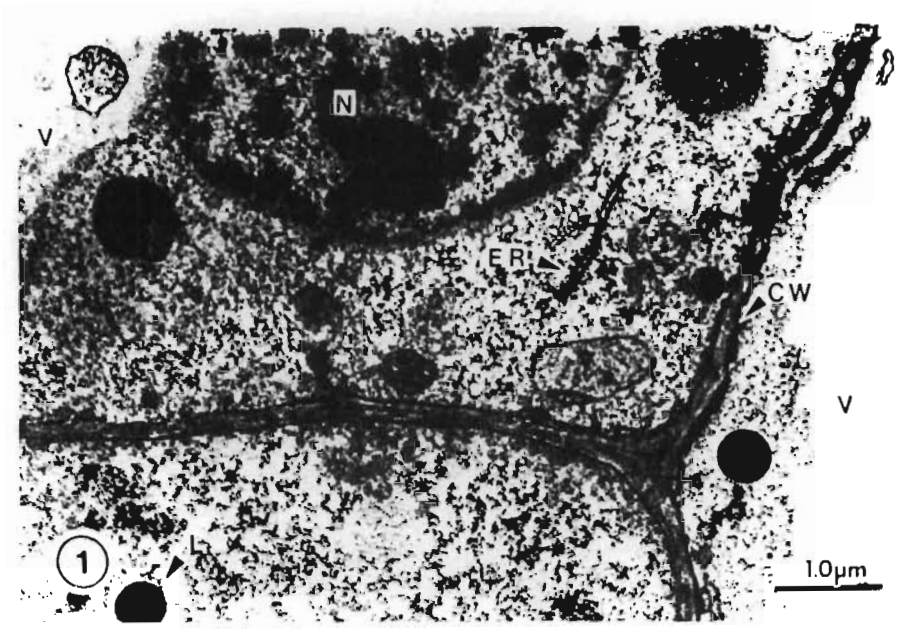
Transmission electron micrographs of callus cells of maize, *Zea mays*, grown for six weeks on culture medium (Green & Phillips, 1975) containing 1.0 mg/l fumonisin B,

FIGURE 1 Cell showing a nucleus with chromatin, lipid bodies and endoplasmic reticulum. Please note the lipid bodies at the cell wall/ plasmalemma edge

FIGURE 2 Cell showing enlarged proplastid with strains of stromal lamellae. Please note the clumping of the cytoplasm which might indicate that this cell is dead

FIGURE 3 Cell with large vacuole containing phenolic substances

CW = Cell Wall
ER = Endoplasmic Reticulum
L = Lipid body
M = Mitochondrion
N = Nucleus
P = Proplastid
Ph = Phenolic substance
V = Vacuole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V
ER
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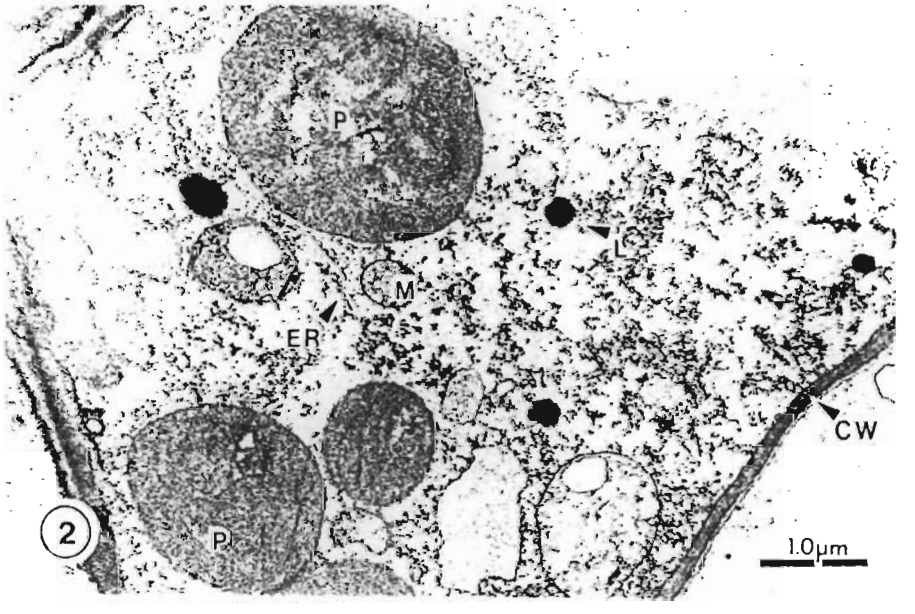
V
L
1
1.0μm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ER
CW
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2
1.0μm

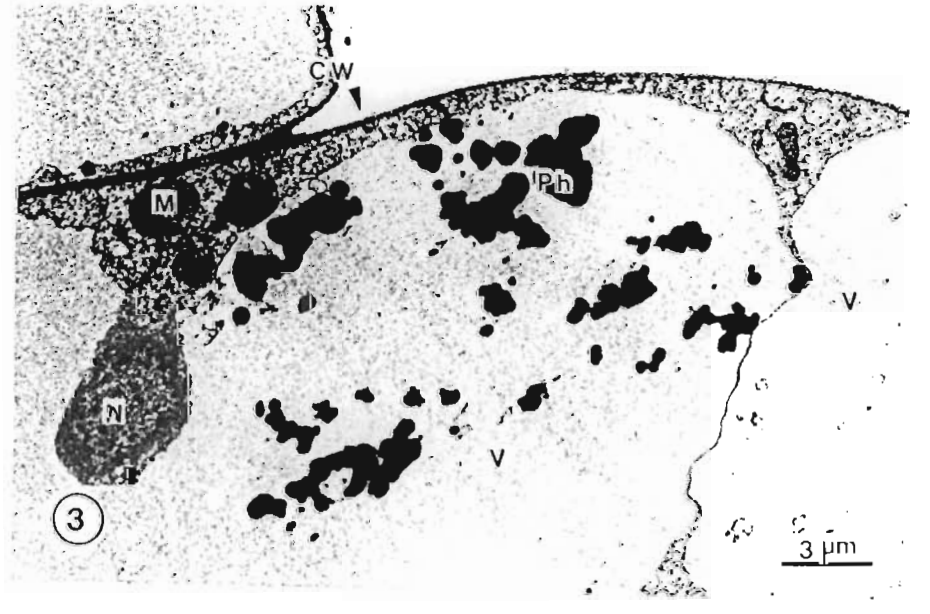

CW
M
Ph
V
N
V
3
3 μm

PLATE 5

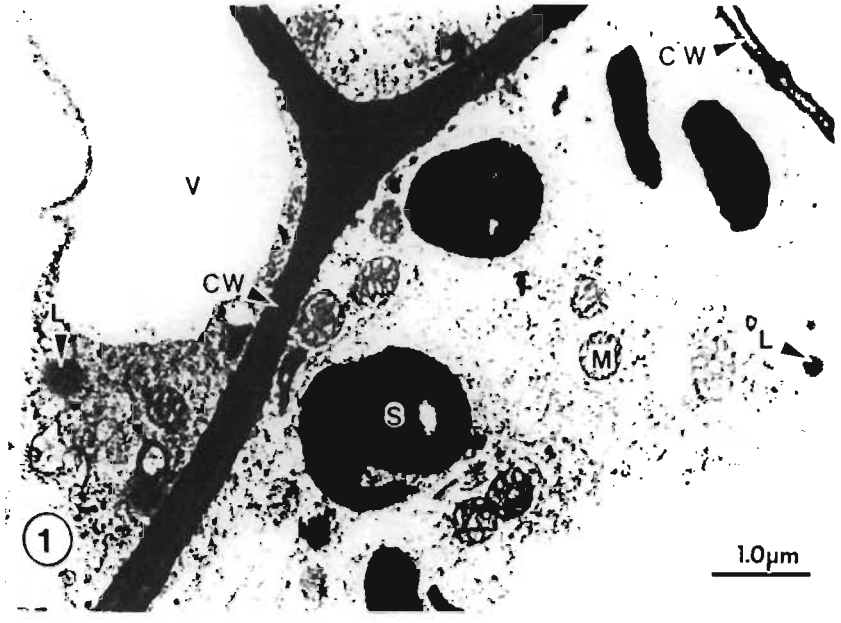
Transmission electron micrographs of callus cells of maize, *Zea mays*, grown for six weeks on culture medium (Green & Phillips, 1975) containing 10 mg/l fumonisin B_1

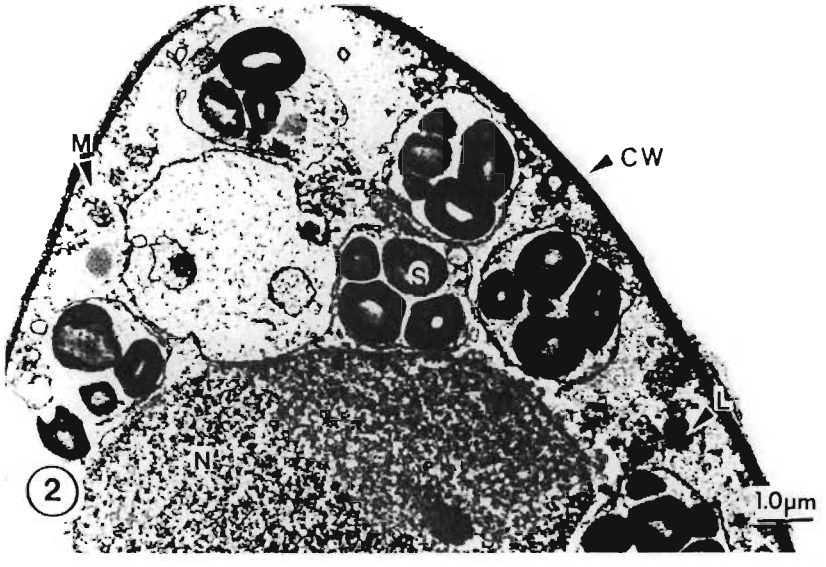
FIGURE 1 Cells showing amyloplasts with starch grains, mitochondria, lipid bodies, and large vacuoles

FIGURE 2 Cell showing a nucleus, amyloplasts filled with large amounts of starch grains, and lipid bodies

FIGURE 3 Cell showing large amounts of starch grains

CW = Cell Wall
L = Lipid body
M = Mitochondrion
N = Nucleus
S = Starch (in amyloplast)
V = Vacuole


CW
V
CW
L
M
L
S
1
1.0μm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CW
S
L
N
2
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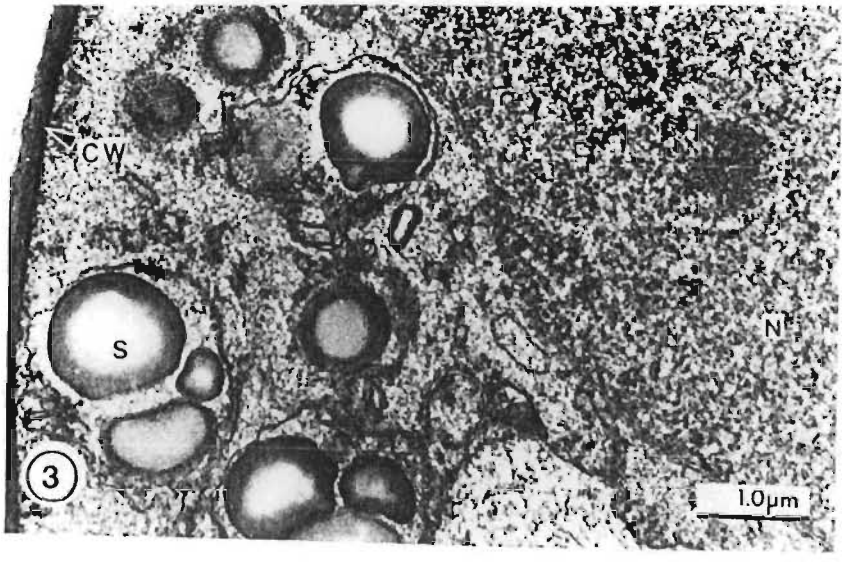

CW
S
N
3
1.0μm

PLATE 6

Transmission electron micrographs of callus cells of maize, *Zea mays*, grown for six weeks on culture medium (Green & Phillips, 1975) containing 100 mg/l fumonisin B_1. Please note the enlarged cell walls and the absence of the granular cytoplasm

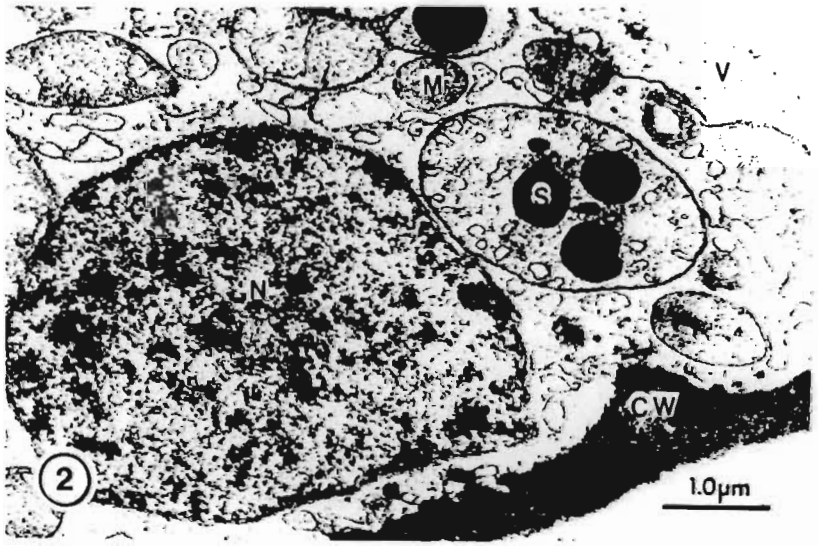
FIGURE 1 Cell showing a nucleus, amyloplasts, a mitochondrion, and large amounts of endoplasmic reticulum and ribosomes

FIGURE 2 Cell showing a nucleus, amyloplasts, and membrane structures

FIGURE 3 Cell showing a nucleus, amyloplasts and mitochondria. Please note the splitting of the nuclear membrane (arrows), and the total lack of cytoplasm

CW = Cell Wall
ER = Endoplasmic Reticulum
Go = Golgi system
M = Mitochondrion
N = Nucleus
S = Starch (in amyloplast)
V = Vacuole


M
CW
N
Go
ER
S
1
1.0μm


M
V
S
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CW
2
1.0μm

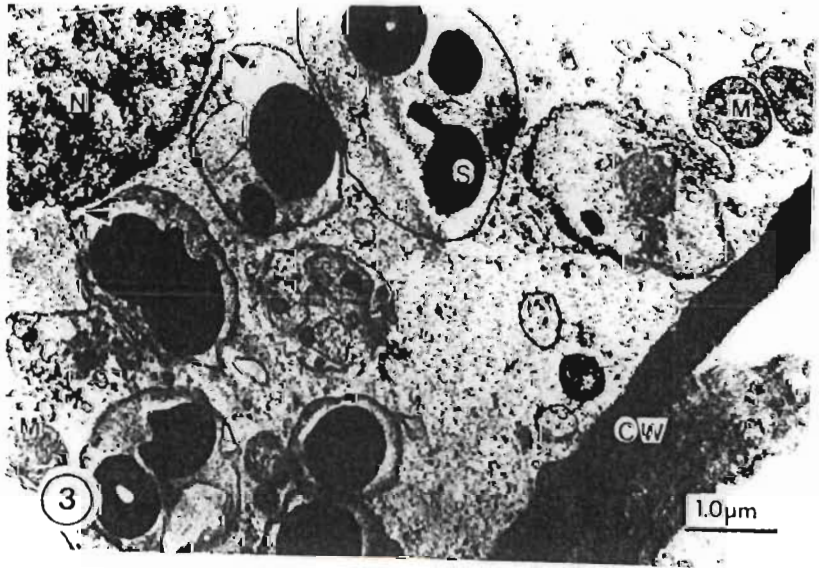

N
S
M
M
CW
3
1.0μm

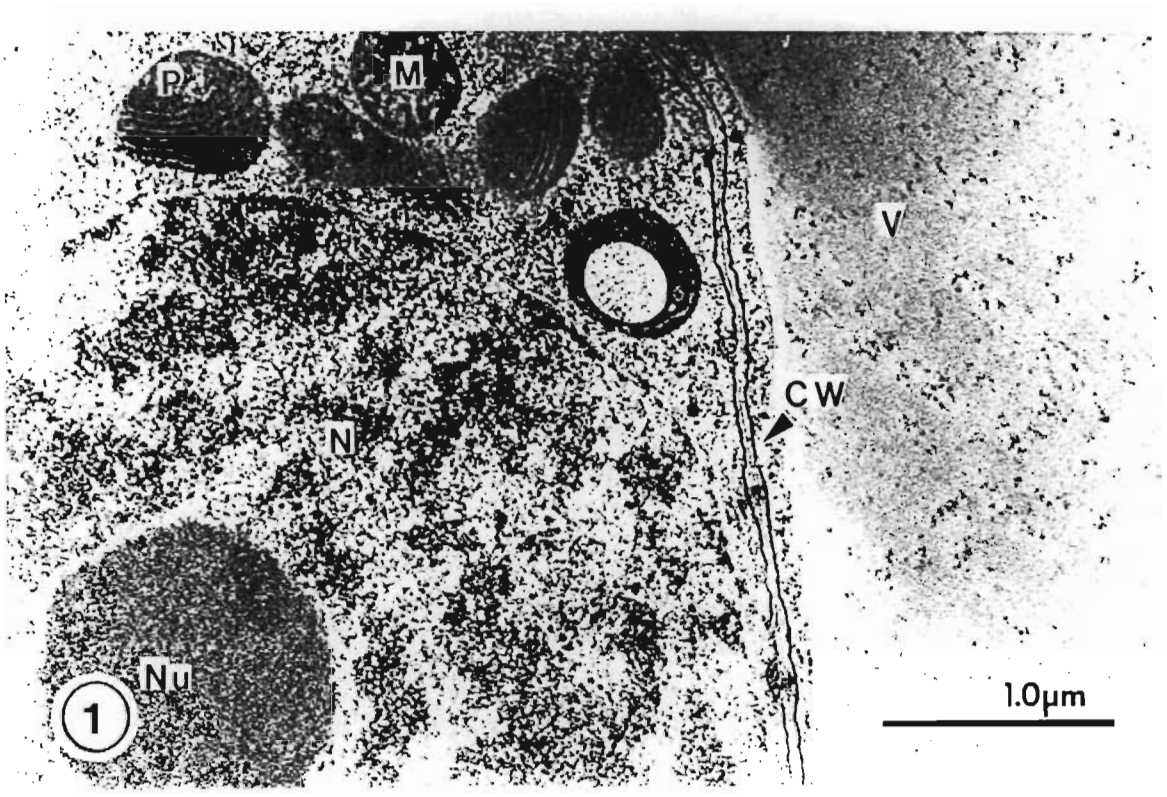
PLATE 7

Transmission electron micrographs of callus cells of maize, *Zea mays*, grown for six weeks on culture medium (Green & Phillips, 1975) containing no fumonisin B_1 (**FIGURE 1**) and 100 mg/l fumonisin B_1 (**FIGURE 2**). Please note the cell wall difference between the two treatments (both micrographs have the same magnification)

FIGURE 1 Cell showing a nucleus with nucleolus, a mitochondrion, and proplastids with stromal lamellae. Please note the large vacuole of the adjacent cell

FIGURE 2 Cell showing a nucleus and an amyloplast. Please note the complete degradation of the cytoplasm and the abundance of membrane structure

CW = Cell Wall
M = Mitochondrion
N = Nucleus
Nu = Nucleolus
P = Proplastid
S = Starch (in amyloplast)
V = Vacuole


P
M
V
CW
N
Nu
1
1.0μm

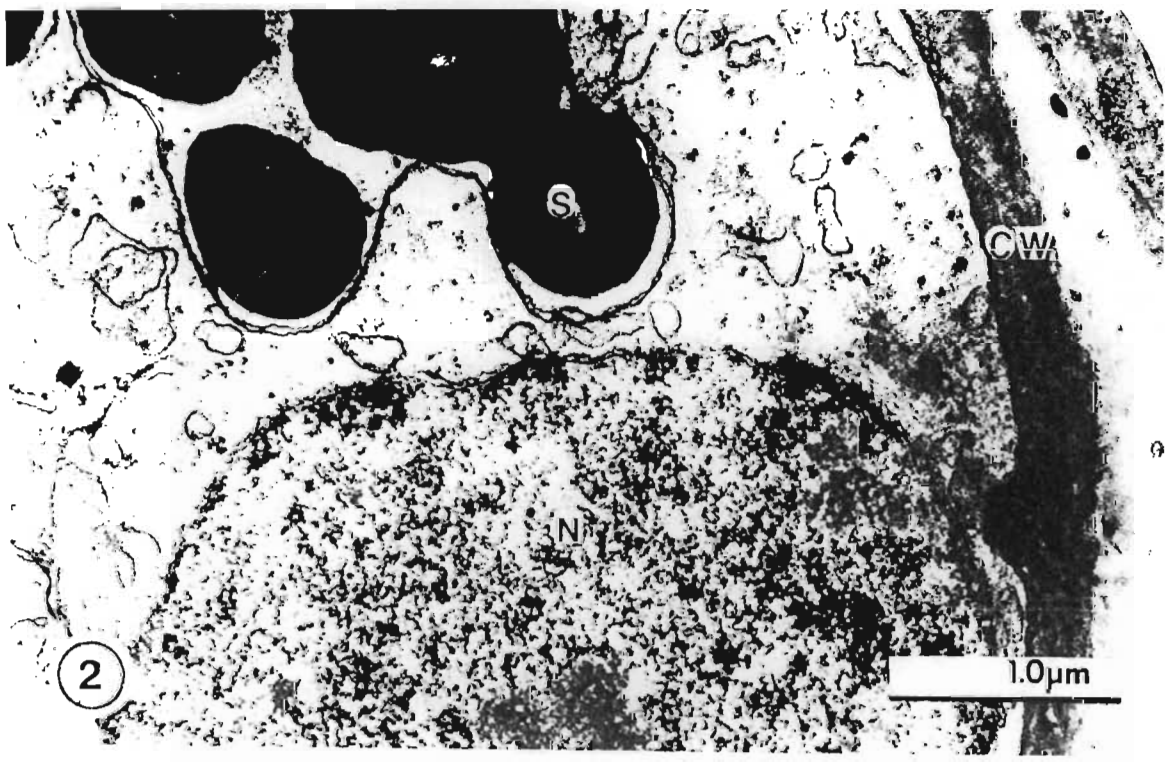

S
CW
N
2
1.0μm

PLATE 8

Photographs of seven-week-old maize, *Zea mays*, seedlings injected with 0.1 ml of a 10 g/l fumonisin B_1 solution, or deionised water, at the base of the stalk at 21 days

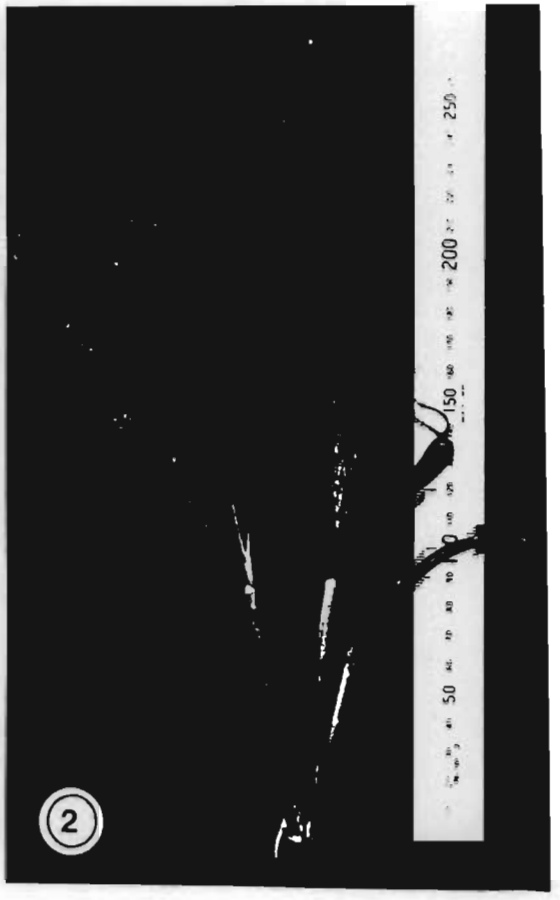
FIGURE 1 Comparison of two seedlings; a water-injected control plant (left) and a toxin-injected plant (right). Please note the difference in length and leaf width.

FIGURE 2 Formation of side shoots at the stalk base of a toxin-treated seedling

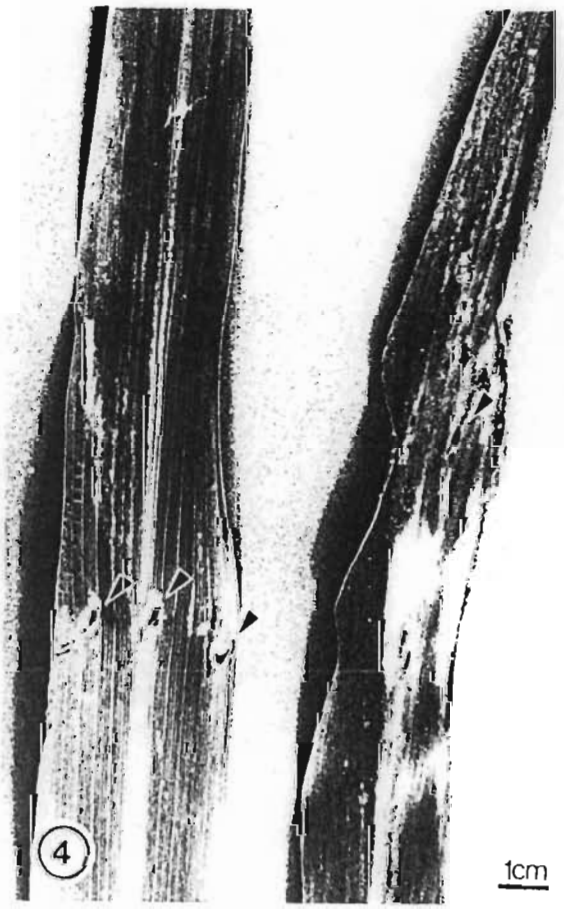
FIGURE 3 Comparison of the stalk base of two seedlings; a toxin-injected plant (left) and a water-injected control plant (right). Please note the internodal stunting of the toxin-treated plant

FIGURE 4 Comparison of the leaves of two seedlings; a water-injected control plant (left) and a toxin-injected plant (right). Please note that the control plant does not show any chlorotic spots, while the toxin-treated plants has a large chlorotic spot. The holes (arrows) in the leaves are caused by the injection needle during the treatment


1
4 cm


2


3
1 cm


4
1cm

effect on the structure of the chloroplasts of the leaves, but more data need to be collected before a conclusion can be drawn.

DISCUSSION

The mass increase tests showed a distinct effect of fumonisin B_1 (FB_1) on maize callus, which became more obvious as the concentration of the toxin increased. However, the callus was still alive after six weeks of exposure to all concentrations of the toxin. The regrowth tests showed a complete recovery of the callus at all concentrations except the highest (100 mg FB_1/l). At this level, the callus is still alive after the treatment with the toxin, but after seven weeks on toxin-free culture medium, it does not seem to have recovered completely from the effect of the toxin.

This slow recovery might be caused by the break-down of the structure of the cytoplasm in most cells, resulting in cell death. It is therefore concluded that only the few cells, which have not been affected to such an extent that cell death has occurred, have the ability to recover and divide again. TEM studies on the intermediate toxin levels (0.1, 1.0 and 10 mg FB_1/l) showed an increased level of cell disorganisation as the toxin level increased. The concentration range used in this experiment could shed no light on the exact concentration of FB_1, which will cause immediate cell death.

In the regrowth period (week 6-14 in Table 2), the lower values of the growth rate of the control and the 0.1 mg FB_1/litre were caused by their higher callus mass at the start of the regrowth phase of the experiment. This is due to their enhanced growth during the preceding experimental phase.

The TEM study revealed an increase in the thickness of the cell wall with an increased toxin level. Leach & Rowell (1966) described appositions resulting in a thickening of the cell wall when maize leaves were inoculated with wheat stem rust, *Puccinia graminis* f.sp. *tritici* Pers., a non-pathogen of maize. Cell-free

exudates from germinating urediospores of wheat stem rust induced the same response upon infiltration into maize leaf tissue (Leach & Rowell, 1969). Heath (1971) described similar cell wall thickening in the leaf cells of beans, *Phaseolus vulgaris* L., after infection with cow pea rust, *Uromyces phaseoli* (Pers.) Wint. f.sp. *vignae* (Barcl.) Arth., to which bean is a non-host. These deposits against the cell wall, under various names, are among the most common responses of toxin-treated plants (Luke *et al.*, 1966; Aist, 1976). Wheeler (1974) suggested that deposits in disease or toxin-treated plants may function as a protective barrier over damaged areas of the plasma membrane. Crystalline deposits and appositions were found in cell walls of oat leaves treated with uranyl salts or victorin, and thus appositions may function to sequester toxic materials (Easton & Hanchey, 1972). In the present study only an increase in the thickness of the cell wall was observed, and no appositions were found. However, the function of the thicker cell wall might serve the same purpose as the deposits.

Studies, by McLean *et al.* (1990), of the effects of aflatoxin B_1, a mycotoxin of *Aspergillus flavus* (Link) Fr., on the ultrastructure of maize callus cells showed an increase in the degree of vacuolation of the cells, a more pronounced and irregular chromatin clumping in the nucleus, and a loss of cytoplasmic integrity. These researchers have also found that with an increasing toxin level, cellular disorganisation became more severe, membranes appeared to lose their osmiophilic properties and lipid bodies increased in size. Although in the present study lipid bodies were found in the 1.0 and the 10 mg/l treatment, they were not observed in the cells grown at the highest concentration of FB_1. Neither were changes in the chromatin of the nucleus, and an increased degree of vacuolation noted. Hanchey (1981) stated that with most toxins there is little evidence of nuclear damage until fairly late stages of cellular damage.

Park *et al.* (1981) observed veinal necrosis in tomato leaves after 30 hours of exposure to a 10 mg/l solution of AL-toxin (or AAL-toxin), a mycotoxin of *Alternaria alternata* (Fr.) Keissler f.sp. *lycopersici* with an almost identical structural formula as fumonisin B (Marasas *et al.*, 1988b) (see APPENDIX 2.12). TEM studies by these researchers showed that this toxin (at 10 mg/l) had an effect

on the mitochondria and rough endoplasmic reticulum (ER) of tomato leaves 24 hours after treatment. Mitochondria in the toxin-treated leaves were swollen, the matrix was leached to the same density of the cytoplasm, and there was a reduction in number of cristae. Swollen and vesiculated cellular-structures were found in the toxin-treated leaf cells. These structures were identical with ER because ribosomes attached to their surface. These effects on mitochondria were very similar to those of *Bipolaris maydis* (Nisik. & Miyake) Shoem. T-toxin on mitochondria in T-cytoplasm maize (Aldrich *et al.*, 1971, cited by Nishimura & Kohmoto, 1983). Although these studies indicate that both these host-specific toxins have an effect on the mitochondria, in the present study no change in the structure of the mitochondria was observed. The increase in the amount of endoplasmic reticulum in the cells has sometimes been interpreted as an indicator of increased protein synthesis, however since it also occurs in rather abnormal conditions, it could also mean exactly the opposite (Hanchey, 1981).

McFarland (1981) and Gilchrist (1983) (both cited by Fuson & Pratt, 1988) suggested that AAL-toxin inhibited aspartate carbamoyltransferase (ACTase), an enzyme involved in the pyrimidine biosynthesis and located in the chloroplasts of plant cells. However, Fuson & Pratt (1988) concluded from the results of their study that AAL-toxin might have another (as yet unknown) site of action in addition to/ or instead of the suggested ACTase. In the present study the site of action of the FB_1 toxin could not be identified.

When both resistant and susceptible tomato leaf pieces were tested with AAL-toxin, it was found that toxin-insensitivity was not linked with resistance to *A. alternata* f.sp. *lycopersici* demonstrated by the parental plant (Witsenboer *et al.*, 1988). Toxin-insensitive cultures were found to derive from both resistant and susceptible cultivar origins. This implies that the mode of action of AAL-toxin *in vitro* is different to the effect of toxins produced by the fungus during the infection process in susceptible tomato plants (*in vivo*). The reaction of host callus tissue to other host-specific toxins, e.g. *Bipolaris victoriae* (Meehan & Murphy) Shoem. toxin (Rines & Luke, 1985), or *B. maydis* T-toxin (Brettell *et al.*, 1980), is highly correlated with the reaction demonstrated by the source of the tissue. It would be

interesting to test the reaction of callus derived from *Fusarium*-resistant maize lines.

Phenolic substances have been found to suppress toxin production in *Fusarium oxysporum* f.sp. *lycopersici* (Sacc.) Snyd. et Hans., but did not detoxify the *Fusarium*-toxins (Chet *et al.*, 1978). Other authors have reported that phenolics can detoxify toxins produced by pathogens (Sridhar & Mahadevan, 1979; Krishanamohan & Vidhyasekaran, 1986, both cited in Vidhyasekaran, 1988). The role of phenolic substances is not clear and has been correlated with disease resistance or incompatible reactions (Vidhyasekaran, 1988). Steinkamp *et al.* (1979) found phenolic substances in lesions in the leaves of beet, *Beta vulgaris* L., inoculated with *Cercospora beticola* Speg., or CB-toxin, but could not find a similar response when beet leaves were inoculated with cercosporin, another toxin from *C. beticola* (Steinkamp *et al.*, 1981). In the present study, the occurrence of phenolics at the 1.0 mg/l FB_1 concentration only, could have resulted from the fact that, at this level, callus cells were exposed to a concentration of fumonisin to which they were able to respond. At the higher levels, the effect of the toxin was much more severe, while at the lower levels the toxin had hardly any influence on the cell metabolism.

Amyloplasts function as starch storage vesicles in plant cells (Wolfe, 1981). Disturbances in cell metabolism may cause an overproduction of starch (Cameron, 1952). Increased starch accumulation could result from increase mobilization of lipids, stimulation of starch synthesis, or decreased translocation of sucrose (Hanchey, 1981). A lack of inorganic phosphate reduces the exchange of triose-phosphate through the membranes of the proplastids, and 3-phosphoglycerinealdehyde (3-PGAld), produced in the Calvin reaction, is transformed into starch grains.

The stunting of plants found after treatment with tentoxin, a toxin produced by *Alternaria alternata*, suggests that the decreased translocation of sucrose is at least partly responsible for the accumulation of starch (Templeton *et al.*, 1967). This idea has later been supported by the findings of Schadler *et al.* (1976), and also in the present study a decrease in plant length and starch accumulation

supports this hypothesis.
Starch grains can be produced as a reaction to a disturbance in the cell metabolism after the Calvin reaction, or may result from a specific effect of the toxin on the permeability of the membranes and/or the exchange of inorganic phosphate.

Since a significant reduction in plant height was recorded without a concomitant difference in the number of leaves on the seedling, it is clear that the toxin has an effect on the elongation of the stalk. However, the reduction in the size of the leaves indicates a more general toxic action. The subsequent reduction in dry mass of the plants was to be expected from the recorded height difference and the smaller leaves. Internodal stunting of maize plants has been described by Cole *et al*. (1973) for plants treated with moniliformin, a mycotoxin produced by other *Fusarium* species. Although, moniliformin and fumonisin B_1 have a completely different molecular structure (see APPENDIX 2.12 and 2.13), their mode of action may be similar.

The phytotoxic effect of the toxin on maize seedlings was obvious at both FB_1-concentrations used. This proved that the toxic effects of FB_1 on callus cells can be reproduced in *in vivo* situations, and it can therefore be concluded that these maize callus bio-assays do represent the *in vivo* situation.

It cannot be stated with certainty that the ultrastructural changes discussed in this chapter were, in fact, directly induced by fumonisin B_1. The toxin has a very large molecular structure and it is therefore unlikely that the molecule is transported through the cell wall and/or plasmalemma. The effect of the mycotoxin can be the induction of a series of events, which might include hormonal changes and accumulation of other toxic compounds such as phytoalexins. However, this research shows a clear effect of the toxin concentration on the ultrastructure of the callus cells and further research is necessary to establish whether this is a direct or an indirect effect.

This research shows that fumonisin B_1 is not only highly toxic to animals, but it also has a direct phytotoxic effect on the growth of callus cells. An increasing

concentration of the toxin causes a break-down of cell structure, which finally can result in cell death. Although FB_1 is very similar in structure to the AAL-toxin of *A. alternata* f.sp. *lycopersici*, further research is necessary to establish whether fumonisin can also be classified as a host-specific toxin. This could then result in the use of this toxin in *in vitro* selection of toxin-insensitive cell cultures.

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CHAPTER 3

EVALUATION OF THE RELATIVE PHYTOTOXIC POTENCY OF FUMONISIN B_1, USING A MAIZE CALLUS BIOASSAY

INTRODUCTION

Pathotoxins, in the form of crude and/or purified toxins, have been recognized as useful tools for the induction and selection of toxin-insensitive mutants in cell culture, which proved to be disease-resistant (Wenzel, 1985; Daub, 1986). However, numerous disease-resistant mutants have been obtained without any form of selection pressure (e.g. Evans & Sharp, 1983; Umbeck & Gengenbach, 1983). Research by Armstrong (1986) showed that plants regenerated from both organogenic and embryogenic maize tissue culture frequently possessed genetic and/or cytogenetic abnormalities. However, Rines & Luke (1985) found no induction of resistance in callus without treatment with a pathotoxin. They postulated that the possibility of induction of resistance is depending on the genetic nature of the resistance.

Mycotoxins are, according to Graniti (1972, cited by Reiss, 1978), substances that are produced by fungi on foods and feeds, and that can bring about specific intoxication symptoms in animals and very probably also in man. Although many mycotoxins may be toxic to plants, they are never involved in the development of plant diseases. Because of this fact the mycotoxins may be distinguished from the phytotoxins, which are metabolites of phytopathogenic fungi which intoxicate the host plant (Graniti, 1972, cited by Reiss, 1978). These definitions overlap considerably, and one of the main reasons for maintaining the separate labels is the lack of information about the phytotoxic effects on plants of the so-called mycotoxins.

Toxicologically, fumonisin B_1 (FB_1), a mycotoxin of *Fusarium moniliforme* Sheldon, is considered to be the most potent of the fumonisin group and although research has proven that only small amounts (0.875 mg/kg body mass/day,

which amounted to a total dose of 276 mg) of this toxin can cause leuko-encephalomalacia in horses (Marasas *et al.*, 1988), nothing is known about the phytotoxic potential of this secondary metabolite. To evaluate the potency of this toxin, a comparison was made between the effect of FB_1 and two other potent mycotoxins of *Fusarium* spp., moniliformin and T-2 toxin, on the mass increase of maize (*Zea mays* L.) callus, by using different levels of the toxin in the culture medium. Results of these tests were compared with the effects of toxin extracts of *Exserohilum turcicum* (Pass.) Leonard & Suggs on maize callus growth.

Moniliformin was first isolated from a strain of *Fusarium moniliforme* (Cole *et al.*, 1973), although later reports indicated that this toxin is not frequently produced by *F. moniliforme* (Marasas *et al*, 1984). The moniliformin used in the present experiments, originated from a strain of *F. subglutinans* (Wollenw. & Reink.) Nelson, Toussoun & Marasas. The moniliformin-producing ability of *F. subglutinans* is associated with the geographical origin of the isolates (Marasas *et al.*, 1979). Moniliformin has been shown to be highly poisonous to a range of laboratory animals (Marasas *et al.*, 1984). Although synthetic derivatives of moniliformin with specific herbicidal and plant growth regulatory properties have been found (Fisher & Bellus, 1979; Bellus *et al.*, 1980), it appears that the phytotoxic effects of moniliformin itself have hardly been studied since the initial work of Cole *et al.* (1973), and a recent review article by Gilbert (1989) only quoted this article for the effect of the toxin on plants.

T-2 toxin was first isolated from *F. tricinctum* (Corda) Sacc., strain T-2, by Bamburg (1968, cited by Marasas *et al.*, 1984), and Bamburg *et al.* (1968), but since then it has been found in mycotoxic extracts of various other *Fusarium* species (Marasas *et al.*, 1984). It has been shown that T-2 toxin is responsible for alimentary toxic aleukia (ATA) (Mirocha & Pathre, 1973), one of the best-documented accounts of the effects of *Fusarium* toxins on man resulting from ingestion of overwintering cereals. However in nature, acute toxicity due to ingestion of T-2 toxin is rare (Mirocha, 1984). For these experiments a toxin originating from a culture of *F. tricinctum* was used. The phytotoxic effects of T-2 toxin have been reasonably well researched (e.g. Marasas *et al.*, 1971;

Helgeson *et al.*, 1973; Linnainmaa *et al.*, 1979).

Although the phytotoxins of *Bipolaris* species have been well researched, little is known about the toxic compounds in the related *E. turcicum*. In the present study, no attempt was made to characterize the toxic substances produced by this fungus, but it was intended to establish whether a crude extract of the mycelium contained toxic properties. This toxic extract could then be employed in the *in vitro* selection of resistant maize tissue. Stable heritable resistance has been found using this method with several *Bipolaris* species, e.g. *B. maydis* (Nisik & Miyake) Shoem. race T in maize (Gengenbach *et al.*, 1977, cited by Brettell *et al.*, 1980), *B. victoriae* (Meehan & Murphy) Shoem. in oats, *Avena sativa* L. (Rines & Luke, 1985), and *B. oryzae* (Breda de Haan) Shoem. in rice, *Oryza sativa* L. (Ling *et al.*, 1985).

MATERIALS AND METHODS

Callus initiation. Maize callus was initiated from the scutella of immature embryos (Green & Phillips, 1975). From previous research (Hughes, 1984), it was known that maize inbred line B14 was responsive to tissue culture, and the cultivation capacity over longer periods was excellent. However, the shoot-forming capacity of this line is poor (Hughes, 1984), and it has been established that this genotype is not suitable for plant regeneration (Van Asch, unpublished data). Since no better genotypes were available at the start of this research, and regeneration was only a secondary objective, the tests were performed with this line.

The culture medium, as described by Green & Phillips (1975), contained the inorganic components of Murashige & Skoog (1962) medium, 7.7 mg L-glycine, 1.98 g L-asparagine, 1.3 mg niacin, 0.25 mg thiamine-HCl, 0.25 mg pyridoxine-HCl, 0.25 mg Ca-pantothenate, 20 g sucrose, 8 g agar and 2.0 mg 2,4-D (all quantities given per litre medium). The pH was adjusted to 6.0 with 0.1 N NaOH before autoclaving at 115 °C (or 0.75 kgf/cm^2) for 15 minutes.

The cultures were grown in incubators at 26 °C with a 16-hour photoperiod. The callus was maintained by transferring small pieces of approximately 20 mg to fresh

medium every 4-6 weeks.

Toxin acquisition and isolation

Fumonisin B_1, moniliformin and T-2 toxin. These three mycotoxins of *Fusarium* spp. were obtained from Prof. W.F.O. Marasas of the Medical Research Council, Tygerberg, South Africa.

HT-toxin. *Exserohilum turcicum* was isolated from maize plants grown at Cedara Agricultural College, Cedara, South Africa, in March 1989. An extract (hereafter referred to as "HT-toxin") was made according to the method of Turner & Martinson (1972) for *B. maydis*. The culture of *E. turcicum* was incubated in 40 petri dishes, containing a total of 500 ml of PDA, at 24 °C in the dark for 18 days. The fungal mycelium and the agar were macerated and immersed in 1 litre of methanol for two hours. This extract was filtered through Whatman ® No.2 filter paper in a Buchner funnel and the filtrate was reduced under vacuum to dryness. The remaining volume was adjusted to 115 ml with distilled water and stored at -4 °C until the time of usage. Before use, the stock solution was melted at room temperature.

Callus mass increase tests

Fumonisin B_1 and moniliformin. Both FB_1 and moniliformin are water-soluble mycotoxins. The two highest concentrations (100 and 10 mg/l) were weighed out directly, while the two lowest concentrations (1.0 and 0.1 mg/l) were taken from a stock solution of 5 mg FB_1 or moniliformin in 50 ml deionised water. Because of their non-labile nature, both toxins were added to the culture medium before autoclaving.

T-2 toxin. This mycotoxin is not water-soluble and was dissolved in 1 ml methanol before adding it to 500 ml of culture medium prior to autoclaving. The two highest concentrations were weighed out, while the two lowest were taken from a stock solution in which 5 mg T-2 toxin was dissolved in 1 ml methanol and added to deionised water to total 50 ml of liquid.

HT-toxin. Since the HT-toxin was dissolved in deionised water, no problems were encountered in preparing the toxin concentrations required. The 100 ml/l concentration contained 50 ml of the HT-toxin stock solution per 500 ml culture

medium. The remaining concentrations were obtained by making a dilution series. The HT-toxin solution was added to the culture medium before autoclaving, since preliminary results had shown that after autoclaving a phytotoxic effect still existed.

For testing the phytotoxic effect of the toxin on the mass increase of the callus, pre-weighed pieces of maize callus (average approximately 0.14 g), were placed on 6.5 ml of culture medium in flat-bottomed test tubes (100 mm x diam. 24 mm). To evaluate the effect of the solvent, a concentration of 2 ml methanol per litre was used in a second control series in the test with T-2 toxin. Per treatment, 49 pieces of callus were used and the tests with moniliformin, T-2 toxin, and HT-toxin were repeated twice, while the fumonisin B_1 tests were done three times. The callus was incubated for six weeks in an incubator at 26 °C with a 16-hour photoperiod. After this time, the callus pieces were weighed to determine their mass increase. To eliminate differences between the replications, the statistical analysis of the average values was done on the relative mass increase to the control.

To establish the mass increase of callus per week during the six weeks of the toxin treatment, ten pieces of callus were randomly taken, at weekly intervals, weighed under sterile conditions and returned to the culture medium. This proved to be a very laborious method and the results obtained from the first tests did not warrant a continuation of this approach. Therefore only the mass increase per week results of one replication of FB_1, moniliformin, and HT-toxin are available.

RESULTS

Fumonisin B_1. Although the results of the FB_1 experiments have already been presented and discussed in detail in Chapter 2 of this thesis, they are included here, to enable a comparison between the results of the various toxins to be made. The mass increase of the callus differed significantly ($P < 0.05$) between the treatments with a low toxin concentration (0 and 0.1 mg FB_1/l), the intermediate concentrations (1.0 and 10 mg FB_1/l), and the highest concentration of 100 mg FB_1/l (Table 1).

TABLE 1 Average mass increase (g) of callus of maize, *Zea mays*, grown for six weeks on culture medium (Green & Phillips, 1975) containing different amounts of fumonisin B_1 (see also APPENDIX 2.1)

concentration (mg/l)	replication 1	2	3	average
0 (control)	0.300 a	0.704 a	0.428 a	0.443 a
0.1	0.203 b	0.696 a	0.416 a	0.402 a
1.0	0.058 c	0.382 b	0.275 b	0.242 b
10	0.062 c	0.317 b	0.164 c	0.177 b
100	-0.027 d	0.078 c	0.055 d	0.035 c

Figures in a column followed by the same letter do not differ significantly ($P < 0.05$) in a LSD test

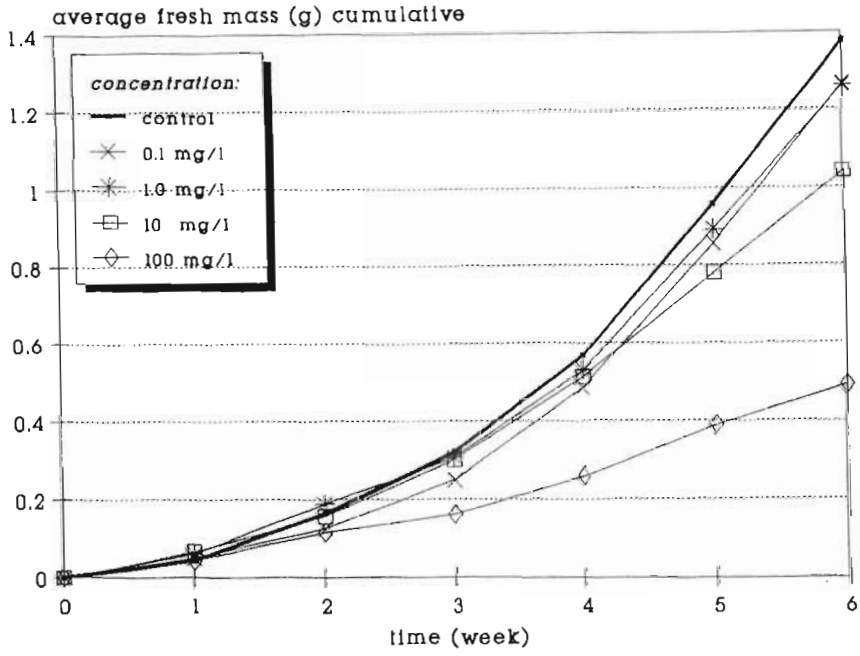

FIGURE 1 Average weekly callus mass increase (g) of maize, *Zea mays*, callus grown on culture medium (Green & Phillips, 1975) containing different amounts of moniliformin (see also APPENDIX 3.1)

Moniliformin. The mass increase per week of the callus over the six weeks of the experiment is graphically presented in Figure 1 (data in Appendix 3.1). To obtain a clear distinction between the various concentrations, the data are

TABLE 2 Average mass increase (g) of callus of maize, *Zea mays*, grown for six weeks on culture medium (Green & Phillips, 1975) containing different amounts of moniliformin (see also APPENDIX 3.2)

concentration (mg/l)	replication 1	2	average	growth rate
0 (control)	0.400 a	0.415 a	0.408 a	3.5 ab
0.1	0.385 a	0.409 a	0.397 a	4.0 a
1.0	0.274 b	0.367 a	0.320 ab	3.1 ab
10	0.134 c	0.262 b	0.197 bc	2.6 ab
100	0.018 d	0.101 c	0.059 c	1.6 b

Figures in a column followed by the same letter do not differ significantly ($P < 0.05$) in a LSD test

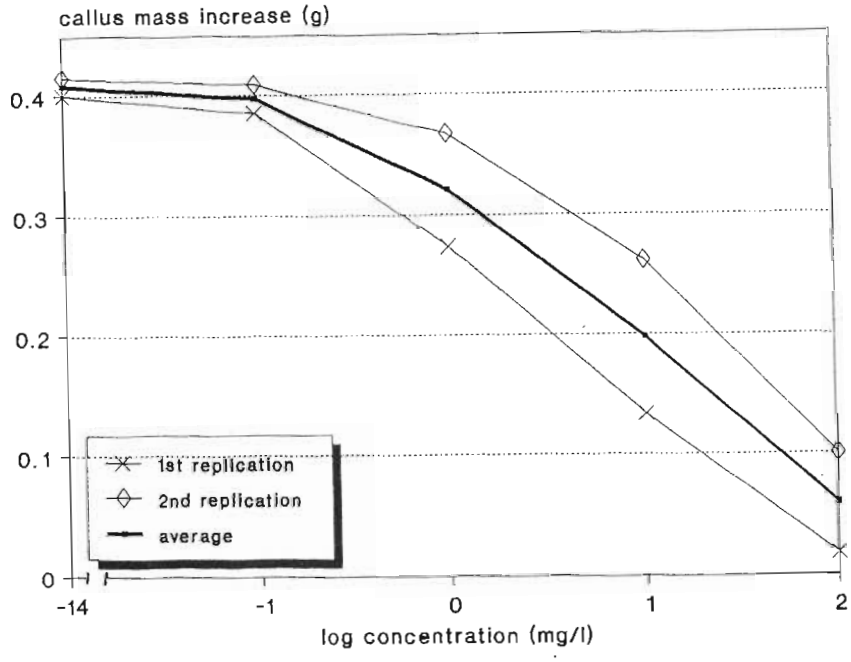

FIGURE 2 Average mass increase (g) of maize, *Zea mays*, callus grown for six weeks on culture medium (Green & Phillips, 1975) containing different amounts of moniliformin

presented cumulatively. For the first two weeks of the experiment, no statistical differences ($P < 0.05$) could be found between the mass increase of callus grown at the various toxin concentrations. At three, four and five weeks the mass increase at the highest concentration is significantly ($P < 0.05$) less than the control and the 0.1 mg/l level. The differences after six weeks are also shown in Table 2 and Figure 2 (replication 2).

After six weeks, the callus of all treatments appeared healthy and only a size

TABLE 3 Average mass increase (g) of callus of maize, *Zea mays*, grown for six weeks on culture medium (Green & Phillips, 1975) containing different amounts of T-2 toxin (see also APPENDIX 3.5)

concentration (mg/l)	replication 1	2	average	growth rate
0 (control)	0.588 a	0.315 a	0.456 a	4.1 a
0.1	0.533 ab	0.293 ab	0.412 ab	4.1 a
1.0	0.510 b	0.228 bc	0.370 bc	3.5 a
10	0.425 c	0.185 cd	0.327 c	3.5 a
100	0.284 d	0.131 d	0.219 d	2.8 a

Figures in a column followed by the same letter do not differ significantly ($P < 0.05$) in a LSD test

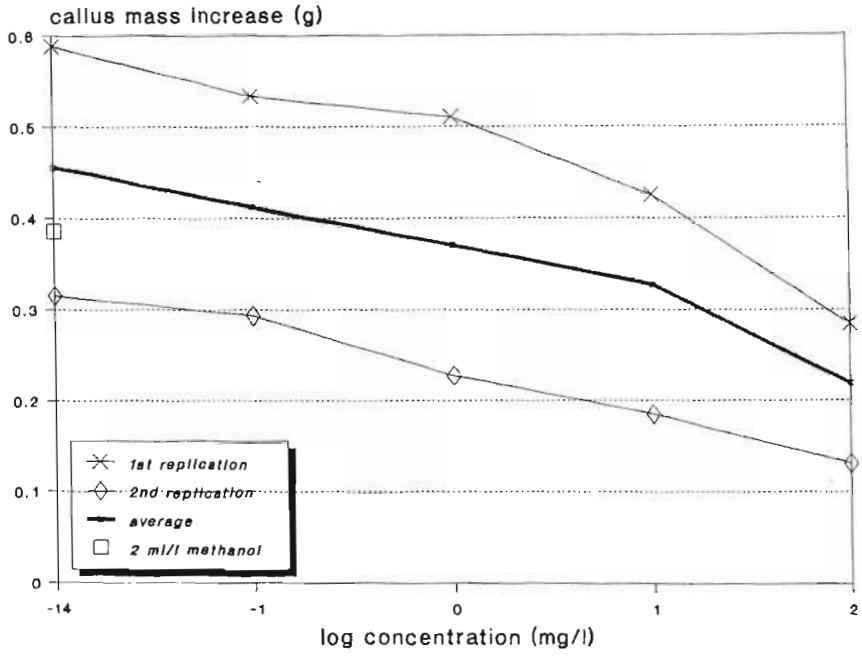

FIGURE 3 Average mass increase (g) of maize, *Zea mays* callus grown for six weeks on culture medium (Green & Phillips, 1975) containing different amounts of T-2 toxin

difference was observed. These differences are reflected in the callus mass increase (Table 2), and it is therefore not necessary to furnish a more detailed description of the callus.

The curve, obtained when these data are displayed graphically, shows a distinct separation between the toxin concentrations and a regular decline in callus mass increase with increasing toxin concentration (Fig. 2). Significant statistical differences ($P < 0.05$) of the average mass increase were found between the

control and 0.1 mg/l and the two highest concentrations, and between the 1.0 mg/l and the 100 mg/l treatment.

To eliminate effects of the callus mass at the start of the experiment the growth rate of the callus, i.e. the final mass divide by the initial mass, was calculated. Although the growth rate decreased with an increasing moniliformin concentration, only the 0.1 mg/l treatment was significantly ($P < 0.05$) different from the highest concentration (Table 2).

T-2 toxin. In this test only small size differences between the pieces of callus could be observed, the appearance of most callus pieces was identical. The data on callus mass increase are presented in Table 3 and Figure 3.

A significant ($P < 0.05$) decline in callus mass was found with increasing toxin concentration. The control was significantly ($P < 0.05$) higher than all concentrations higher than 1.0 mg/l, and the 0.1 concentration was significantly ($P < 0.05$) different from both the 10 and 100 mg/l. The highest concentration of T-2 toxin was significantly different ($P < 0.05$) from all other concentrations (Table 3).

The growth rate did not give any significant differences ($P < 0.05$) between the treatments, although it decreased with an increasing toxin level (Table 3).

The average mass increase of the callus in the second control, containing 2 ml methanol per litre culture medium, was 0.384 g (see also APPENDIX 3.5). This is not statistically different ($P < 0.05$) from the average of the methanol-free controls. It can therefore be concluded that the methanol did not affect the mass increase of callus.

HT-toxin. The callus mass increase per week is graphically presented in Figure 4. The mass increase, over six weeks, is taken cumulatively to obtain a distinct graph. The shape of the curves of the various toxin levels is logarithmic, and no statistical differences ($P < 0.05$) could be found between the mass increase of the different treatments. The only significant difference ($P < 0.05$) existed between the weekly mass increase in the third week of the control and the highest concentration (APPENDIX 3.9).

No visible differences in callus appearance were observed between the different

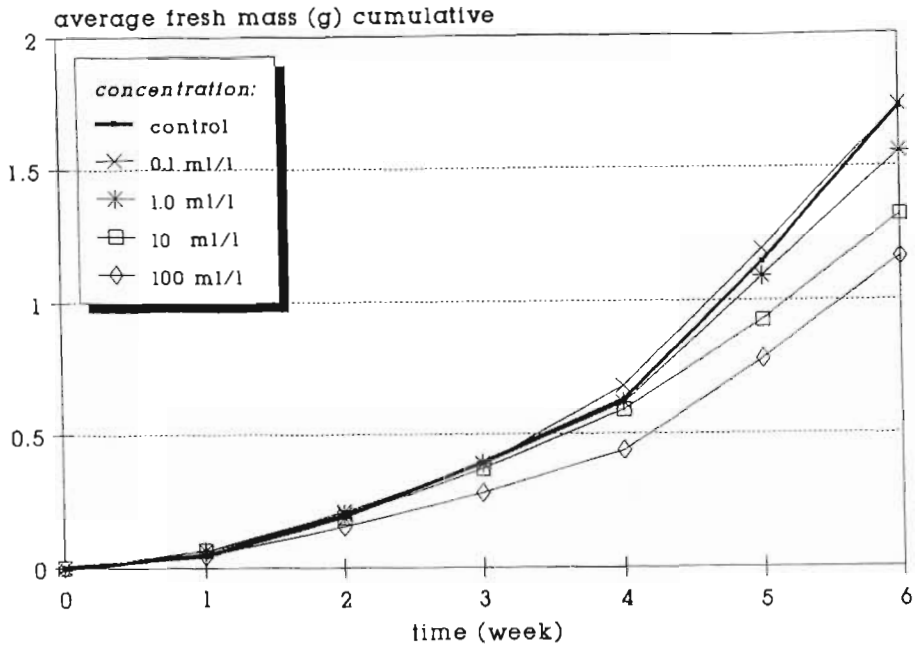

FIGURE 4 Average callus mass (g) increase of maize, *Zea mays*, callus grown on culture medium (Green & Phillips, 1975) containing different amounts of HT-toxin (see also APPENDIX 3.9)

concentrations. The results of the two replications are presented in Table 4 and Figure 5. The increase in callus mass became less with an increasing toxin concentration, however this decrease was only statistically significant ($P < 0.05$) between the control and both the 10 and 100 ml/l concentration (Table 4). The graphical presentation of these results shows a continuous drop in callus mass increase with increasing toxin concentration (Fig. 5).

Although the growth rate showed a decrease with increasing toxin level, this was

TABLE 4 Average mass increase (g) of callus of maize, *Zea mays*, grown for six weeks on culture medium (Green & Phillips, 1975) containing different amounts of HT-toxin (see also APPENDIX 3.10)

concentration (ml/l)	replication 1	replication 2	mean	growth rate
0 (control)	0.578 a	0.632 a	0.606 a	4.8 a
0.1	0.537 a	0.569 a	0.553 ab	5.3 a
1.0	0.463 ab	0.547 ab	0.504 ab	5.3 a
10	0.391 b	0.494 b	0.443 b	3.9 a
100	0.378 b	0.472 b	0.422 b	3.6 a

Figures in a column followed by the same letter do not differ significantly ($P < 0.05$) in a LSD test

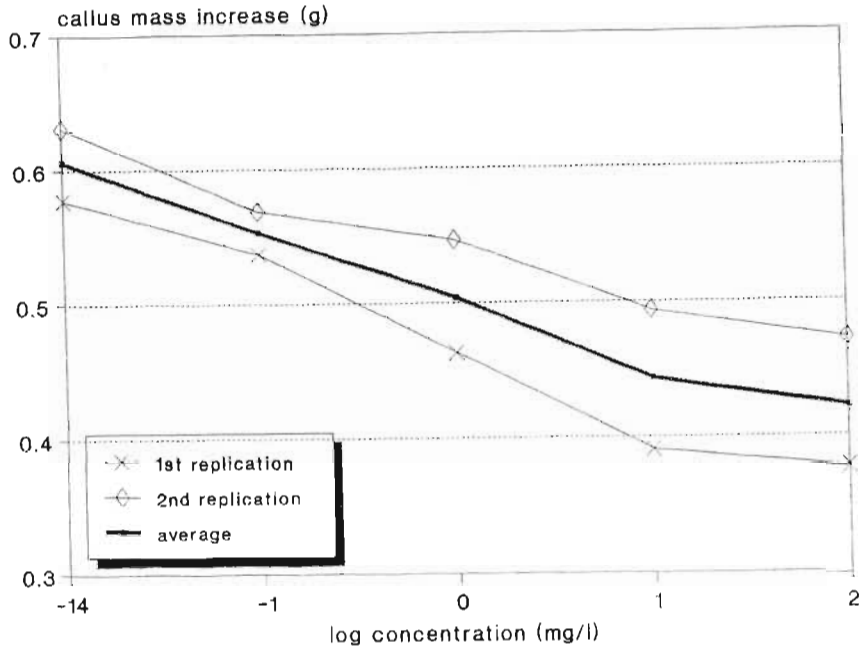

FIGURE 5 Average mass increase (g) of maize, *Zea mays*, callus grown for six weeks on culture medium (Green & Phillips, 1975) containing different amounts of HT-toxin

not supported statistically ($P < 0.05$) (Table 4).

DISCUSSION

Fumonisin. The results of FB_1 have been discussed in chapter 2 of this thesis. However, the aim of this component of the research was to compare the effect of this toxin on callus mass increase with that of other fungal toxins, and therefore the graphical presentation of the data obtained with FB_1 is included in Figure 6 (see also Table 5).

Moniliformin. This mycotoxin caused a gradual reduction of the increase of callus mass as the concentration of the toxin was increased. This growth rate was very regular during the six weeks of the experiment and it can be concluded that the toxin has a constant effect on the callus. The final callus mass increase at the highest toxin concentration (100 mg/l) was not significantly different from that of FB_1 at the same concentration (Table 5), although the callus of the moniliformin

tests looked much healthier than the callus treated with FB_1. When the callus was placed onto toxin-free culture medium after the toxin treatment, regrowth of callus from all concentrations occurred.

After the work done by Cole *et al.* (1973), when they described toxic effects on plants and animals caused by moniliformin, little evidence of research on the phytotoxic effects of this mycotoxin was found. These workers demonstrated a 24 and 57 percent growth inhibition (when compared to the control) of wheat, *Triticum aestivum* L., coleoptiles, partly submerged in a 20 and 200 ppm toxin solution respectively. Both tobacco, *Nicotiana tabacum* L., and maize plants, treated with either 20, 200 or 2000 mg/l moniliformin, showed various morphological disorders, e.g. necrosis, interveinal chlorosis, distortion of the leaf shape and thickening of the midrib (Cole *et al.*, 1973). In the same article it was also found that this mycotoxin has a detrimental effect on the apical dominance of the plants, resulting in rosette-shape tobacco plants. In maize, the toxin caused internodal stunting, which resulted in significantly shorter plants. However, six weeks after the application the plants seemed to have overcome the effect of the toxin and no differences could be demonstrated between the treated and the control plants (Cole *et al.*, 1973). After a cytological study, Styer & Cutler (1984) concluded that 0.001 M moniliformin had an disruptive effect on the spindle apparatus and consequent C-mitotis of maize root tip cells.

T-2 toxin. In the present study, the results show a clear a reduction of the mass increase of maize callus as the concentration of the toxin increased. This was also found in other studies which dealt with phytotoxic effects of this mycotoxin. Marasas *et al.* (1971) demonstrated a reduction in the length and mass of pea, *Pisum sativum* L., seedlings for concentrations of T-2 toxin as low as 0.1 mg/l. In a cytological study, Linnainmaa *et al.* (1979) observed severe destruction, chromosomal aberrations and cytogenetic abnormalities, in the root tips of onion, *Allium cepa* L., four to nine hours after a one-hour treatment in a 100 ppm T-2 toxin solutions. Other surveys demonstrated that T-2 toxin caused, e.g. a reversible reduction in growth of tobacco callus tissues (Helgeson *et al.*, 1973), a reduction in the germ tube length of germinating tobacco pollen (Siriwardana & Lafont, 1978) and a reduction of the auxin-promoted elongation (length) of

soybean, *Glycine max* (L.) Merr., hypocotyles, while the cytokinin-promoted elongation (width) was not affected (Stahl *et al.*, 1973).
The difference in the concentration effect, with e.g. the results of Marasas *et al.* (1971), might be caused by a difference in response between dicotyledonous and monocotyledonous plants.

HT-toxin. The extract from *Exserohilum turcicum* had a distinct phytotoxic effect on the callus, and a reduction in callus mass increase with increasing toxin concentrations was observed. The callus mass increase per week was constant over the six weeks of the experiment. The statistical difference found in the third week was probably a result of the random selection of the callus, and this difference was not observed in any of the following weeks.
Pelcher *et al.* (1975) reported that an increasing concentration of T-toxin of *B. maydis* caused a more severe reaction of protoplasts of susceptible maize lines. At the lowest of 0.1 mg/l no difference with protoplasts derived from resistant lines was observed, while a 1.0 mg/l concentration gave only a slight difference. The susceptible response was distinct at the higher concentrations of 2.0, 10 and 100 mg/l of the toxin. In the present research such a distinct influence of the toxin concentration on the reaction of the callus was not noticed, but protoplasts are much more sensitive than whole callus pieces, nor were susceptible and resistant callus origins compared. The method used for extraction might not have been optimal for a high yield of toxic products and this might have resulted in reduced toxic effects. It is also possible that the autoclaving of the extract influenced its phytotoxic properties. Or lastly, HT-toxin is not as potent as T-toxin of *B. maydis*.
No specific reports were found about the use of *E. turcicum*-extracts, like HT-toxin, for *in vitro* selection of resistant tissue. This could be explained by the fact that *E. turcicum* is a tropical disease and most work on *in vitro* selection is done in the temperate zones, where the related *B. maydis* and *B. oryzae* are more prevalent.

Comparison of the toxins used. All toxins tested caused a reduction in callus mass increase when the toxin concentration was increased.
Statistical differences between the control values of moniliformin and HT-toxin do

TABLE 5 Mass increase of maize, *Zea mays*, callus, grown for six weeks on culture medium (Green & Phillips, 1975) containing different amounts of mycotoxins, expressed as percentage of the control

concentration (mg/l or ml/l)	mycotoxin			
	FB_1	moniliformin	T-2	HT
0 (control)	100.0	100.0	100.0	100.0
0.1	87.9 a	97.4 a	91.8 a	91.5 a
1.0	45.9 a	78.5 a	79.6 a	83.3 a
10	34.7 a	48.3 ab	65.5 ab	72.9 b
100	5.0 a	14.4 a	44.9 b	70.0 b

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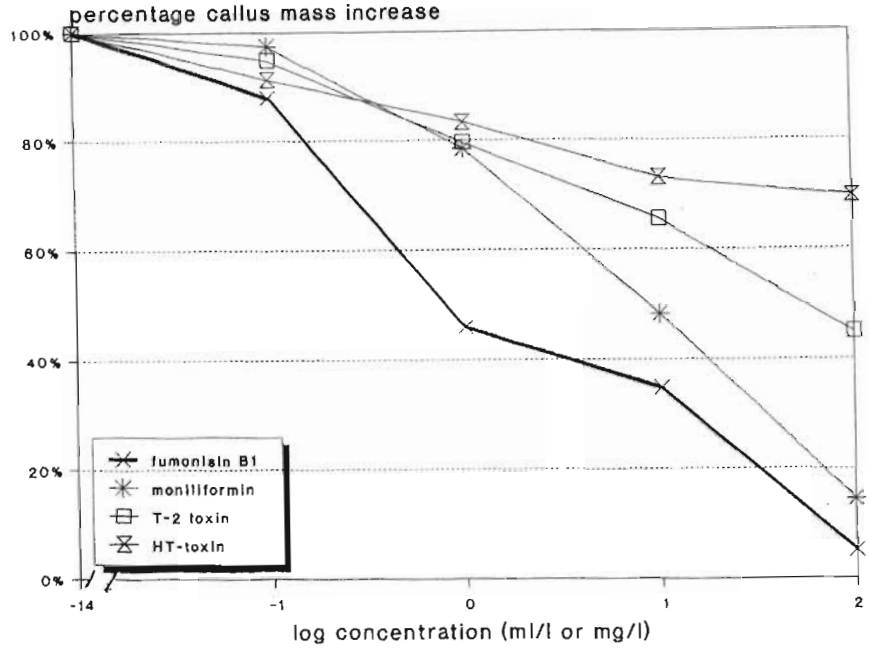

FIGURE 6 Comparison of mass increase of maize, *Zea mays*, callus grown on culture medium (Green & Phillips, 1975) containing different amounts of different mycotoxins, expressed as percentage of the control

not allow a comparison of the values as presented in Table 1 to 4. Therefore the relative values of the callus mass increase to the control were taken, the data of this transformation are presented in Table 5 and Figure 6.

In Figure 6, differences between the four toxins become clear. At the lowest toxin level, no differences between the effects of the various toxins could be observed. FB_1 gave a more severe reduction of callus mass increase at the 1.0 mg/l than any of the other toxin. However, this difference was not statistically significant ($P <$

0.05). At the 10 mg/l concentration, the difference between FB_1 and HT-toxin was significant ($P < 0.05$). The highest toxin concentration caused a severe mass increase reduction when callus was grown on medium with FB_1 or moniliformin (5.0 and 14.4% of the control). T-2 toxin caused a reduction of more than 50 percent (44.9), while HT-toxin reduced the callus mass increase to 70% of the control value. The differences between both FB_1 and moniliformin, and the two other toxins were statistically significant ($P < 0.05$).

The concentration at which the callus mass increase was reduced to under 50% of the control varied between the toxins. For FB_1, this was already at the 1.0 mg/l concentration, while moniliformin and T-2 toxin caused this at 10 and 100 mg/l respectively. HT-toxin did not reduce the callus mass increase with more than 30%. With all of the toxins, the reduction in mass increase was reversible, and when callus pieces were transferred to toxin-free medium after toxin treatment, size increase was observed of callus grown at all concentrations of all toxins (Van Asch, unpublished data).

When using this maize bioassay, the effect of Fumonisin B_1 on maize callus increase is very clear. From these results it can be concluded that FB_1 is more phytotoxic than both moniliformin and T-2 toxin. These two mycotoxins cause cytological disruptions (Styer & Cutler, 1984; Linnainmaa *et al.*, 1979), and the results of the present study suggest that FB_1 may also have an effect on cell division. However, cytological studies are necessary to support this hypothesis. The toxin levels used in this chapter will probably be sufficient for screening for resistant cell material, and even a mild pathotoxin such as HT-toxin might be aggressive enough to allow for selection of toxin-insensitive callus pieces. Although much effort was spent on regeneration induction, no regeneration of maize plants has been achieved from any of the treatments, and it was therefore impossible to support this theory with practical evidence.

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CHAPTER 4

THE USE OF *STENOCARPELLA MACROSPORA* PATHOTOXINS FOR *IN VITRO* SELECTION OF DISEASE RESISTANCE IN MAIZE

INTRODUCTION

In the tropical and sub-tropical areas of the world, *Stenocarpella macrospora* (Earle) Sutton is a pathogen of maize, *Zea mays* L., responsible for blight of leaves, and cob and stalk rot (Cutler *et al.*, 1980a). This fungus was recorded for the first time in South Africa in 1975 (Marasas & Van der Westhuizen, 1979), and since then it has become increasingly important to the local maize industry, especially in the Natal mist belt. A recent survey by McLennan (1990) showed a spread of the disease from the moist and hot conditions of Natal to the Southern and Eastern Transvaal, and the Eastern Orange Free State. Although presently still at low levels, a continuation of the wet weather cycle will probably result in a further spread to the western maize growing areas (McLennan, 1990).

To prevent further spread of this disease, selection for disease resistance is presently one of the main aims of the plant breeding industry. Although resistance to *S. macrospora* has been found in local inbred lines, this resistance was not inherited in a dominant way, and it may be the result of several genes with a strong additive action (McLennan, 1990). Selection for such resistance is a slow and long-term procedure.

Various studies, using host-specific toxins, have been conducted to select for resistance *in vitro*. The effects of T-toxin, a pathotoxin produced by *Bipolaris maydis* (Nisik & Miyake) Shoem., have been well researched, and the use of this toxin has yielded maize lines with a stable resistance to *B. maydis* (Brettell *et al.*, 1980). Latterell & Rossi (1983) stated that the ability of *S. macrospora* to blight green plant tissue was an effect of phytotoxins produced by the fungus. Following the presumed involvement of *S. macrospora* in animal and human diseases,

several mycotoxins have been isolated from cultures of this fungus (Chalmers *et al.*, 1978; Cutler *et al.*, 1980a & 1980b; Probst & Tamm, 1982). Since the primary interest of all these investigations was the identification of mycotoxins, only one of these toxins, chaetoglobosin K, was tested for its effect on maize plants, but no phytotoxic effects were observed (Cutler *et al.*, 1980b). It was the present author's opinion that the crude pathotoxin extract, used by these researchers, might contain one or more phytotoxins, which were lost in the purification methods employed to obtain the mycotoxins. These phytotoxins then could play a role in the selection for disease resistance in maize callus cultures.

The effects of the crude pathotoxin extract from *S. macrospora* (SM-toxin) on callus cells were established by using different levels of the toxin in the culture medium of maize callus. Regrowth tests on toxin-free culture medium after seven weeks of growth on SM-toxin-containing medium were done to measure the regrowth capacity of the treated callus. Transmission electron microscopy (TEM) studies were conducted on the callus after six weeks of growth on the toxic culture medium, in order to study the damage at cellular level. Seedling tests were performed to observe the effect of the pathotoxin extract on both *S. macrospora* -susceptible and -resistant inbred lines.

MATERIALS AND METHODS

Callus initiation. Maize callus was initiated from the scutella of immature embryos (Green & Phillips, 1975). From previous research (Hughes, 1984), it was known that the maize inbred line B14 was responsive to tissue culture, and the growing capacity over longer periods was excellent. However the shoot-forming capacity of this line was poor (Hughes, 1984), and it has been established that this genotype is not suitable for regeneration (Van Asch, unpublished data). Unfortunately no better genotypes were available at the start of this research.
The culture medium, as described by Green & Phillips (1975), contained the inorganic components of Murashige & Skoog (1962) medium, 7.7 mg L-glycine, 1.98 g L-asparagine, 1.3 mg niacin, 0.25 mg thiamine-HCl, 0.25 mg pyridoxine-

HCl, 0.25 mg Ca-pantothenate, 20 g sucrose, 8 g agar, and 2.0 mg 2,4 D (all quantities given per litre medium). The pH was adjusted to 6.0 with 0.1 N NaOH before autoclaving at 115 °C (or 0.75 kgf/cm^2) for 15 minutes.
The cultures were grown in incubators at 26 °C with a 16 hour photoperiod. The callus was maintained by transferring small pieces of approximately 20 mg to fresh medium every 4-6 weeks.

Toxin isolation. A pathotoxin extract from *Stenocarpella macrospora* (SM-toxin) was obtained using an adapted method from Cutler *et al.* (1980a). Cultures of *S. macrospora*, isolate NH of the collection of the Department of Microbiology and Plant Pathology, University of Natal, South Africa, were grown in two 1-litre erlenmeyer flasks, each containing 50 g of shredded wheat, 100 ml of mycological broth (1 g soytone and 4 g dextrose in 50 ml of deionised water, with the pH adjusted to 4.8), 2 g yeast extract, and 20 g sucrose (Kirksey & Cole, 1974). The flasks were incubated for 20 days at 25 °C in the dark. After this period, 300 ml of acetone was added to each flask, the contents were macerated in a blender, filtered through a Whatman® No.1 filter on a Buchner funnel, and the combined clarified filtrate was reduced to dryness under vacuum at 50 °C. The aqueous phase was extracted twice with ethyl acetate; each volume of the solvent double the volume of the aqueous portion. The ethyl acetate fraction was dried with anhydrous sodium sulphate and reduced under vacuum to dryness at 50 °C. The remaining material, a crude pathotoxin extract, was dissolved in 12 ml of ethyl acetate, and stored at -4 °C until the time of usage. To obtain the required amount of pathotoxin for the experiments, this extraction was done twice.

Callus mass increase tests. SM-toxin was insoluble in water, but became water-soluble after a small amount of absolute ethanol was added. For testing the effect of the pathotoxin extract on the mass increase of the callus, pre-weighed pieces of maize callus (average approximately 0.11 gram), were placed on 6.5 ml of culture medium in flat-bottomed test tubes (100 mm x diam. 24 mm). Either 0, 0.01, 0.1, 1.0 or 10.0 ml of SM-toxin per litre was added to the culture medium. Preliminary tests indicated that SM-toxin had still phytotoxic effects after heat-treatment, and therefore the toxin was added to the medium before autoclaving.

To evaluate the effect of the solvent, a second control series with culture medium containing ethyl acetate was used. A different concentration of ethyl acetate, 2.0, 20.0, and 10.0 ml per litre, was used in replication 1, 2 and 3 respectively. The 10 ml/l treatment contained the same amount of ethyl acetate as the highest SM-toxin concentration.

Per treatment, 49 pieces of callus were used and each treatment was repeated three times. The callus was incubated for seven weeks in an incubator at 26 °C with a 16 hour photoperiod. After this time period, the callus pieces were weighed to determine the mass increase. After weighing, the pieces of callus were placed onto culture medium without SM-toxin to observe regrowth.

To establish the mass increase of the callus during the treatment with SM-toxin, ten pieces of callus were randomly taken, at weekly intervals, weighed under sterile conditions, and returned to the culture medium. This proved to be a very laborious method, and the results obtained from this test did not warrant a continuation of this approach in the two subsequent experiments.

Regrowth. After seven weeks on SM-toxin-free medium, ten pieces of callus were selected randomly from each toxin level and weighed, to determine whether differences in growth rate existed. The growth rate was calculated by dividing the final callus mass, i.e. the mass at the end of the regrowth period, by the initial mass, i.e. the mass at the beginning of the regrowth experiment. The callus mass increase per day was also calculated.

Pieces of callus were photographed every two weeks to visualize differences in regrowth.

Transmission electron microscopy. Callus pieces, taken randomly from each treatment after the six weeks of exposure to toxin-containing medium, were fixed in a 3% glutaraldehyde in 0.05 M sodium cacodylate buffer (pH 6.8-7.2), washed twice in the same buffer, and post-fixed for 2 h in 2% buffered OsO_4. After washing in 0.05 M sodium cacodylate buffer (pH 6.8-7.2) twice, the material was dehydrated in an ethanol series and embedded in Spurr's epoxy medium (Spurr, 1969) under high vacuum. The specimen blocks were sectioned and the sections were stained for 10 minutes with 2% uranyl acetate, washed twice with double-

distilled water, post-stained in lead citrate (Reynolds, 1963) for 10 min, and washed again in double-distilled water. The sections were viewed with a Jeol® 100 CX transmission electron microscope at 80 kV.

Seedling tests. The effect of the toxin was tested on twelve 21-day-old maize seedlings of both inbred line I137TN WP87/17->19 (I137TN) and F2834T x B383Y 07/53x54 (F2834T) (supplied by Dr. H.O. Gevers, Summer Grain Sub Centre, Pietermaritzburg, South Africa). These lines are susceptible and resistant to *S. macrospora*, respectively. The plants were injected at the base of the stalk with 0.1 ml of a 50 ml/l SM-toxin solution. Two sets of control plants were used: one set injected with a 50 ml ethyl acetate per litre deionised water solution at the stalk base, while the second set was injected with deionised water. This experiment was replicated three times.

All seedling tests were performed under greenhouse conditions, and the plants were allowed to grow for four weeks after treatment. To establish the effect of the toxin, all plants were carefully examined for necrotic spots or lesions. The height of the plants, from stalk base to the tip of the longest leaf, was measured. After these assessments, the above-ground parts of the plants were dried in an oven at 100 °C for seven days, and the dry mass of the combined sample was recorded.

RESULTS

Callus mass increase tests. For all replications, the data showed a significant ($P < 0.05$) reduction in callus mass increase at the highest SM-toxin level (Table 1). The mass increase of callus grown at the 1.0 ml/l concentration was in all tests significantly ($P < 0.05$) larger than that of the 0.1 and the 0.01 ml/l concentrations, while in both replication 2 and 3 it was also significantly larger than the mass increase of callus grown without the toxin. To eliminate the differences between the replications, statistical analysis was done on the mass increase relative to the control. These data (Table 1) do not show many statistically significant ($P < 0.05$) differences. Only the differences between control and 10 ml/l, 1.0 ml/l and 10.0

TABLE 1 Average mass increase (g) of maize, *Zea mays*, callus grown for six weeks on culture medium (Green & Phillips, 1975) containing different amounts of SM-toxin (see also APPENDIX 4.1)

concentration (ml/l)	replication 1	2	3	average
0 (control)	0.271 a	0.457 ab	0.372 a	0.369 ab
0.01	0.099 b	0.397 b	0.371 a	0.299 bc
0.1	0.140 b	0.504 a	0.389 a	0.345 abc
1.0	0.219 a	0.665 c	0.628 b	0.487 a
10	0.007 c	0.318 d	0.120 c	0.122 c

Figures in a column followed by the same letter do not differ significantly ($P < 0.05$) in a LSD test

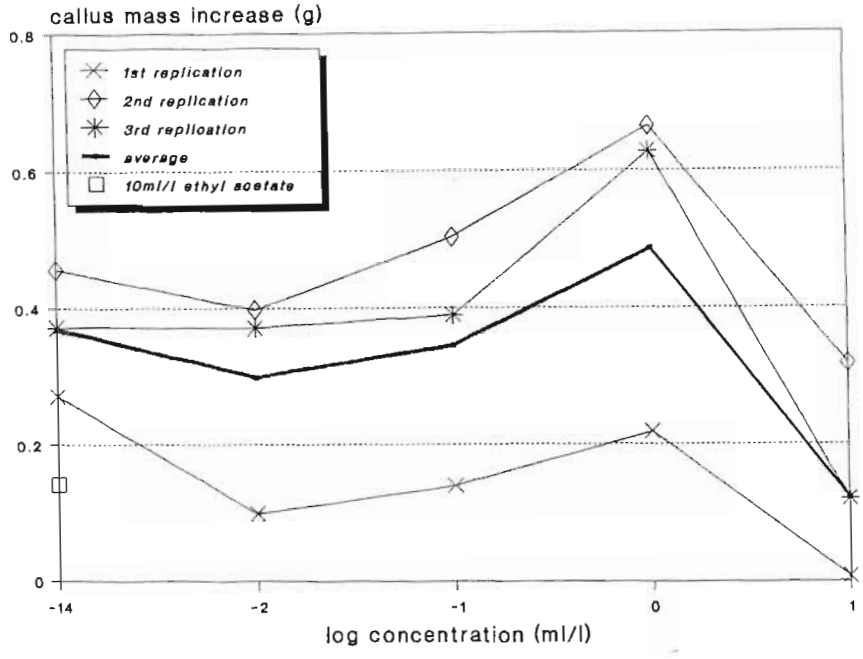


FIGURE 1 Average mass increase (g) of maize, *Zea mays*, callus grown for six weeks on culture medium (Green & Phillips, 1975) containing different amounts of SM-toxin

ml/l, and 0.01 ml/l and 1.0 ml/l treatment were found to be significant ($P < 0.05$). Although differences existed between the replications of the same treatment, a general trend becomes clear when the results are displayed graphically (Fig. 1). A slight drop in callus mass increase occurred at the 0.01 ml/l compared to the control, while the 0.1 ml/l treatment had approximately the same mass increase as the control. Compared to the 0.01 and 0.1 ml/l levels of the test, the 1.0 ml/l had a higher value for callus mass increase. In both replication 2 and 3 the mass increase at the 0.1 and 1.0 levels was actually higher than the mass increase of

the control. In all replications the highest concentration of 10 ml/l gave a significant ($P < 0.05$) reduction of callus mass increase when compared with all other treatments.

With an increasing ethyl acetate concentration a distinct decrease in callus mass increase was observed. The lowest concentration (2 ml/l) ethyl acetate used did not influence the callus mass increase at all (0.276 $\pm$ 0.212 g for the ethyl acetate compared to 0.271 g for the control). The amount of ethyl acetate (10 ml/l) used at the highest concentration SM-toxin gave a significant reduction in callus mass increase (0.166 $\pm$ 0.213 g) compared to the control (0.372 $\pm$ 0.288 g). At the highest level of 20 ml/l a direct phytotoxic effect was noticeable, the average callus mass decreased with 0.005 $\pm$ 0.013 gram over the experimental period (see also APPENDIX 4.1).

The mass increase of callus during the six weeks of replication 1 was logarithmic for all treatments with SM-toxin, except the 10 ml/l concentration, which showed a more linear trend (Fig. 2, see also APPENDIX 4.6). Callus grown on culture medium containing ethyl acetate (2 ml/l) also displayed a logarithmic growth curve (Fig. 2).

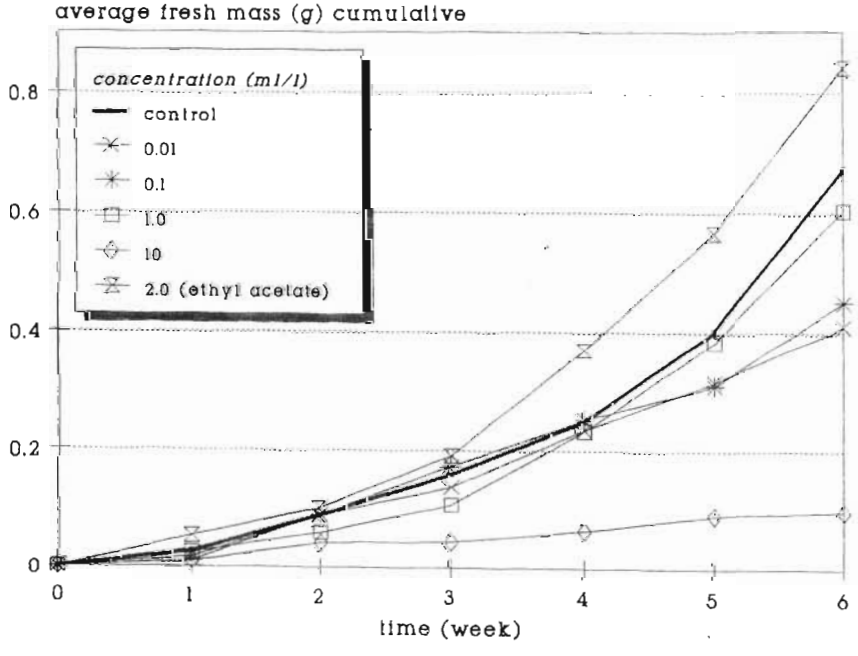


FIGURE 2 Cumulative average fresh mass increase (g) of maize, *Zea mays*, callus grown on culture medium (Green & Phillips, 1975) containing different amounts of SM-toxin over a six week interval

Although the growth rate of the callus grown on medium containing 10 ml SM-toxin per litre was much lower than that of the control (Table 2), this difference was not statistically significant ($P < 0.05$). However, the growth rate of the highest concentration was significantly different from that of the 0.1 and the 1.0 ml/l levels. Adding 10 ml/l ethyl acetate to the growing medium did not seem to effect the growth rate of the callus, only a statistical ($P < 0.05$) difference with the 1.0 ml/l SM-toxin concentration was found.

After seven weeks, the callus of the control treatment and that grown at the intermediate SM-toxin concentrations of 0.1 and 1.0 ml/l appeared very healthy and had increased considerably in size (Table 1, and column 1/row A, C and D resp. in Plate 1). The calli treated with the lowest toxin level (0.01 ml SM-toxin/l) were slightly less well developed, but had visibly grown (Table 1, and column 1/row B in Plate 1). However, the callus treated with the highest toxin level (10 ml SM-toxin/l) had visibly grown less than that at all other concentrations (Table 1, and column 1/row E in Plate 1). Callus grown on medium containing 10 ml/l ethyl acetate, did not show any difference in development from that of the control (column 1/row F in Plate 1).

Regrowth. After seven weeks of toxin treatment, calli from all SM-toxin concentration levels were transferred to toxin-free medium. After six weeks, no change in the visual appearance of the callus derived from the various SM-toxin concentrations could be observed (Plate 1).

Again the growth rate of the 10 ml/l SM-toxin concentration was lower than that of all other treatments, but only significant differences ($P < 0.05$) were found with the 0.1 and 1.0 ml/l levels (Table 2). During the seven-week regrowth period, the growth rate of the 1.0 ml/l concentration was also significantly ($P < 0.05$) different than that of the control, 0.01 ml/l, and ethyl acetate treatment. It would appear as if the growth rates, induced by the toxin treatment, were retained even after the callus was transferred to toxin-free medium, and significant differences found in the toxin-treatment were also observed during the regrowth period.

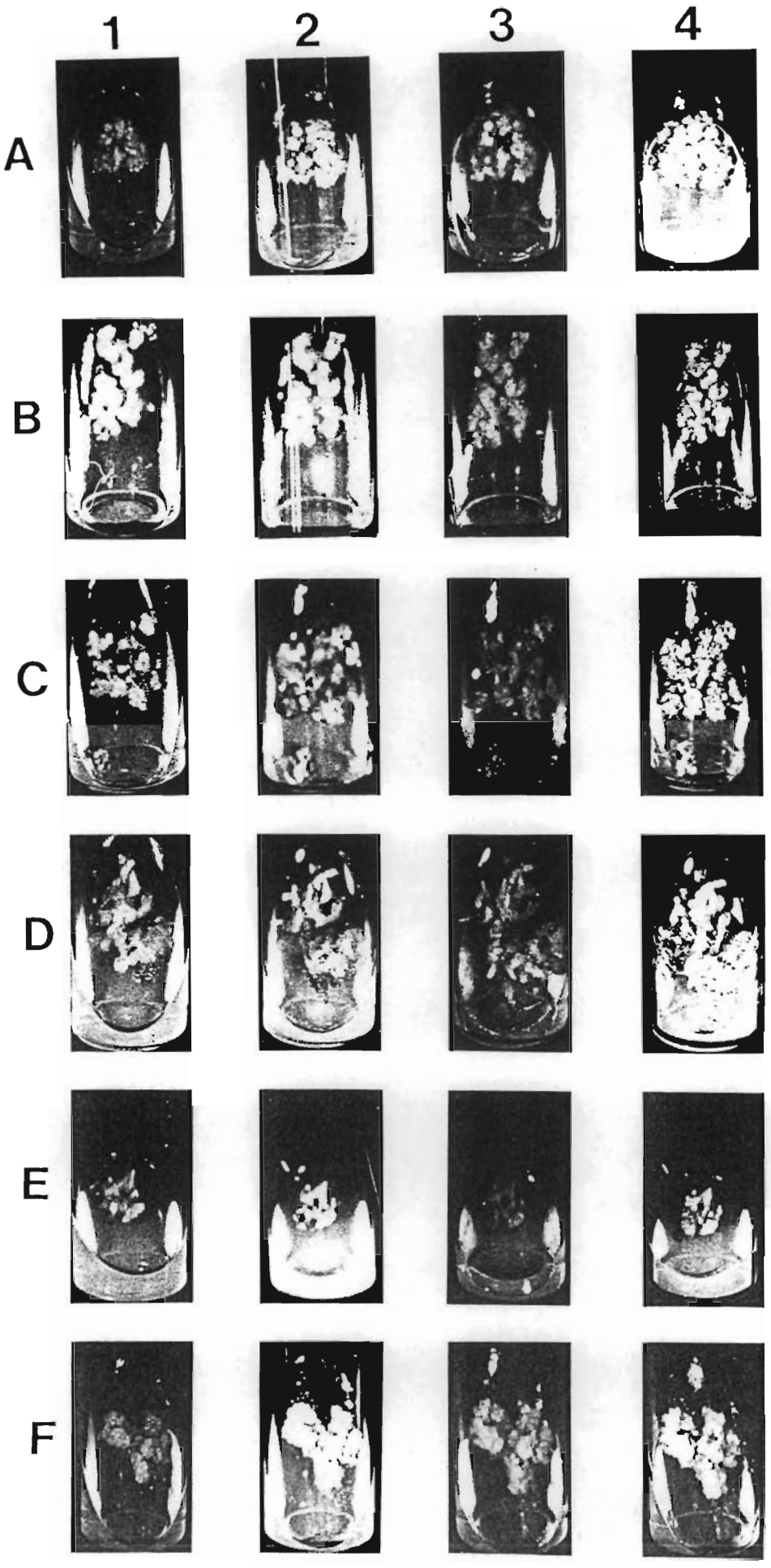
The only significant differences ($P < 0.05$) in growth rate over the duration of both the growth period on toxin-containing and toxin-free medium (week 0-14) were found between both the 0.1 and 1.0 ml/l treatment and the 10 ml SM-toxin per litre

PLATE 1

Regrowth over a six week period (columns) of maize, *Zea mays*, callus pieces growing on culture medium (Green & Phillips, 1975) after treatment with different concentrations (rows) of SM-toxin, a pathotoxin extract of *Stenocarpella macrospora* (tube-width is 24 mm)

A. Control, no SM-toxin added to the culture medium
B. 0.01 ml/l SM-toxin in the culture medium
C. 0.1 ml/l SM-toxin in the culture medium
D. 1.0 ml/l SM-toxin in the culture medium
E. 10 ml/l SM-toxin in the culture medium
F. 10 ml/l ethyl acetate in the culture medium

1 Week 0; start of the experiment, the ending of the callus growth test
2 Week 2; two weeks after week 0
3 Week 4; two weeks after week 2
4 Week 6; two weeks after week 4


1
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E
F

TABLE 2 Growth rate of maize, *Zea mays*, callus growing on culture medium, 1975) during (week 0-7) and after (week 7-14) treatment with different concentrations of SM-toxin (see also APPENDIX 4.7)

concentration (ml/l)	growth rate week 0- 7	week 7-14	week 0-14
0 (control)	5.65 abc	1.39 ab	9.79 ab
0.01	5.05 abc	1.57 ab	9.79 ab
0.1	6.98 bc	1.75 bc	15.92 b
1.0	8.68 c	2.09 c	17.98 b
10	1.86 a	1.15 a	2.14 a
ethyl acetate*	4.54 ab	1.55 ab	9.46 ab

Figures in a column followed by the same letter do not differ significantly in a LSD test ($P < 0.05$)
*ethyl acetate concentration was 10 ml/l

(Table 2). No difference in growth rate was found between the control, 0.01 ml/l level and the 10 ml/l ethyl acetate treatment. Although the 10 ml/l SM-toxin concentration had a much lower growth rate (2.14), it was not statistically ($P < 0.05$) different from the treatments with a growth rate of approximately 9.5 (week 0-14 in Table 2).

The increase in mass per day over the regrowth period (week 7-14) showed significant differences between all treatments, except the 0.1 ml/l, and the 1.0 ml/l (Table 3). All significant differences ($P < 0.05$) between the SM-toxin levels, found

TABLE 3 Average mass increase per day (g) of maize, *Zea mays*, callus (n = 10) growing on culture medium, during (week 0-7) and after (week 7-14) treatment with SM-toxin (See also APPENDIX 4.7)

concentration (ml/l)	mass increase (g per day) week 0- 7	week 7-14	week 0-14
0 (control)	0.008 ab	0.006 ab	0.007 ab
0.01	0.007 ab	0.007 ab	0.007 ab
0.1	0.009 bc	0.010 bc	0.010 bc
1.0	0.014 c	0.015 c	0.014 c
10	0.002 a	0.001 a	0.001 a
ethyl acetate*	0.006 ab	0.005 ab	0.005 ab

Figures in a column followed by the same letter do not differ significantly in a LSD test ($P < 0.05$)
*ethyl acetate concentration was 10 ml/l

during the toxin treatment (week 0-7), were also retained in the regrowth period (week 7-14). Even though the mass increase of the 10 ml/l concentration was only 0.001 g/day, this was not significantly ($P < 0.05$) lower than that of the control. The mass increase per day of the ethyl acetate treatment was slightly, but not significantly ($P < 0.05$), less than that of the control.

Transmission electron microscopy. Untreated maize callus cells had nuclei with complete nuclear envelopes, mitochondria, large vacuoles, and, in the cytoplasm, proplastids and occasionally chloroplasts were observed (Plate 2, Fig. 1). The walls of these cells were approximately 0.11 μm thick.

Compared to the control, with an increasing level of SM-toxin an increasing amount of starch grains was observed in the proplastids (Plate 2, Figs. 2 and 3; Plate 3, Figs. 1 and 2). The cristae in the mitochondria appeared to be absent in all SM-toxin treatments. No effect of the pathotoxin was observed on the nucleus, or the thickness of the cell walls.

The ultrastructure of callus grown on medium with 10 ml/l ethyl acetate did not differ from the control, although occasionally the nuclear envelope was distorted (Plate 3, Fig. 3).

Seedling tests. In all plants, lesions surrounded by a small necrotic area appeared at the site of injection. Occasionally flecking of the leaves was observed, however, this seemed to be caused by pressure of the injected liquid rather than as an effect of the toxin. No phytotoxic effects were noticed.

In two replications, SM-toxin-treated plants of the *S. macrospora*-susceptible line, I137TN, were found to be significantly ($P < 0.05$) shorter than the control plants (Table 4). Plants of this line, which were injected with ethyl acetate, showed significant ($P < 0.05$) height differences with the control in one replication.

To eliminate differences between the replications, the height relative to the control was used to assess height differences. These values did not show any significant differences ($P < 0.05$) between plants injected with SM-toxin and plants injected with ethyl acetate (Table 5). Both treatments were significantly ($P < 0.05$) shorter than untreated plants, the reduction in height was 81% and 85% for SM-toxin and

PLATE 2

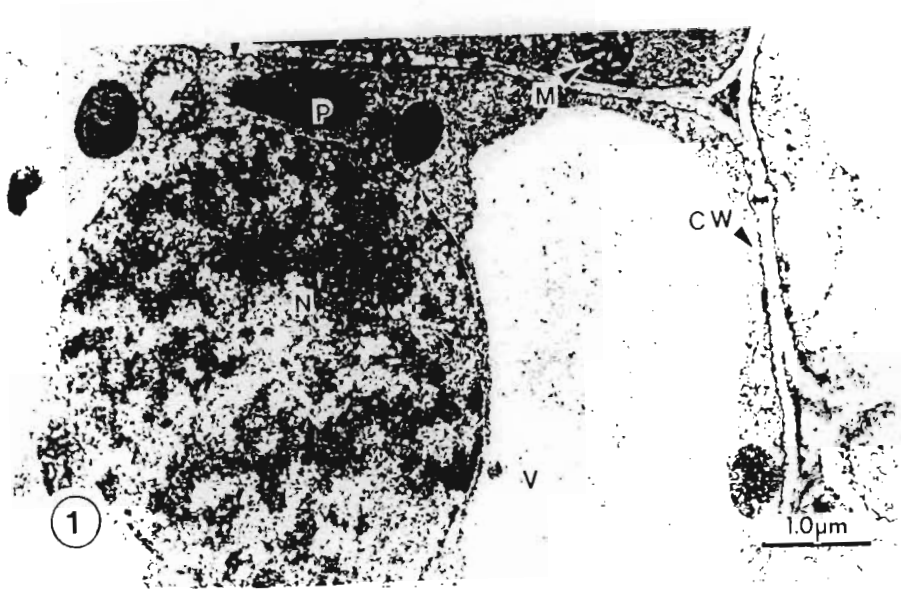
Transmission electron micrographs of callus cells of maize, *Zea mays*, grown for six weeks on culture medium (Green & Phillips, 1975) containing different amounts of SM-toxin, a pathotoxin extract of *Stenocarpella macrospora*

FIGURE 1 Callus cells grown on medium without SM-toxin (control). Please note that no starch is present in the proplastids, and the presence of cristae in the mitochondrion

FIGURE 2 Callus cell grown on medium containing 0.01 ml/l SM-toxin. Please note the starch in the proplastids, and the lack of cristae in the mitochondria

FIGURE 3 Callus cells grown on medium containing 0.1 ml/l SM-toxin. Please note the increase size of the starch grains compared to the 0.01 ml/l concentration (Fig. 2)

CW = Cell Wall
ER = Endoplasmic reticulum
M = Mitochondrion
N = Nucleus
Nu = Nucleolus
P = Proplastid
V = Vacuole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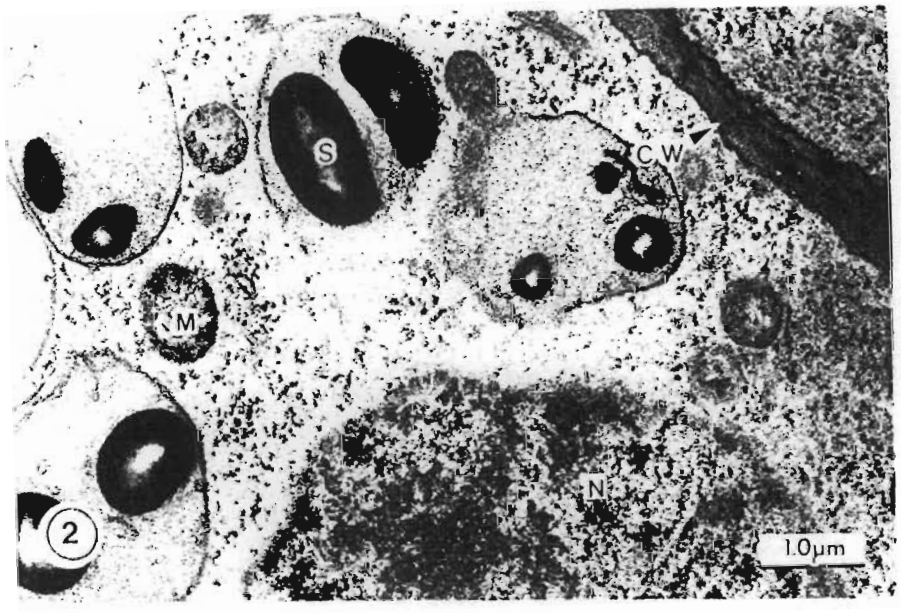
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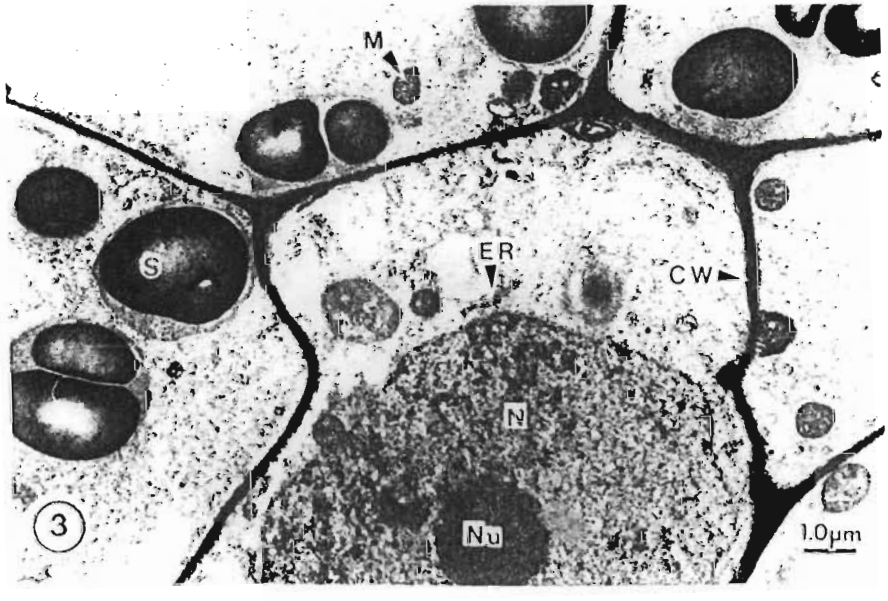
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PLATE 3

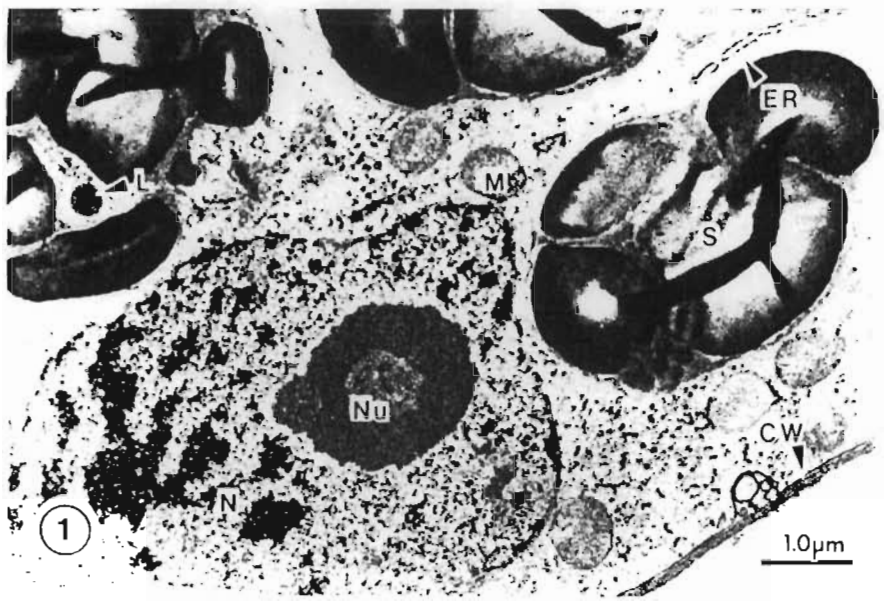
Transmission electron micrographs of callus cells of maize, *Zea mays*, grown for six weeks on culture medium (Green & Phillips, 1975) containing different amounts of SM-toxin, a pathotoxin extract of *Stenocarpella macrospora*

FIGURE 1 Callus cell grown on medium containing 1.0 ml/l SM-toxin. Please note the large amounts of starch

FIGURE 2 Callus cell grown on medium containing 10 ml/l SM-toxin. Please note that there is no obvious difference with the 1.0 ml/l treatment (Fig. 1)

FIGURE 3 Callus cell grown on medium containing 10 ml/l ethyl acetate (no SM-toxin). Please note the distortion of the nuclear envelope (arrows)

CW = Cell Wall
ER = Endoplasmic reticulum
M = Mitochondrion
N = Nucleus
Nu = Nucleolus
P = Proplastid
V = Vacuole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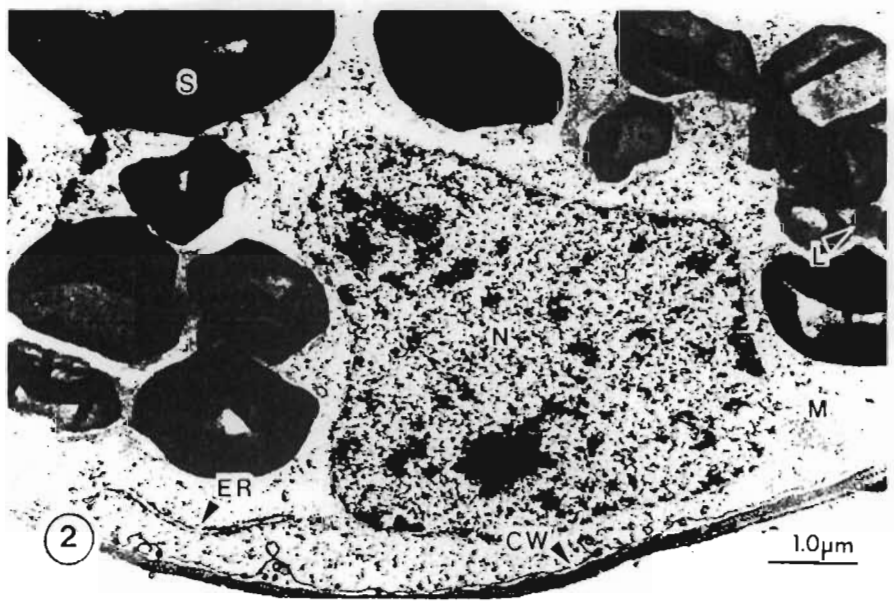
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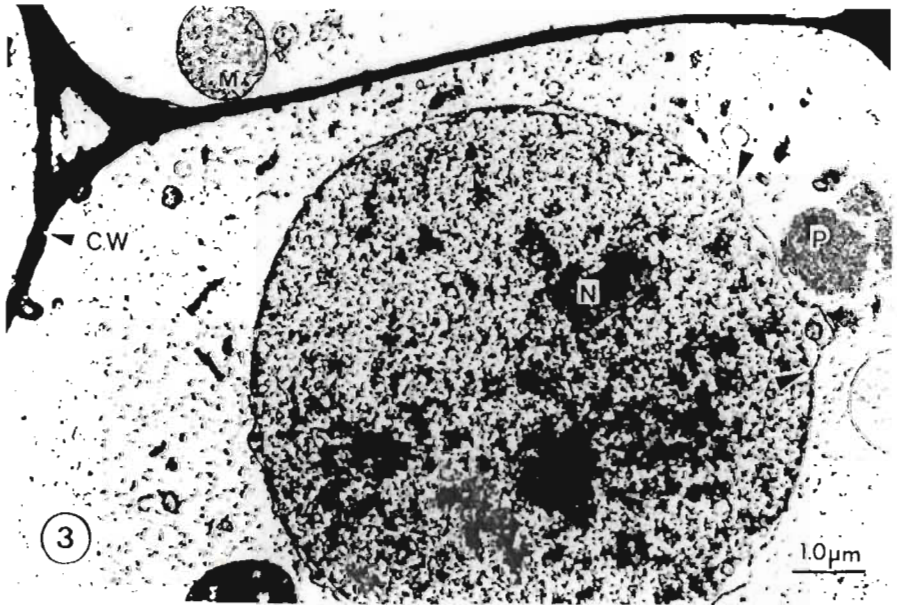
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TABLE 4 Average height (cm) of maize, *Zea mays*, seedlings, injected with 0.1 ml of a 50 ml/l solution of either SM-toxin or ethyl acetate at the base of the stalk at 21-days, four weeks after treatment. The control was injected with 0.1 ml deionised water (see also APPENDIX 4.9 and 4.11)

line	treatment	replication 1	2	3	average
I137TN	SM-toxin	49.1 a	37.0 a	29.9 a	39.2 a
	ethyl acetate	50.9 a	32.9 a	36.2 ab	41.2 a
	control	55.8 a	43.5 b	42.0 b	48.3 a
F2834	SM-toxin	57.6 a	33.1 a	32.0 a	38.8 a
	ethyl acetate	56.8 a	33.2 a	46.9 b	45.4 a
	control	57.2 a	31.3 a	47.3 b	45.3 a

Figures of an inbred line in a column followed by the same letter do not differ significantly in a LSD test ($P < 0.05$)

TABLE 5 Average height of maize, *Zea mays*, seedlings, injected with 0.1 ml of a 50 ml/l solution of either SM-toxin or ethyl acetate at the base of the stalk at 21 days, four weeks after treatment, expressed as a percentage of the height of the control (plants injected with 0.1 ml deionised water)

line	treatment	replication 1	2	3	average
I137TN	SM-toxin	88.0	85.1	71.2	81.3 a
	ethyl acetate	91.2	75.6	86.2	85.2 a
	control	100.0	100.0	100.0	100.0 b
F2834	SM-toxin	100.7	105.8	67.7	87.7 a
	ethyl acetate	99.3	106.1	99.2	100.2 a
	control	100.0	100.0	100.0	100.0 a

Figures of an inbred line in a column followed by the same letter do not differ significantly in a LSD test ($P < 0.05$)

ethyl acetate injected plants respectively.

Only in one replication did SM-toxin have a significant ($P < 0.05$) effect on the height of the *S. macrospora*-resistant line, F2834T (Table 4). The height of plants of this inbred line was not affected by the injection with ethyl acetate.

The values relative to the control showed an approximately 12% height reduction of the seedlings treated with SM-toxin. However, this was caused by the height reduction in replication 3, and it could not be statistically supported ($P < 0.05$).

In both inbred line I137TN and F2834, no significant ($P < 0.05$) differences in dry mass were found between any of the treatments (Table 6). However, when the values relative to the control are used for statistical analysis, the dry mass of the above-ground parts of the *S. macrospora*-susceptible inbred line injected with SM-toxin was found to be statistically ($P < 0.05$) less than that of the control, or those treated with ethyl acetate (Table 7).

TABLE 6 Average dry mass (g) of the above-ground parts of maize, *Zea mays*, seedlings, injected with 0.1 ml of a 50 ml/l solution of either SM-toxin or ethyl acetate at the base of the stalk at 21 days, four weeks after treatment. The control was injected with 0.1 ml deionised water (see also APPENDIX 4.9 and 4.11)

line	treatment	replication 1	2	3	average
I137TN	SM-toxin	0.42	0.30	0.26	0.33 a
	ethyl acetate	0.43	0.38	0.38	0.40 a
	control	0.62	0.42	0.40	0.48 a
F2834	SM-toxin	0.54	0.35	0.37	0.42 a
	ethyl acetate	0.52	0.36	0.66	0.51 a
	control	0.63	0.23	0.65	0.50 a

Means of an inbred line followed by the same letter do not differ significantly in a LSD test ($P < 0.05$)

TABLE 7 Average dry mass (g) of the above-ground parts of maize, *Zea mays*, seedlings, injected with 0.1 ml of a 50 ml/l solution of either SM-toxin or ethyl acetate at the base of the stalk at 21 days, four weeks after treatment, expressed as a percentage of the dry mass of the control (plants injected with 0.1 ml deionised water)

line	treatment	replication 1	2	3	average
I137TN	SM-toxin	67.7	71.4	65.0	66.0 a
	ethyl acetate	69.4	90.5	95.0	80.0 b
	control	100.0	100.0	100.0	100.0 b
F2834	SM-toxin	85.7	152.2	56.9	82.0 a
	ethyl acetate	82.5	156.5	101.5	104.0 a
	control	100.0	100.0	100.0	100.0 a

Means of an inbred line followed by the same letter do not differ significantly in a LSD test ($P < 0.05$)

DISCUSSION

The pathotoxin extract from *Stenocarpella macrospora* did show phytotoxic effects, although they were often not statistically significant. One of the most characteristic features was the distinct growth-stimulating effect of the extract at the 1.0 ml/l concentration. Since no chemical analysis was done on the toxic concentrate, the substance responsible remains unknown.

The phytotoxic effects of substances at the low toxin concentrations (0.01 ml/l) were apparently counteracted by a stimulatory compound, which became clear at a toxin concentration of 1.0 ml/l. The stimulatory effect of this compound is, in turn, counteracted by toxic effects at the highest toxin concentration. It is not known whether the toxic or stimulatory substances occur singly or whether several act in concert, neither is it clear whether the toxic factor(s) at low toxin concentrations is/are the same as that/those expressed at the highest SM-toxin concentration.

Although the callus mass increase test with 10 ml/l ethyl acetate indicated that this solvent caused a reduction in callus mass increase, the values of the growth rate and the callus mass increase per day do not support this finding. The lower initial callus mass of the ethyl acetate treatment (APPENDIX 4.1) might be responsible for the lower mass increase, in which case it can be concluded that 10 ml/l ethyl acetate does not have an effect on callus mass increase.

The regrowth period (week 7-14), when callus was placed on SM-toxin-free medium after seven weeks of growth of toxin containing medium, did not alter the order of the growth rates, or the callus mass increase per day. This means that the effects of the toxin were permanent, or at least, long-lasting, and once the growth had been affected by the toxin no change in growth rate could be observed during the regrowth period on toxin-free medium. This is an effect different from that found in the tests with fumonisin B_1 (Chapter 2 of this thesis) where the growth of all toxin levels, but the highest, recovered to the same level after transferring the callus pieces to toxin-free medium.

A change in the ultrastructure of mitochondria, after treatment with a pathotoxin, was also observed in other studies, e.g. AL-toxin of *Alternaria alternata* (Fr.) Keissler f.sp *lycopersici* (Park *et al.*, 1981), and cercosporin, a toxin produced by *Cercospora beticola* Speg. (Steinkamp *et al.*, 1981).
The increasing amount of starch, with an increasing level of toxin, could be explained by an increase in the mobilization of lipids, stimulation of starch synthesis, or decreased translocation of sugars (Hanchey, 1981). The stunting of seedlings, found after treatment with tentoxin, a toxin produced by *A. alternata*, suggests that the decreased translocation of sucrose is, at least partly, responsible for the accumulation of starch (Templeton *et al.*, 1967). This hypothesis has been supported by later findings of Schadler *et al.* (1976), and the results with fumonisin B_1 in the present study (Chapter 2).

Although no statistical ($P < 0.05$) differences could be found between treatments in the height or the dry mass of above-ground parts of plants of the *S. macrospora* -resistant inbred line, treatment with both SM-toxin and ethyl acetate caused a significant ($P < 0.05$) reduction in height of susceptible seedlings. In this line no significant difference was found between the dry mass of ethyl acetate-injected plants and the control. The SM-toxin-treated *S. macrospora*-susceptible plants did have a significant ($P < 0.05$) reduction in plant height and a significantly ($P < 0.05$) lower dry mass of the above-ground parts.
These results indicate that the pathotoxin extract of *S. macrospora* has a host-specific effect on seedlings: susceptible lines are found to be susceptible to SM-toxin, and resistant lines are less or not susceptible. Similar host-specific toxins are found, *inter alia*, in *Bipolaris* spp., e.g. *B. maydis* (Nisik & Miyake) Shoem. (Brettell *et al.*, 1980), and in the formae speciales of *A. alternata* (Nishimura & Kohmoto, 1983).
The growth-stimulating properties of SM-toxin were not observed in the seedlings, but this might be caused by the higher concentration of toxin used in this experiment.
No adequate explanation could be found for the difference in reaction of the two inbred lines to ethyl acetate.

From these results it can be concluded that a crude pathotoxin extract from *S. macrospora* might not only contain the mycotoxins found by Chalmers *et al.* (1978), Cutler *et al.* (1980a & 1980b) and Probst & Tamm (1982), but also phytotoxins which cause growth inhibition of callus and seedlings.
To those wishing to pursue this research, it is recommended that the solubility of the pathotoxin extract may be improved by dissolving the crude extract in methanol instead of ethyl acetate. Previous research has shown that 2 ml/l methanol has no noticeable effect on maize callus (see Chapter 3).

The trends found in the research with maize seedlings indicate that the toxin-extract used in these studies can be classified as host-specific toxin. Host specific toxins have been used for selection of disease resistance (e.g. Rines & Luke, 1985; Daub, 1986; Witsenboer *et al.*, 1988), and the results obtained in this study open the possibility of the use of SM-toxin in *in vitro* selection of toxin-insensitive cell cultures of maize lines. Although regeneration was not achieved with the inbred line used in this study, regeneration studies of South African inbred lines show a promising future for *in vitro* selection for disease resistance in local inbreds (Woodward & Furze, 1988; Van Asch, unpublished data).

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Statistical analysis of latent period data for inoculation with 3SA126 on Morocco, for inoculation with 3SA86 only on SST 25 (single), and for inoculation with 3SA86 on SST 25 at different time intervals after inoculation with 3SA126 (double). Data in APPENDIX 1.2, 1.3, 1.4 and 1.5

	time interval	rep.	n =	mean	standard deviation	value minimum	value maximum	range
Morocco	1 day	1	20	204.9	2.7	201.4	211.0	9.6
		2	39	212.4	7.6	199.3	231.4	32.1
	4 days	1	35	203.9	8.7	191.7	222.1	30.4
		2	24	207.7	4.6	196.7	215.3	18.6
	7 days	1	37	216.8	8.7	196.4	234.0	37.6
		2	40	217.2	6.7	203.6	228.9	25.3
	10 days	1	24	207.6	10.9	191.9	233.5	41.6
	MEAN		7	210.0	5.4	203.9	217.2	13.3
SST 25 single	1 day	1	21	203.1	4.0	193.5	211.0	17.5
		2	118	208.4	8.3	198.1	234.8	36.7
	4 days	1	85	214.6	5.9	199.3	226.8	27.5
		2	34	212.0	4.6	202.7	222.7	20.0
	7 days	1	91	211.8	11.6	170.5	234.5	64.0
		2	112	215.2	8.4	201.6	234.4	32.8
	10 days	1	62	209.4	10.4	192.2	233.5	41.3
	MEAN		7	210.6	4.1	203.1	215.2	12.1
SST 25 double	1 day	1	28	202.4	4.3	194.7	210.5	15.8
		2	87	205.0	8.7	174.0	232.9	58.9
	4 days	1	86	218.7	8.2	199.9	235.5	35.6
		2	20	215.4	7.0	207.4	228.5	21.1
	7 days	1	41	213.3	11.0	190.9	234.5	43.6
		2	49	221.2	8.8	202.0	234.4	32.4
	10 days	1	42	210.7	10.5	192.5	234.0	41.5
	MEAN		7	212.4	6.9	202.4	221.2	18.8

Latent period data of the tests with a one-day-interval: for inoculation with 3SA126 on Morocco, for inoculation with 3SA86 only on SST 25 (single), and for inoculation with 3SA86 on SST 25 one day after inoculation with 3SA126 (double).

	replication 1			replication 2					
	Morocco single	SST 25 single	SST 25 double	Morocco single	SST 25 single	SST 25 single	SST 25 single	SST 25 double	SST 25 double
1	201.4	193.5	194.7	199.3	198.1	204.9	218.2	174.0	205.1
2	201.8	198.6	197.1	199.4	198.4	205.0	218.4	179.8	205.4
3	201.8	198.7	197.1	200.8	198.8	205.3	218.9	189.8	206.0
4	202.1	199.3	197.3	200.8	198.9	205.4	219.7	191.1	206.0
5	202.4	200.0	198.5	202.0	199.3	205.5	220.4	193.4	206.4
6	202.9	200.7	198.7	203.1	199.5	205.8	221.0	194.3	206.4
7	203.5	200.7	198.8	206.6	199.5	205.9	221.4	196.8	206.7
8	203.8	201.2	199.3	206.8	199.7	205.9	223.7	196.9	206.9
9	204.3	203.5	200.1	207.2	200.1	205.9	223.9	197.0	207.2
10	204.5	203.6	200.1	207.3	200.1	205.9	224.8	198.7	207.5
11	204.7	203.7	200.3	207.7	200.2	206.2	225.4	198.8	207.6
12	205.1	203.8	200.5	207.7	200.2	206.3	226.2	198.8	207.7
13	205.5	204.1	200.6	207.7	200.2	206.4	226.5	199.0	208.3
14	205.6	204.3	200.8	208.1	200.2	206.5	226.9	199.3	208.9
15	206.0	205.0	201.1	208.1	200.3	206.9	227.1	199.3	209.2
16	206.6	205.2	201.4	209.7	200.3	207.2	227.7	199.8	209.2
17	206.9	205.5	202.9	210.4	200.3	207.3	229.7	200.0	209.3
18	207.8	206.6	204.5	210.6	200.4	207.4	234.8	200.1	209.4
19	210.5	206.7	205.6	211.0	200.5	208.4	(n=118)	200.2	209.8
20	211.0	209.6	206.0	211.3	200.8	208.5		200.3	210.3
21	(n=20)	211.0	206.5	213.0	200.9	208.6		200.7	210.8
22		(n=21)	206.5	213.5	200.9	208.8		200.8	211.4
23			206.7	214.3	201.1	208.9		200.8	212.2
24			207.6	215.1	201.1	209.0		201.1	213.0
25			208.0	215.6	201.1	209.1		201.1	213.0
26			208.4	216.2	201.5	209.3		201.3	213.5
27			208.6	217.0	201.5	209.4		201.3	213.5
28			210.5	218.0	201.6	209.4		201.4	213.6
29			(n=28)	218.5	201.6	209.9		201.4	215.7
30				218.7	201.7	210.1		201.5	215.8
31				219.0	201.8	210.2		201.6	219.9
32				219.1	201.8	210.2		202.0	221.0
33				220.4	201.8	210.3		202.3	221.2
34				220.4	202.0	210.5		202.3	221.8
35				220.5	202.3	211.3		202.4	223.1
36				221.3	202.3	211.5		202.6	225.0
37				221.3	202.4	211.6		202.7	232.9
38				226.4	202.5	211.8		202.8	(n=87)
39				231.4	202.7	211.8		203.2	
40				(n=39)	202.9	212.5		203.6	
41					203.0	213.5		203.6	
42					203.1	213.7		203.6	
43					203.1	214.2		203.6	
44					203.4	214.3		204.2	
45					204.3	215.6		204.4	
46					204.3	215.6		204.5	
47					204.5	216.2		204.8	
48					204.8	216.4		205.0	
49					204.8	216.8		205.0	
50					204.9	216.9		205.1	

Latent period data of the tests with a four-day-interval: for inoculation with 3SA126 on Morocco, for inoculation with 3SA86 only on SST 25 (single), and for inoculation with 3SA86 on SST 25 one day after inoculation with 3SA126 (double).

	replication 1					replication 2 (flagleaf)		
	Morocco single	SST 25 single	SST 25 single	SST 25 double	SST 25 double	Morocco single	SST 25 single	SST 25 double
1	191.7	199.3	217.3	199.9	221.7	196.7	202.7	207.4
2	192.0	202.5	217.3	200.6	221.7	202.6	203.4	207.9
3	192.2	203.2	217.4	203.0	222.1	203.2	203.8	208.4
4	193.4	203.5	217.5	203.5	222.1	203.9	206.1	208.5
5	194.7	203.9	217.6	204.3	222.4	204.1	206.6	208.6
6	194.8	205.0	217.9	204.7	222.6	204.2	207.9	209.9
7	195.2	205.8	217.9	205.5	222.7	204.3	208.7	210.3
8	195.6	206.3	218.0	205.5	222.8	205.2	209.2	212.2
9	195.8	206.6	218.0	205.5	223.0	205.7	209.4	212.6
10	195.8	206.7	218.1	205.5	223.5	206.0	209.5	214.3
11	196.2	206.9	218.1	207.6	223.6	206.0	209.7	214.4
12	197.8	206.9	218.2	207.7	224.1	206.2	211.1	215.4
13	198.3	207.4	218.7	207.9	224.3	206.6	211.7	216.0
14	198.9	207.7	218.8	208.6	224.3	208.6	211.7	216.0
15	200.3	207.7	219.5	209.7	224.4	208.8	211.9	218.3
16	200.8	208.4	219.5	210.9	224.8	209.0	211.9	219.3
17	202.2	209.0	219.5	211.3	225.0	209.7	212.1	225.7
18	202.5	209.3	219.7	211.4	225.4	212.4	212.1	225.8
19	203.9	209.6	219.8	212.9	225.8	212.5	212.5	228.5
20	206.3	209.6	220.1	213.4	226.0	212.9	212.5	228.5
21	207.5	209.6	220.3	213.7	226.3	213.0	212.6	(n=20)
22	208.6	209.9	220.3	214.1	226.4	213.2	212.9	
23	208.7	210.2	220.3	214.1	226.7	215.1	213.5	
24	209.7	210.9	221.0	214.2	226.8	215.3	213.9	
25	209.9	211.1	221.0	214.4	227.0	(n=24)	214.0	
26	210.7	211.6	221.2	214.9	227.3		214.2	
27	210.7	211.6	221.2	215.2	227.9		214.4	
28	211.1	211.7	221.7	215.4	227.9		214.5	
29	211.6	212.0	221.9	215.5	228.7		215.6	
30	212.0	212.3	222.3	215.6	229.0		216.2	
31	212.3	212.8	222.3	215.7	229.1		219.0	
32	217.2	212.9	223.3	216.3	229.4		219.1	
33	218.0	213.1	223.8	217.3	231.8		220.1	
34	219.6	213.4	224.8	217.6	231.9		222.7	
35	222.1	213.8	226.8	218.0	235.0		(n=34)	
36	(n=35)	214.0	(n=85)	218.1	235.5			
37		214.5		219.0	(n=86)			
38		214.7		219.5				
39		215.1		219.5				
40		215.2		219.5				
41		215.4		219.8				
42		215.5		220.0				
43		215.6		220.0				
44		216.2		220.4				
45		216.2		220.5				
46		216.3		220.8				
47		216.9		220.9				
48		217.1		221.0				
49		217.2		221.4				
50		217.3		221.5				

Latent period data of the tests with a seven-day-interval: for inoculation with 3SA126 on Morocco, for inoculation with 3SA86 only on SST 25 (single), and for inoculation with 3SA86 on SST 25 one day after inoculation with 3SA126 (double).

	replication 1				replication 2				
	Morocco single	SST 25 single	SST 25 single	SST 25 double	Morocco single	SST 25 single	SST 25 single	SST 25 single	SST 25 double
1	196.4	170.5	215.6	190.9	203.6	201.6	212.8	226.4	202.0
2	197.3	172.8	215.6	192.3	204.8	202.0	213.0	226.7	205.8
3	205.2	175.4	215.7	193.3	205.7	202.3	213.7	227.9	205.8
4	206.3	175.8	216.1	197.0	205.8	202.6	213.8	228.1	206.5
5	206.8	177.2	216.1	197.5	206.6	202.6	214.0	228.2	206.6
6	207.6	191.5	216.4	201.7	208.0	202.7	215.0	229.1	206.6
7	207.9	200.3	216.7	202.0	209.6	202.9	215.1	229.5	208.0
8	209.6	200.4	216.8	202.0	209.6	202.9	215.2	230.3	209.7
9	211.0	202.2	217.0	204.5	210.7	203.3	215.9	230.9	212.1
10	211.2	202.2	217.1	206.8	212.6	204.0	216.0	233.3	213.5
11	212.2	202.6	217.3	207.4	212.8	204.1	216.3	233.6	214.1
12	212.8	203.4	217.8	207.7	214.4	204.4	216.5	234.4	214.8
13	213.0	203.6	217.9	208.0	215.0	204.9	216.8	(n=112)	216.3
14	213.1	203.8	218.0	208.4	216.0	205.1	216.9		217.4
15	214.6	203.9	218.2	209.2	216.9	205.2	217.3		217.4
16	217.6	204.2	218.4	209.3	217.0	205.3	217.7		217.9
17	217.8	204.5	218.5	211.5	217.3	205.3	217.8		218.1
18	217.9	205.6	218.7	211.5	217.3	205.3	218.1		218.6
19	217.9	205.7	218.7	212.0	217.6	205.6	218.1		219.2
20	218.0	206.3	218.8	213.1	217.8	206.0	218.1		219.8
21	218.7	206.8	219.0	213.3	217.9	206.3	218.3		220.4
22	219.3	206.9	219.2	213.4	218.9	206.5	218.6		221.3
23	219.5	207.2	219.2	213.5	219.0	206.6	218.7		221.5
24	219.6	207.8	219.3	214.0	219.5	206.6	218.9		221.5
25	220.7	207.8	219.9	215.7	219.7	206.9	219.0		221.7
26	221.4	208.2	220.1	217.4	220.0	207.1	219.0		221.8
27	222.0	208.7	220.5	219.0	220.6	207.3	219.6		222.6
28	222.3	208.7	221.9	219.4	221.5	207.4	219.7		223.2
29	223.0	208.9	222.0	220.9	221.8	207.5	219.8		223.8
30	223.6	209.3	222.1	221.1	222.0	208.0	219.9		224.4
31	224.0	209.4	222.5	221.6	222.3	208.5	219.9		225.4
32	224.4	210.0	223.0	221.8	222.5	208.8	220.0		226.0
33	224.9	211.1	223.0	222.8	223.4	209.7	220.1		226.0
34	227.4	211.5	223.0	222.8	224.2	210.4	220.4		226.3
35	228.8	212.1	223.4	224.6	224.5	210.4	220.8		226.8
36	233.9	212.4	223.4	224.9	224.9	210.7	221.8		226.9
37	234.0	212.7	223.8	227.9	224.9	210.8	221.9		226.9
38	(n=37)	212.8	223.8	229.0	226.4	211.0	221.9		228.1
39		212.8	224.5	230.0	227.5	211.1	222.2		229.1
40		213.6	228.3	231.3	228.9	211.1	222.7		230.7
41		213.6	234.5	234.5	(n=40)	211.2	223.0		231.3
42		213.9	(n=91)	(n=41)		211.3	223.6		231.6
43		213.9				211.4	223.8		231.6
44		214.3				211.9	224.4		231.8
45		214.3				212.1	225.3		232.3
46		214.4				212.2	225.3		232.7
47		214.4				212.5	225.7		234.0
48		214.9				212.7	225.8		234.0
49		215.0				212.7	226.0		234.4
50		215.3				212.8	226.4		(n=49)

Latent period data of the tests with a ten-day-interval: for inoculation with 3SA126 on Morocco, for inoculation with 3SA86 only on SST 25 (single), and for inoculation with 3SA86 on SST 25 one day after inoculation with 3SA126 (double).

replication 1

	Morocco single	SST 25 single	SST 25 single	SST 25 double
1	191.9	192.2	220.5	192.5
2	195.4	192.8	221.3	193.0
3	195.6	194.1	221.3	197.0
4	197.8	194.3	221.5	197.3
5	197.9	196.4	221.5	197.3
6	199.2	196.8	222.4	200.3
7	199.3	197.0	222.8	200.5
8	200.2	197.3	225.5	200.6
9	200.6	198.4	227.1	200.6
10	200.9	198.6	227.5	203.4
11	202.0	198.9	227.7	203.5
12	206.5	199.1	233.5	203.9
13	207.0	199.2	(n=62)	204.5
14	209.0	199.7		204.5
15	209.5	200.1		205.0
16	209.5	200.2		205.2
17	211.3	200.7		205.4
18	212.3	200.7		206.9
19	214.8	200.7		207.8
20	217.1	201.9		208.5
21	218.4	201.9		209.0
22	225.2	202.1		209.5
23	227.7	203.6		209.5
24	233.5	204.2		210.9
25	(n=24)	204.4		213.1
26		204.7		213.6
27		205.5		213.6
28		205.8		215.6
29		206.5		215.6
30		207.5		216.7
31		207.9		216.9
32		209.0		217.7
33		209.0		218.3
34		209.5		218.8
35		210.7		220.0
36		210.8		223.8
37		212.6		224.3
38		213.1		224.6
39		213.3		225.8
40		214.3		230.4
41		214.3		231.5
42		215.5		234.0
43		216.0		(n=42)
44		216.9		
45		217.4		
46		217.6		
47		218.8		
48		219.2		
49		220.0		
50		220.0		

Statistical analysis of infection frequency data for inoculation with 3SA126 on Morocco, and for inoculation with 3SA86 only on SST 25 (single). Data in APPENDIX 1.7, 1.8, 1.9, 1.10, 1.11, 1.12 and 1.13. N.D. = Not Determined

	time interval	rep.	n =	mean	standard deviation	value minimum	value maximum	range
Morocco	1 day	1	4	0.5	1.0	0.0	2.0	2.0
		2	4	14.0	19.2	1.5	42.3	40.8
	4 days	1	4	33.9	4.5	27.8	37.3	9.5
		2	4	38.2	19.6	17.4	56.1	38.7
	7 days	1	2	19.1	1.6	17.9	20.2	2.3
		2	3	15.9	5.2	10.0	19.7	9.7
	10 days	1	2	30.6	35.1	5.8	55.4	49.6
	AVERAGE		7	21.7	13.2	0.5	38.2	37.7
SST 25 single	1 day	1	8	2.3	1.7	0.9	6.0	5.1
		2	8	17.9	3.3	12.8	23.9	11.1
	4 days	1	8	26.6	6.3	17.7	35.0	17.3
		2	8	15.7	3.7	10.6	21.2	10.6
	7 days	1	8	22.5	12.0	1.4	36.8	35.4
		2	8	37.6	6.6	28.1	50.2	22.1
	10 days	1	6	21.2	21.0	7.6	63.0	55.4
	AVERAGE		7	20.5	10.8	2.3	37.6	35.3

Infection frequency data for inoculation with 3SA126 on Morocco, for inoculation with 3SA86 only on SST 25 (single), and for inoculation with 3SA86 on SST 25 one day (replication 1) after the inoculation with 3SA126 (double). Values in this table are the mean of: n=3 for spores counted, n=4 for pustules/cm^2 for inoculation with Morocco, and n=6 for pustules/cm^2 for both SST 25 tests. N.D. = Not Determined

	tray no.	race	weight (mg)	spores counted	spores/ cm^2	germinated spores/cm^2	pustules/ cm^2	INFECTION FREQUENCY
Morocco single		3SA126	2.32	149	173	65	0.0	0.0
		3SA126	2.26	353	411	153	0.0	0.0
		3SA126	2.80	343	399	149	0.0	0.0
		3SA126	2.26	196	228	85	1.7	2.0
SST 25 single		3SA86	2.41	236	275	264	3.8	1.5
		3SA86	2.21	279	325	313	4.7	1.5
		3SA86	2.42	337	392	377	7.0	1.9
		3SA86	2.56	156	182	175	10.5	6.0
		3SA86	2.58	272	317	305	5.0	1.6
		3SA86	2.43	216	252	242	3.0	1.2
		3SA86	2.52	265	309	297	2.7	0.9
		3SA86	2.14	223	260	250	9.7	3.9
SST 25 double	1	3SA126	2.28	218	254	94	N.D.	-
	2	3SA126	2.30	489	569	212	N.D.	-
	3	3SA126	2.55	547	637	237	N.D.	-
	4	3SA126	2.56	459	534	199	N.D.	-
	5	3SA126	2.80	98	114	42	N.D.	-
	6	3SA126	2.35	126	147	55	N.D.	-
	7	3SA126	2.66	420	489	182	N.D.	-
	8	3SA126	2.82	523	609	227	N.D.	-
	1	3SA86	2.26	227	264	254	20.0	7.9
	2	3SA86	2.67	252	293	282	5.3	1.9
	3	3SA86	2.68	341	397	382	13.7	3.6
	4	3SA86	2.47	265	309	297	0.7	0.2
	5	3SA86	2.16	254	296	285	8.5	3.0
	6	3SA86	2.84	260	303	291	7.5	2.6
	7	3SA86	2.41	223	260	250	0.2	0.1
	8	3SA86	2.81	168	196	188	1.3	0.7

Statistical analysis of infection frequency data for inoculation with 3SA86 on SST 25 at various time intervals after inoculation with 3SA126 (double). Data in APPENDIX 1.7, 1.8, 1.9, 1.10, 1.11, 1.12 and 1.13. N.D. = Not Determined

	time interval	rep.	n =	mean	standard deviation	value minimum	value maximum	range
SST 25 double	1 day	1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
		2	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	4 days	1	8	38.1	7.9	25.7	47.9	22.2
		2	2	22.7	1.4	21.7	23.7	2.0
	7 days	1	8	19.8	4.1	13.8	27.3	13.5
		2	8	19.3	7.7	10.3	31.8	21.5
	10 days	1	8	38.0	29.5	4.0	86.0	82.0
	AVERAGE		5	27.6	9.6	19.3	38.1	18.8
	1 day	1	8	2.5	2.5	0.1	7.9	7.8
		2	8	15.6	7.5	8.4	31.2	22.8
	4 days	1	8	16.5	2.9	13.8	23.0	9.2
		2	8	9.5	6.3	2.1	20.7	18.6
	7 days	1	8	8.8	4.1	4.9	15.5	10.6
		2	8	20.7	8.8	13.8	40.7	26.9
	10 days	1	8	8.6	10.1	0.0	31.4	31.4
	AVERAGE		7	11.7	6.1	2.5	20.7	18.2

Infection frequency data for inoculation with 3SA126 on Morocco, for inoculation with 3SA86 only on SST 25 (single), and for inoculation with 3SA86 on SST 25 one day (replication 2) after the inoculation with 3SA126 (double). Values in this table are the mean of: n=3 for spores counted, n=4 for pustules/cm^2 for inoculation with Morocco, and n=6 for pustules/cm^2 for both SST 25 tests. N.D. = Not Determined

	tray no.	race	weight (mg)	spores counted	spores/ cm^2	germinated spores/cm^2	pustules/ cm^2	INFECTION FREQUENCY
Morocco single		3SA126	2.99	324	377	191	80.7	42.3
		3SA126	2.33	262	305	154	2.3	1.5
		3SA126	2.53	455	530	268	6.3	2.4
		3SA126	2.50	330	384	194	18.7	9.6
SST 25 single		3SA86	2.95	386	449	232	38.3	16.5
		3SA86	3.50	403	470	243	51.5	21.2
		3SA86	2.30	404	470	383	91.5	23.9
		3SA86	2.75	347	404	329	59.0	17.9
		3SA86	2.21	356	415	338	43.3	12.8
		3SA86	2.44	389	453	369	64.0	17.3
		3SA86	2.77	391	455	429	71.3	16.6
		3SA86	2.64	355	413	390	66.5	17.1
SST 25 double	1	3SA126	2.74	285	332	289	N.D.	-
	2	3SA126	2.23	162	189	164	N.D.	-
	3	3SA126	2.20	274	319	278	N.D.	-
	4	3SA126	2.27	367	427	372	N.D.	-
	5	3SA126	2.28	291	339	295	N.D.	-
	6	3SA126	2.32	290	338	294	N.D.	-
	7	3SA126	2.53	338	394	199	N.D.	-
	8	3SA126	2.75	452	526	266	N.D.	-
	1	3SA86	2.46	347	404	381	118.9	31.2
	2	3SA86	2.14	291	339	320	62.4	19.5
	3	3SA86	2.29	352	410	334	31.4	9.4
	4	3SA86	2.28	375	437	356	40.9	11.5
	5	3SA86	3.02	360	419	342	56.7	16.6
	6	3SA86	2.99	365	425	346	29.2	8.4
	7	3SA86	3.31	408	475	246	42.2	17.2
	8	3SA86	2.98	392	456	236	25.2	10.7

Infection frequency data for inoculation with 3SA126 on Morocco, for inoculation with 3SA86 only on SST 25 (single), and for inoculation with 3SA86 on SST 25 four days (replication 1) after the inoculation with 3SA126 (double). Values in this table are the mean of: n=3 for spores counted, n=4 for pustules/cm^2 for inoculation with Morocco, and n=6 for pustules/cm^2 for both SST 25 tests. N.D. = Not Determined

	tray no.	race	weight (mg)	spores counted	spores/ cm^2	germinated spores/cm^2	pustules/ cm^2	INFECTION FREQUENCY
Morocco single		3SA126	2.39	277	323	259	96.3	37.2
		3SA126	2.38	275	321	258	85.5	33.2
		3SA126	2.69	311	362	291	81.0	27.8
		3SA126	2.38	275	321	258	96.0	37.3
SST 25 single		3SA86	2.76	286	333	303	85.5	28.2
		3SA86	2.83	228	265	241	42.8	17.7
		3SA86	2.46	209	243	221	43.8	19.8
		3SA86	2.34	173	201	183	64.3	35.0
		3SA86	2.27	167	194	177	47.0	26.6
		3SA86	2.30	205	239	217	75.3	34.6
		3SA86	2.31	213	248	225	62.8	27.8
		3SA86	2.87	186	216	197	45.3	23.0
SST 25 double	1	3SA126	2.46	285	332	266	127.5	47.9
	2	3SA126	2.42	280	326	262	118.8	45.3
	3	3SA126	2.32	268	313	251	119.0	47.4
	4	3SA126	2.51	290	338	272	88.8	32.7
	5	3SA126	2.28	264	307	247	87.3	35.4
	6	3SA126	2.45	284	330	265	93.8	35.4
	7	3SA126	2.53	293	341	274	70.3	25.7
	8	3SA126	2.40	278	323	260	91.3	35.1
	1	3SA86	2.47	210	245	223	33.5	15.0
	2	3SA86	2.31	173	201	183	25.5	13.9
	3	3SA86	2.70	256	298	272	46.3	17.0
	4	3SA86	2.60	166	193	176	27.8	15.8
	5	3SA86	2.77	171	199	181	25.0	13.8
	6	3SA86	2.61	187	218	199	45.8	23.0
	7	3SA86	2.85	165	193	175	28.3	16.1
	8	3SA86	2.34	131	152	138	24.0	17.3

Infection frequency data for inoculation with 3SA126 on Morocco, for inoculation with 3SA86 only on SST 25 (single), and for inoculation with 3SA86 on SST 25 four days (replication 2, flagleaf) after the inoculation with 3SA126 (double). Values in this table are the mean of: n = 3 for spores counted, n = 4 for pustules/cm^2 for inoculation with Morocco, and n = 6 for pustules/cm^2 for both SST 25 tests. N.D. = Not Determined

	tray no.	race	weight (mg)	spores counted	spores/ cm^2	germinated spores/cm^2	pustules/ cm^2	INFECTION FREQUENCY
Morocco single		3SA126	2.63	304	354	294	51.0	17.4
		3SA126	2.55	295	344	285	153.0	53.7
		3SA126	2.56	296	345	286	73.0	25.5
		3SA126	2.68	310	361	299	168.0	56.1
SST 25 single		3SA86	2.74	317	369	314	51.0	16.2
		3SA86	2.78	322	375	318	33.7	10.6
		3SA86	2.39	277	322	274	58.0	21.2
		3SA86	2.21	256	298	253	48.5	19.2
		3SA86	2.40	278	323	275	39.7	14.4
		3SA86	2.01	233	271	230	39.3	17.1
		3SA86	2.38	275	321	273	43.0	15.8
		3SA86	2.33	270	314	267	29.7	11.1
SST 25 double	1	3SA126	2.17	251	292	242	57.5	23.7
	2	3SA126	2.37	274	319	265	57.5	21.7
	3	3SA126	2.16	250	291	241	N.D.	-
	4	3SA126	2.18	252	294	244	N.D.	-
	5	3SA126	2.48	287	334	277	N.D.	-
	6	3SA126	2.61	302	352	292	N.D.	-
	7	3SA126	2.57	297	346	287	N.D.	-
	8	3SA126	2.61	302	352	292	N.D.	-
	1	3SA86	2.34	271	315	268	22.3	8.3
	2	3SA86	2.42	280	326	277	18.7	6.7
	3	3SA86	2.83	328	381	324	14.0	4.3
	4	3SA86	2.31	267	311	265	15.7	5.9
	5	3SA86	2.21	256	298	253	31.0	12.2
	6	3SA86	2.55	295	344	292	60.5	20.7
	7	3SA86	2.57	297	346	294	47.0	16.0
	8	3SA86	2.41	279	325	276	5.8	2.1

Infection frequency data for inoculation with 3SA126 on Morocco, for inoculation with 3SA86 only on SST 25 (single), and for inoculation with 3SA86 on SST 25 seven days (replication 1) after the inoculation with 3SA126 (double). Values in this table are the mean of: n = 3 for spores counted, n = 4 for pustules/cm^2 for inoculation with Morocco, and n = 6 for pustules/cm^2 for both SST 25 tests. N.D. = Not Determined

	tray no.	race	weight (mg)	spores counted	spores/ cm^2	germinated spores/cm^2	pustules/ cm^2	INFECTION FREQUENCY
Morocco single		3SA126	2.65	376	437	331	67.0	20.2
		3SA126	2.91	561	653	494	88.5	17.9
		3SA126	2.72	332	387	293	N.D.	-
		3SA126	2.83	635	740	560	N.D.	-
SST 25 single		3SA86	2.29	155	180	69	7.3	10.6
		3SA86	2.24	161	187	71	1.0	1.4
		3SA86	2.25	265	308	117	25.5	21.8
		3SA86	2.67	346	402	153	44.3	29.0
		3SA86	2.44	257	300	114	33.8	29.7
		3SA86	2.35	224	261	99	36.5	36.8
		3SA86	2.69	408	475	180	32.8	18.2
		3SA86	2.83	220	256	97	31.5	32.4
SST 25 double	1	3SA126	2.94	434	505	383	83.7	21.9
	2	3SA126	2.76	390	455	344	93.8	27.3
	3	3SA126	2.74	407	474	359	77.3	21.5
	4	3SA126	2.74	509	593	449	84.0	18.7
	5	3SA126	3.16	482	561	425	58.8	13.8
	6	3SA126	3.40	534	622	471	100.3	21.3
	7	3SA126	2.72	544	633	479	84.3	17.6
	8	3SA126	2.83	441	514	389	64.3	16.5
	1	3SA86	2.74	353	411	156	10.8	6.9
	2	3SA86	2.59	374	435	165	12.3	7.4
	3	3SA86	2.39	259	302	115	8.5	7.4
	4	3SA86	2.61	275	320	122	18.8	15.5
	5	3SA86	2.05	195	227	86	4.2	4.9
	6	3SA86	2.40	353	411	156	11.5	7.4
	7	3SA86	2.26	302	351	133	7.8	5.8
	8	3SA86	2.24	170	198	75	11.3	15.0

Infection frequency data for inoculation with 3SA126 on Morocco, for inoculation with 3SA86 only on SST 25 (single), and for inoculation with 3SA86 on SST 25 seven days (replication 2) after the inoculation with 3SA126 (double). Values in this table are the mean of: n = 3 for spores counted, n = 4 for pustules/cm^2 for inoculation with Morocco, and n = 6 for pustules/cm^2 for both SST 25 tests. N.D. = Not Determined

	tray no.	race	weight (mg)	spores counted	spores/ cm^2	germinated spores/cm^2	pustules/ cm^2	INFECTION FREQUENCY
Morocco single		3SA126	2.74	350	408	345	62.3	18.1
		3SA126	2.72	417	485	410	41.0	10.0
		3SA126	3.36	544	633	536	105.5	19.7
		3SA126	2.94	455	530	448	N.D.	-
SST 25 single		3SA86	1.97	112	131	96	37.8	39.4
		3SA86	2.23	167	194	143	56.3	39.5
		3SA86	2.22	165	192	141	48.8	34.7
		3SA86	1.75	144	168	123	43.0	35.0
		3SA86	1.88	200	233	171	48.0	28.1
		3SA86	1.81	140	163	119	39.5	33.1
		3SA86	2.22	170	198	145	59.0	40.7
		3SA86	2.24	103	120	88	44.3	50.2
SST 25 double	1	3SA126	2.68	360	419	354	106.0	29.9
	2	3SA126	3.21	382	444	376	61.3	16.3
	3	3SA126	2.63	408	475	402	63.7	15.8
	4	3SA126	2.97	549	640	541	55.5	10.3
	5	3SA126	2.86	475	553	468	96.0	20.5
	6	3SA126	2.77	432	503	426	135.5	31.8
	7	3SA126	2.64	396	461	390	50.7	13.0
	8	3SA126	2.77	483	562	475	80.3	16.9
	1	3SA86	2.20	103	120	88	18.0	20.5
	2	3SA86	2.31	79	92	68	16.5	24.4
	3	3SA86	1.92	152	177	129	17.8	13.8
	4	3SA86	1.82	127	147	108	15.5	14.3
	5	3SA86	1.78	103	120	88	13.8	15.7
	6	3SA86	2.25	117	137	100	19.3	19.3
	7	3SA86	2.12	79	92	67	27.3	40.7
	8	3SA86	2.03	154	180	132	22.3	16.9

Infection frequency data for inoculation with 3SA126 on Morocco, for inoculation with 3SA86 only on SST 25 (single), and for inoculation with 3SA86 on SST 25 ten days (replication 1) after the inoculation with 3SA126 (double). Values in this table are the mean of: n = 3 for spores counted, n = 4 for pustules/cm² for inoculation with Morocco, and n = 6 for pustules/cm² for both SST 25 tests. N.D. = Not Determined

	tray no.	race	weight (mg)	spores counted	spores/ cm²	germinated spores/cm²	pustules/ cm²	INFECTION FREQUENCY
Morocco single		3SA126	1.65	100	117	87	48.0	55.4
		3SA126	1.71	80	94	69	4.0	5.8
SST 25 single		3SA86	2.43	276	321	244	26.0	10.6
		3SA86	2.20	281	327	249	34.3	13.8
		3SA86	1.61	177	206	157	33.3	21.2
		3SA86	1.87	22	26	21	13.0	63.0
		3SA86	2.17	280	326	248	27.0	10.9
		3SA86	1.90	234	273	208	15.7	7.6
		3SA86	1.89	273	318	242	N.D.	-
		3SA86	2.09	195	227	173	N.D.	-
SST 25 double	1	3SA126	1.50	75	88	65	56.0	86.0
	2	3SA126	1.06	67	78	58	31.0	53.6
	3	3SA126	1.70	119	139	103	51.0	49.6
	4	3SA126	1.64	69	80	59	36.0	60.7
	5	3SA126	2.25	87	101	75	6.0	8.0
	6	3SA126	1.87	85	99	73	7.0	9.5
	7	3SA126	2.20	86	101	75	3.0	4.0
	8	3SA126	2.15	125	146	108	35.0	32.4
	1	3SA86	1.99	233	272	207	8.7	4.2
	2	3SA86	1.63	347	404	307	3.7	1.2
	3	3SA86	2.10	275	321	244	0.0	0.0
	4	3SA86	1.77	165	192	146	4.0	2.7
	5	3SA86	2.30	240	279	212	17.7	8.3
	6	3SA86	1.82	276	321	245	21.0	8.6
	7	3SA86	1.69	146	170	129	15.7	12.2
	8	3SA86	2.34	111	130	99	31.0	31.4

Statistical analysis of the average mass (g) of maize, *Zea mays*, callus pieces grown on culture medium (Green & Phillips, 1975) containing different concentrations of fumonisin B_1, a mycotoxin of *Fusarium moniliforme*. "initial" is mass at the start of the experiment, "final" is mass after six weeks of growth. Data of the replications in APPENDIX 2.2, 2.3 and 2.4

CONCENTRATION		n =	MASS (gram) initial	std.dev.*	final	std.dev.	increase**	std.dev.
0 mg/l (control)	rep 1	44	0.140	0.048	0.440	0.240	0.300	0.223
	rep 2	27	0.135	0.037	0.839	0.263	0.704	0.257
	rep 3	49	0.140	0.050	0.569	0.257	0.428	0.249
	average		0.138	0.002	0.616	0.166	0.477	0.169
	sum	120					(0.443)	
0.1 mg/l	rep 1	45	0.159	0.059	0.362	0.260	0.203	0.258
	rep 2	28	0.132	0.046	0.828	0.313	0.696	0.283
	rep 3	49	0.151	0.046	0.567	0.207	0.416	0.184
	average		0.147	0.011	0.586	0.191	0.438	0.202
	sum	122					(0.402)	
1.0 mg/l	rep 1	41	0.139	0.048	0.197	0.111	0.058	0.107
	rep 2	42	0.153	0.039	0.535	0.228	0.382	0.218
	rep 3	49	0.121	0.049	0.396	0.143	0.275	0.127
	average		0.138	0.013	0.376	0.139	0.238	0.135
	sum	132					(0.242)	
10 mg/l	rep 1	47	0.141	0.057	0.203	0.074	0.062	0.056
	rep 2	43	0.161	0.038	0.478	0.120	0.317	0.119
	rep 3	48	0.114	0.029	0.278	0.094	0.164	0.093
	average		0.139	0.019	0.320	0.116	0.181	0.105
	sum	138					(0.177)	
100 mg/l	rep 1	46	0.193	0.051	0.167	0.042	-0.027	0.037
	rep 2	45	0.136	0.034	0.215	0.057	0.078	0.042
	rep 3	48	0.151	0.055	0.206	0.063	0.055	0.040
	average		0.160	0.024	0.196	0.021	0.035	0.045
	sum	139					(0.035)	

* std.dev. = standard deviation

** the figure given in brackets is the average mass increase of all the data of both rep. 1, 2 & 3

Mass (g) of individual maize, *Zea mays*, callus pieces grown on culture medium (Green & Phillips, 1975) containing different concentrations of fumonisin B_1, a mycotoxin of *Fusarium moniliforme* (replication 1). "initial" is mass at the start of the experiment, "final" is mass after six weeks of growth

CONCENTRATION:

	0 mg/l (control)			0.1 mg/l			1.0 mg/l			10 mg/l			100 mg/l		
	initial	final	increase	initial	final	increase	initial	final	increase	initial	final	increase	initial	final	increase
1	0.12	0.11	-0.01	0.20	0.15	-0.05	0.23	0.19	-0.04	0.16	0.11	-0.05	0.32	0.19	-0.13
2	0.04	0.04	0.00	0.13	0.10	-0.03	0.18	0.14	-0.04	0.25	0.21	-0.04	0.23	0.13	-0.10
3	0.13	0.14	0.01	0.26	0.24	-0.02	0.11	0.09	-0.02	0.09	0.06	-0.03	0.27	0.17	-0.10
4	0.16	0.17	0.01	0.10	0.08	-0.02	0.21	0.19	-0.02	0.13	0.10	-0.03	0.23	0.14	-0.09
5	0.15	0.18	0.03	0.16	0.14	-0.02	0.16	0.15	-0.01	0.04	0.03	-0.01	0.28	0.21	-0.07
6	0.08	0.12	0.04	0.13	0.12	-0.01	0.17	0.16	-0.01	0.14	0.14	0.00	0.22	0.15	-0.07
7	0.11	0.16	0.05	0.08	0.07	-0.01	0.17	0.16	-0.01	0.18	0.18	0.00	0.29	0.22	-0.07
8	0.12	0.18	0.06	0.09	0.08	-0.01	0.23	0.22	-0.01	0.14	0.15	0.01	0.16	0.10	-0.06
9	0.12	0.22	0.10	0.16	0.16	0.00	0.06	0.05	-0.01	0.16	0.17	0.01	0.19	0.13	-0.06
10	0.15	0.25	0.10	0.21	0.21	0.00	0.15	0.14	-0.01	0.32	0.33	0.01	0.29	0.23	-0.06
11	0.10	0.21	0.11	0.10	0.11	0.01	0.07	0.07	0.00	0.13	0.15	0.02	0.17	0.12	-0.05
12	0.20	0.31	0.11	0.11	0.12	0.01	0.11	0.11	0.00	0.20	0.22	0.02	0.23	0.18	-0.05
13	0.09	0.21	0.12	0.19	0.20	0.01	0.12	0.12	0.00	0.06	0.09	0.03	0.19	0.14	-0.05
14	0.19	0.32	0.13	0.11	0.13	0.02	0.18	0.18	0.00	0.13	0.16	0.03	0.24	0.19	-0.05
15	0.10	0.24	0.14	0.15	0.17	0.02	0.17	0.18	0.01	0.06	0.10	0.04	0.23	0.19	-0.04
16	0.17	0.31	0.14	0.24	0.26	0.02	0.04	0.05	0.01	0.15	0.19	0.04	0.14	0.11	-0.03
17	0.13	0.28	0.15	0.15	0.18	0.03	0.09	0.10	0.01	0.16	0.21	0.05	0.14	0.11	-0.03
18	0.12	0.29	0.17	0.21	0.25	0.04	0.18	0.19	0.01	0.28	0.33	0.05	0.17	0.14	-0.03
19	0.14	0.35	0.21	0.17	0.22	0.05	0.17	0.19	0.02	0.09	0.14	0.05	0.20	0.17	-0.03
20	0.15	0.38	0.23	0.21	0.27	0.06	0.20	0.22	0.02	0.15	0.20	0.05	0.25	0.22	-0.03
21	0.17	0.41	0.24	0.24	0.32	0.08	0.06	0.08	0.02	0.15	0.20	0.05	0.14	0.12	-0.02
22	0.12	0.40	0.28	0.11	0.20	0.09	0.08	0.10	0.02	0.13	0.19	0.06	0.13	0.11	-0.02
23	0.10	0.40	0.30	0.23	0.33	0.10	0.07	0.10	0.03	0.14	0.20	0.06	0.16	0.14	-0.02
24	0.09	0.40	0.31	0.12	0.23	0.11	0.09	0.12	0.03	0.04	0.10	0.06	0.16	0.14	-0.02
25	0.14	0.46	0.32	0.17	0.29	0.12	0.13	0.16	0.03	0.11	0.17	0.06	0.18	0.16	-0.02
26	0.12	0.46	0.34	0.15	0.27	0.12	0.22	0.26	0.04	0.15	0.22	0.07	0.19	0.17	-0.02
27	0.17	0.53	0.36	0.06	0.19	0.13	0.13	0.18	0.05	0.20	0.27	0.07	0.19	0.17	-0.02
28	0.20	0.57	0.37	0.12	0.26	0.14	0.10	0.16	0.06	0.24	0.31	0.07	0.21	0.19	-0.02
29	0.16	0.54	0.38	0.21	0.37	0.16	0.13	0.19	0.06	0.10	0.18	0.08	0.16	0.15	-0.01
30	0.17	0.57	0.40	0.15	0.33	0.18	0.14	0.20	0.06	0.13	0.21	0.08	0.17	0.16	-0.01
31	0.24	0.66	0.42	0.10	0.31	0.21	0.16	0.22	0.06	0.13	0.21	0.08	0.23	0.22	-0.01
32	0.14	0.59	0.45	0.25	0.47	0.22	0.11	0.18	0.07	0.09	0.17	0.08	0.09	0.08	-0.01
33	0.07	0.52	0.45	0.31	0.53	0.22	0.18	0.25	0.07	0.09	0.18	0.09	0.24	0.23	-0.01
34	0.19	0.66	0.47	0.13	0.58	0.45	0.12	0.21	0.09	0.16	0.25	0.09	0.10	0.10	0.00
35	0.03	0.51	0.48	0.11	0.57	0.46	0.20	0.32	0.12	0.23	0.32	0.09	0.14	0.14	0.00
36	0.14	0.68	0.54	0.25	0.72	0.47	0.16	0.30	0.14	0.11	0.21	0.10	0.15	0.15	0.00
37	0.13	0.68	0.55	0.08	0.55	0.47	0.10	0.25	0.15	0.12	0.22	0.10	0.19	0.19	0.00
38	0.24	0.79	0.55	0.20	0.67	0.47	0.14	0.34	0.20	0.12	0.22	0.10	0.21	0.21	0.00
39	0.11	0.67	0.56	0.18	0.76	0.58	0.12	0.47	0.35	0.10	0.21	0.11	0.14	0.15	0.01
40	0.15	0.78	0.63	0.10	0.69	0.59	0.12	0.53	0.41	0.13	0.24	0.11	0.20	0.21	0.01
41	0.28	0.92	0.64	0.14	0.76	0.62	0.12	0.54	0.42	0.19	0.30	0.11	0.15	0.16	0.01
42	0.14	0.88	0.74	0.21	0.86	0.65				0.11	0.22	0.11	0.22	0.24	0.02
43	0.13	0.88	0.75	0.15	0.84	0.69				0.16	0.29	0.13	0.15	0.17	0.02
44	0.15	0.93	0.78	0.05	0.75	0.70				0.12	0.27	0.15	0.18	0.21	0.03
45				0.16	1.16	1.00				0.07	0.23	0.16	0.23	0.27	0.04
46										0.14	0.33	0.19	0.14	0.19	0.05
47										0.16	0.37	0.21			
48															
49															

Mass (g) of individual maize, *Zea mays*, callus pieces grown on culture medium (Green & Phillips, 1975) containing different concentrations fumonisin B_1, a mycotoxin of *Fusarium moniliforme* (replication 2). "initial" is mass at the start of the experiment, "final" is mass after six weeks of growth

CONCENTRATION:

	0 mg/l (control)			0.1 mg/l			1.0 mg/l			10 mg/l			100 mg/l		
	initial	final	increase	initial	final	increase	initial	final	increase	initial	final	increase	initial	final	increase
1	0.08	0.18	0.10	0.09	0.11	0.02	0.18	0.20	0.02	0.17	0.25	0.08	0.14	0.14	0.00
2	0.12	0.31	0.19	0.12	0.30	0.18	0.09	0.14	0.05	0.13	0.23	0.10	0.23	0.24	0.01
3	0.14	0.38	0.24	0.07	0.36	0.29	0.12	0.21	0.09	0.24	0.35	0.11	0.09	0.10	0.01
4	0.15	0.47	0.32	0.20	0.56	0.36	0.14	0.24	0.10	0.20	0.35	0.15	0.10	0.13	0.03
5	0.10	0.61	0.51	0.06	0.46	0.40	0.27	0.38	0.11	0.16	0.31	0.15	0.10	0.13	0.03
6	0.14	0.66	0.52	0.05	0.46	0.41	0.17	0.29	0.12	0.12	0.28	0.16	0.12	0.15	0.03
7	0.22	0.80	0.58	0.09	0.62	0.53	0.13	0.26	0.13	0.23	0.41	0.18	0.14	0.18	0.04
8	0.12	0.71	0.59	0.13	0.67	0.54	0.17	0.31	0.14	0.10	0.30	0.20	0.17	0.21	0.04
9	0.14	0.78	0.64	0.05	0.61	0.56	0.14	0.29	0.15	0.23	0.45	0.22	0.07	0.11	0.04
10	0.11	0.79	0.68	0.16	0.78	0.62	0.14	0.30	0.16	0.14	0.37	0.23	0.08	0.12	0.04
11	0.15	0.84	0.69	0.10	0.74	0.64	0.15	0.31	0.16	0.11	0.36	0.25	0.12	0.16	0.04
12	0.09	0.79	0.70	0.11	0.79	0.68	0.16	0.36	0.20	0.19	0.45	0.26	0.16	0.20	0.04
13	0.13	0.89	0.76	0.11	0.79	0.68	0.12	0.33	0.21	0.09	0.36	0.27	0.10	0.15	0.05
14	0.08	0.85	0.77	0.12	0.82	0.70	0.17	0.41	0.24	0.23	0.50	0.27	0.14	0.19	0.05
15	0.10	0.87	0.77	0.12	0.82	0.70	0.15	0.42	0.27	0.14	0.42	0.28	0.12	0.17	0.05
16	0.16	0.93	0.77	0.14	0.84	0.70	0.04	0.33	0.29	0.14	0.42	0.28	0.18	0.23	0.05
17	0.18	0.98	0.80	0.10	0.81	0.71	0.18	0.48	0.30	0.15	0.44	0.29	0.10	0.16	0.06
18	0.14	0.98	0.84	0.13	0.99	0.86	0.15	0.47	0.32	0.17	0.47	0.30	0.12	0.18	0.06
19	0.19	1.03	0.84	0.17	1.06	0.89	0.16	0.48	0.32	0.16	0.46	0.30	0.16	0.22	0.06
20	0.14	0.99	0.85	0.19	1.10	0.91	0.18	0.50	0.32	0.16	0.46	0.30	0.19	0.25	0.06
21	0.15	1.02	0.87	0.22	1.14	0.92	0.16	0.52	0.36	0.14	0.45	0.31	0.12	0.19	0.07
22	0.19	1.07	0.88	0.16	1.09	0.93	0.09	0.47	0.38	0.19	0.51	0.32	0.20	0.27	0.07
23	0.21	1.12	0.91	0.18	1.12	0.94	0.14	0.52	0.38	0.20	0.52	0.32	0.13	0.21	0.08
24	0.11	1.06	0.95	0.15	1.10	0.95	0.16	0.54	0.38	0.12	0.46	0.34	0.16	0.24	0.08
25	0.11	1.09	0.98	0.18	1.15	0.97	0.07	0.51	0.44	0.15	0.49	0.34	0.08	0.16	0.08
26	0.10	1.16	1.06	0.21	1.29	1.08	0.19	0.65	0.46	0.16	0.50	0.34	0.19	0.27	0.08
27	0.10	1.29	1.19	0.13	1.27	1.14	0.14	0.61	0.47	0.18	0.52	0.34	0.11	0.20	0.09
28				0.15	1.33	1.18	0.15	0.65	0.50	0.15	0.50	0.35	0.14	0.24	0.10
29							0.14	0.68	0.54	0.12	0.48	0.36	0.11	0.21	0.10
30							0.21	0.76	0.55	0.23	0.59	0.36	0.11	0.21	0.10
31							0.16	0.73	0.57	0.18	0.55	0.37	0.18	0.28	0.10
32							0.14	0.72	0.58	0.17	0.56	0.39	0.14	0.25	0.11
33							0.13	0.72	0.59	0.17	0.58	0.41	0.11	0.22	0.11
34							0.15	0.77	0.62	0.19	0.60	0.41	0.15	0.26	0.11
35							0.17	0.79	0.62	0.10	0.51	0.41	0.15	0.26	0.11
36							0.15	0.79	0.64	0.16	0.58	0.42	0.17	0.28	0.11
37							0.15	0.81	0.66	0.21	0.63	0.42	0.12	0.24	0.12
38							0.16	0.83	0.67	0.13	0.56	0.43	0.13	0.25	0.12
39							0.22	0.89	0.67	0.13	0.59	0.46	0.14	0.26	0.12
40							0.13	0.85	0.72	0.16	0.62	0.46	0.14	0.26	0.12
41							0.20	0.98	0.78	0.11	0.61	0.50	0.18	0.31	0.13
42							0.21	0.99	0.78	0.16	0.67	0.51	0.13	0.27	0.14
43										0.16	0.84	0.68	0.12	0.27	0.15
44													0.13	0.30	0.17
45													0.17	0.34	0.17
46															
47															
48															
49															

Mass (g) of individual maize, *Zea mays*, callus pieces grown on culture medium (Green & Phillips, 1975) containing different concentrations of fumonisin B_1, a mycotoxin of *Fusarium moniliforme* (replication 3). "initial" is mass at the start of the experiment, "final" is mass after six weeks of growth

CONCENTRATION:

	0 mg/l (control)			0.1 mg/l			1.0 mg/l			10 mg/l			100 mg/l		
	initial	final	increase	initial	final	increase	initial	final	increase	initial	final	increase	initial	final	increase
1	0.06	0.06	0.00	0.15	0.20	0.05	0.09	0.10	0.01	0.18	0.21	0.03	0.21	0.20	-0.01
2	0.08	0.09	0.01	0.16	0.28	0.12	0.08	0.10	0.02	0.14	0.18	0.04	0.17	0.17	0.00
3	0.10	0.15	0.05	0.11	0.25	0.14	0.06	0.09	0.03	0.09	0.13	0.04	0.20	0.20	0.00
4	0.16	0.21	0.05	0.14	0.31	0.17	0.06	0.10	0.04	0.12	0.16	0.04	0.20	0.20	0.00
5	0.15	0.22	0.07	0.08	0.25	0.17	0.17	0.28	0.11	0.11	0.16	0.05	0.18	0.18	0.00
6	0.06	0.14	0.08	0.14	0.35	0.21	0.19	0.33	0.14	0.15	0.20	0.05	0.20	0.21	0.01
7	0.12	0.20	0.08	0.06	0.27	0.21	0.17	0.32	0.15	0.13	0.19	0.06	0.05	0.06	0.01
8	0.25	0.35	0.10	0.19	0.41	0.22	0.07	0.22	0.15	0.13	0.19	0.06	0.12	0.13	0.01
9	0.17	0.31	0.14	0.11	0.35	0.24	0.12	0.27	0.15	0.11	0.18	0.07	0.15	0.16	0.01
10	0.08	0.22	0.14	0.12	0.39	0.27	0.14	0.31	0.17	0.12	0.19	0.07	0.23	0.25	0.02
11	0.17	0.33	0.16	0.08	0.37	0.29	0.18	0.35	0.17	0.11	0.19	0.08	0.12	0.14	0.02
12	0.19	0.39	0.20	0.14	0.43	0.29	0.09	0.27	0.18	0.12	0.20	0.08	0.12	0.14	0.02
13	0.11	0.34	0.23	0.13	0.42	0.29	0.16	0.35	0.19	0.13	0.22	0.09	0.15	0.18	0.03
14	0.24	0.48	0.24	0.19	0.48	0.29	0.12	0.31	0.19	0.13	0.22	0.09	0.15	0.18	0.03
15	0.22	0.47	0.25	0.16	0.45	0.29	0.15	0.35	0.20	0.08	0.17	0.09	0.14	0.17	0.03
16	0.09	0.34	0.25	0.23	0.52	0.29	0.07	0.28	0.21	0.07	0.17	0.10	0.12	0.15	0.03
17	0.13	0.44	0.31	0.11	0.40	0.29	0.09	0.31	0.22	0.13	0.24	0.11	0.11	0.14	0.03
18	0.17	0.50	0.33	0.19	0.49	0.30	0.06	0.28	0.22	0.11	0.22	0.11	0.12	0.16	0.04
19	0.24	0.59	0.35	0.11	0.43	0.32	0.11	0.35	0.24	0.09	0.21	0.12	0.16	0.20	0.04
20	0.14	0.50	0.36	0.08	0.43	0.35	0.06	0.31	0.25	0.14	0.26	0.12	0.09	0.13	0.04
21	0.16	0.52	0.36	0.21	0.56	0.35	0.09	0.34	0.25	0.11	0.23	0.12	0.16	0.20	0.04
22	0.10	0.49	0.39	0.15	0.51	0.36	0.09	0.36	0.27	0.09	0.22	0.13	0.23	0.27	0.04
23	0.21	0.65	0.44	0.15	0.52	0.37	0.27	0.56	0.29	0.07	0.21	0.14	0.20	0.25	0.05
24	0.09	0.53	0.44	0.09	0.47	0.38	0.11	0.40	0.29	0.15	0.29	0.14	0.13	0.18	0.05
25	0.08	0.54	0.46	0.10	0.49	0.39	0.10	0.39	0.29	0.09	0.25	0.16	0.23	0.28	0.05
26	0.14	0.61	0.47	0.19	0.59	0.40	0.12	0.42	0.30	0.12	0.28	0.16	0.12	0.18	0.06
27	0.13	0.60	0.47	0.20	0.61	0.41	0.09	0.39	0.30	0.10	0.27	0.17	0.12	0.18	0.06
28	0.05	0.53	0.48	0.10	0.52	0.42	0.19	0.50	0.31	0.11	0.30	0.19	0.15	0.21	0.06
29	0.09	0.59	0.50	0.18	0.62	0.44	0.16	0.47	0.31	0.19	0.38	0.19	0.13	0.19	0.06
30	0.21	0.71	0.50	0.17	0.62	0.45	0.07	0.38	0.31	0.08	0.27	0.19	0.21	0.27	0.06
31	0.16	0.70	0.54	0.15	0.62	0.47	0.19	0.50	0.31	0.09	0.29	0.20	0.23	0.30	0.07
32	0.14	0.68	0.54	0.13	0.63	0.50	0.11	0.43	0.32	0.12	0.32	0.20	0.08	0.15	0.07
33	0.09	0.65	0.56	0.18	0.69	0.51	0.12	0.44	0.32	0.08	0.28	0.20	0.11	0.18	0.07
34	0.15	0.75	0.60	0.14	0.67	0.53	0.11	0.44	0.33	0.07	0.29	0.22	0.12	0.19	0.07
35	0.12	0.73	0.61	0.12	0.65	0.53	0.17	0.51	0.34	0.16	0.39	0.23	0.13	0.20	0.07
36	0.18	0.82	0.64	0.15	0.69	0.54	0.11	0.45	0.34	0.14	0.38	0.24	0.12	0.20	0.08
37	0.11	0.76	0.65	0.12	0.66	0.54	0.17	0.51	0.34	0.07	0.31	0.24	0.19	0.27	0.08
38	0.09	0.75	0.66	0.12	0.67	0.55	0.16	0.52	0.36	0.14	0.38	0.24	0.15	0.23	0.08
39	0.13	0.82	0.69	0.17	0.76	0.59	0.09	0.45	0.36	0.09	0.33	0.24	0.06	0.15	0.09
40	0.17	0.87	0.70	0.18	0.78	0.60	0.15	0.52	0.37	0.08	0.33	0.25	0.33	0.43	0.10
41	0.15	0.85	0.70	0.15	0.78	0.63	0.10	0.49	0.39	0.09	0.34	0.25	0.10	0.20	0.10
42	0.18	0.90	0.72	0.13	0.76	0.63	0.11	0.51	0.40	0.18	0.47	0.29	0.09	0.19	0.10
43	0.14	0.87	0.73	0.12	0.75	0.63	0.09	0.50	0.41	0.12	0.42	0.30	0.27	0.38	0.11
44	0.16	0.92	0.76	0.30	0.93	0.63	0.13	0.55	0.42	0.10	0.40	0.30	0.11	0.23	0.12
45	0.10	0.86	0.76	0.19	0.84	0.65	0.10	0.54	0.44	0.09	0.39	0.30	0.11	0.24	0.13
46	0.21	0.99	0.78	0.22	0.92	0.70	0.08	0.56	0.48	0.09	0.43	0.34	0.13	0.26	0.13
47	0.21	1.00	0.79	0.20	0.91	0.71	0.04	0.53	0.49	0.12	0.48	0.36	0.09	0.23	0.14
48	0.11	0.91	0.80	0.23	1.06	0.83	0.12	0.64	0.52	0.12	0.50	0.38	0.14	0.29	0.15
49	0.09	0.93	0.84	0.19	1.02	0.83	0.26	0.83	0.57						

APPENDIX 2.5

Mass (g), taken at weekly intervals, of individual maize, *Zea mays*, callus pieces grown for six weeks on culture medium (Green & Phillips, 1975) containing different concentrations of fumonisin B_1, a mycotoxin of *Fusarium moniliforme* (replication 1)

week	concentration				
	control	0.1	1.0	10	100
1					
	0.01	0.02	-0.01	0.01	-0.04
	0.02	0.03	0.00	0.02	-0.02
	0.02	0.03	0.03	0.03	0.00
	0.02	0.05	0.04	0.04	0.00
	0.04	0.06	0.05	0.05	0.01
	0.05	0.08	0.05	0.06	0.01
	0.08	0.08	0.05	0.07	0.01
	0.09	0.11	0.06	0.07	0.01
	0.14	0.13	0.07	0.09	0.02
	0.22	0.13	0.10	0.17	0.05
2					
	0.01	0.02	0.01	-0.02	-0.05
	0.03	0.03	0.02	0.04	-0.04
	0.04	0.03	0.03	0.05	-0.02
	0.08	0.04	0.04	0.05	-0.02
	0.08	0.06	0.04	0.05	-0.01
	0.09	0.06	0.04	0.05	0.00
	0.11	0.14	0.05	0.07	0.01
	0.13	0.18	0.06	0.09	0.01
	0.17	0.21	0.09	0.12	0.02
	0.17	0.24	0.09	0.17	0.02

week	concentration				
	control	0.1	1.0	10	100
3					
	0.01	-0.03	0.03	-0.06	-0.08
	0.03	-0.01	0.03	-0.01	-0.07
	0.05	0.02	0.03	0.00	-0.04
	0.05	0.06	0.04	0.01	-0.03
	0.06	0.06	0.06	0.04	-0.03
	0.06	0.06	0.08	0.05	0.00
	0.09	0.10	0.09	0.08	0.00
	0.12	0.12	0.12	0.08	0.00
	0.18	0.17	0.15	0.09	0.02
	0.31	0.26	0.20	0.15	0.02
4					
	0.02	-0.02	-0.01	-0.01	-0.08
	0.02	0.00	0.01	-0.01	-0.07
	0.04	0.02	0.04	0.01	-0.06
	0.04	0.03	0.05	0.06	-0.02
	0.05	0.04	0.05	0.06	-0.01
	0.09	0.06	0.06	0.07	0.00
	0.11	0.10	0.06	0.07	0.02
	0.13	0.10	0.08	0.12	0.03
	0.16	0.11	0.17	0.14	0.04
	0.43	0.18	0.28	0.16	0.06

week	concentration				
	control	0.1	1.0	10	100
5					
	0.06	-0.01	-0.02	-0.01	-0.07
	0.08	0.02	0.00	0.01	-0.03
	0.12	0.02	0.01	0.04	-0.03
	0.13	0.03	0.01	0.06	-0.01
	0.27	0.03	0.03	0.07	-0.01
	0.33	0.04	0.04	0.08	0.00
	0.46	0.09	0.09	0.09	0.01
	0.48	0.14	0.29	0.09	0.02
	0.50	0.34	0.37	0.10	0.05
	0.61	0.62	0.38	0.14	0.09

average* and standard deviation**

1 *	0.07	0.07	0.04	0.06	0.01
**	0.06	0.04	0.03	0.04	0.02
2 *	0.09	0.10	0.05	0.07	-0.01
**	0.05	0.08	0.03	0.05	0.02
3 *	0.10	0.08	0.08	0.04	-0.02
**	0.08	0.08	0.06	0.06	0.03
4 *	0.11	0.06	0.08	0.07	-0.01
**	0.12	0.06	0.08	0.06	0.05
5 *	0.30	0.13	0.12	0.07	0.00
**	0.19	0.19	0.15	0.04	0.04

Statistical analysis of the average mass increase (g) of maize, *Zea mays*, callus pieces grown for six weeks on culture medium (Green & Phillips, 1975) containing different concentrations of fumonisin B_1, a mycotoxin of *Fusarium moniliforme*, and of the mass increase (g) when the callus was subsequently transferred to toxin-free medium. Data in APPENDIX 2.7

		MASS (gram)			GROWTH RATE			MASS INCREASE PER DAY (gram)		
		week 0	week 6	week 14	0 to 6	6 to 14	0 to 14	0 to 6	6 to 14	0 to 14
0 mg/l control	average	0.15	0.71	1.37	4.93	1.97	9.66	0.014	0.011	0.012
	std.dev.*	0.04	0.20	0.38	1.65	0.47	3.78	0.005	0.005	0.004
	minimum	0.09	0.44	0.56	2.0	1.3	4.3	0.006	0.002	0.004
	maximum	0.24	1.00	1.88	8.3	3.0	17.1	0.021	0.017	0.018
	average	0.16	0.58	1.17	3.79	2.22	7.98	0.011	0.010	0.010
	std.dev.	0.04	0.19	0.27	1.02	0.76	2.41	0.004	0.004	0.003
	minimum	0.08	0.25	0.81	1.8	1.2	4.7	0.003	0.003	0.007
	maximum	0.22	0.92	1.61	5.5	3.9	13.0	0.018	0.015	0.015
1.0 mg/l	average	0.13	0.43	1.17	3.50	2.80	9.89	0.008	0.013	0.011
	std.dev.	0.04	0.11	0.23	0.90	0.47	3.12	0.002	0.003	0.002
	minimum	0.06	0.27	0.62	2.3	2.3	5.2	0.004	0.006	0.005
	maximum	0.19	0.56	1.48	5.4	3.8	16.2	0.011	0.016	0.013
10 mg/l	average	0.12	0.31	0.91	2.66	3.02	7.84	0.005	0.010	0.008
	std.dev.	0.03	0.08	0.18	0.69	0.76	2.37	0.002	0.003	0.002
	minimum	0.08	0.20	0.66	1.3	2.2	4.8	0.001	0.006	0.006
	maximum	0.18	0.47	1.20	3.7	4.6	12.1	0.008	0.016	0.011
100 mg/l	average	0.16	0.21	0.42	1.41	2.12	3.00	0.001	0.004	0.003
	std.dev.	0.05	0.04	0.12	0.49	0.83	1.52	0.001	0.002	0.001
	minimum	0.06	0.15	0.18	1.0	0.9	0.9	0.000	0.000	0.000
	maximum	0.23	0.27	0.60	2.5	3.8	6.2	0.003	0.007	0.005

* std.dev. = standard deviation

Data of the average mass increase (g) of maize, *Zea mays*, callus pieces grown for six weeks on culture medium (Green & Phillips, 1975) containing different concentrations of fumonisin B_{1}, a mycotoxin of *Fusarium moniliforme*, and the mass increase (g) when the callus was subsequently transferred to toxin-free culture medium

	MASS (gram)			GROWTH RATE*			MASS INCREASE PER DAY (gram)**		
	week 0	week 6	week 14	0 to 6	6 to 14	0 to 14	0 to 6	6 to 14	0 to 14
control	0.09	0.53	0.99	5.9	1.9	11.0	0.011	0.008	0.009
	0.11	0.91	1.88	8.3	2.1	17.1	0.021	0.016	0.018
	0.12	0.73	1.65	6.1	2.3	13.8	0.016	0.016	0.016
	0.13	0.44	0.56	3.4	1.3	4.3	0.008	0.002	0.004
	0.14	0.50	1.52	3.6	3.0	10.9	0.009	0.017	0.014
	0.15	0.75	1.66	5.0	2.2	11.1	0.015	0.015	0.015
	0.16	0.92	1.40	5.8	1.5	8.8	0.019	0.008	0.013
	0.18	0.82	1.44	4.6	1.8	8.0	0.016	0.011	0.013
	0.21	1.00	1.59	4.8	1.6	7.6	0.020	0.010	0.014
	0.24	0.48	1.02	2.0	2.1	4.3	0.006	0.009	0.008
0.1 mg/l	0.08	0.25	0.81	3.1	3.2	10.1	0.004	0.009	0.007
	0.10	0.52	0.96	5.2	1.8	9.6	0.011	0.007	0.009
	0.12	0.66	1.56	5.5	2.4	13.0	0.014	0.015	0.015
	0.15	0.62	1.43	4.1	2.3	9.5	0.012	0.014	0.013
	0.16	0.28	1.10	1.8	3.9	6.9	0.003	0.014	0.010
	0.17	0.62	1.17	3.6	1.9	6.9	0.012	0.009	0.010
	0.18	0.69	0.84	3.8	1.2	4.7	0.013	0.003	0.007
	0.18	0.62	1.16	3.4	1.9	6.4	0.011	0.009	0.010
	0.20	0.61	1.07	3.1	1.8	5.4	0.011	0.008	0.009
	0.22	0.92	1.61	4.2	1.8	7.3	0.018	0.012	0.014
1.0 mg/l	0.06	0.28	0.97	4.7	3.5	16.2	0.006	0.012	0.009
	0.09	0.27	1.02	3.0	3.8	11.3	0.005	0.013	0.009
	0.10	0.54	1.33	5.4	2.5	13.3	0.011	0.013	0.013
	0.11	0.45	1.23	4.1	2.7	11.2	0.009	0.013	0.011
	0.12	0.42	1.30	3.5	3.1	10.8	0.008	0.015	0.012
	0.12	0.27	0.62	2.3	2.3	5.2	0.004	0.006	0.005
	0.16	0.47	1.32	2.9	2.8	8.3	0.008	0.014	0.012
	0.16	0.52	1.24	3.3	2.4	7.8	0.009	0.012	0.011
	0.17	0.51	1.21	3.0	2.4	7.1	0.009	0.012	0.011
	0.19	0.56	1.48	2.9	2.6	7.8	0.009	0.016	0.013

* Growth rate 0 to 6: mass week 6/ mass week 0; Growth rate 6 to 14: mass week 14/ mass week 6;
Growth rate 0 to 14: mass week 14/ mass week 0;

** week 0 to 6 = 39 days; week 6 to 14 = 59 days

Data of the average mass increase (g) of maize, *Zea mays*, callus pieces grown for six weeks on culture medium (Green & Phillips, 1975) containing different concentrations of fumonisin B_1, a mycotoxin of *Fusarium moniliforme*, and the mass increase (g) when the callus was subsequently transferred to toxin-free culture medium

	MASS (gram)			GROWTH RATE*			MASS INCREASE PER DAY (gram)**		
	week 0	week 6	week 14	0 to 6	6 to 14	0 to 14	0 to 6	6 to 14	0 to 14
10 mg/l	0.08	0.27	0.77	3.4	2.9	9.6	0.005	0.008	0.007
	0.09	0.33	1.09	3.7	3.3	12.1	0.006	0.013	0.010
	0.09	0.25	0.97	2.8	3.9	10.8	0.004	0.012	0.009
	0.10	0.27	0.68	2.7	2.5	6.8	0.004	0.007	0.006
	0.12	0.28	0.66	2.3	2.4	5.5	0.004	0.006	0.006
	0.12	0.42	1.05	3.5	2.5	8.8	0.008	0.011	0.009
	0.14	0.26	1.20	1.9	4.6	8.6	0.003	0.016	0.011
	0.15	0.20	0.72	1.3	3.6	4.8	0.001	0.009	0.006
	0.16	0.39	0.91	2.4	2.3	5.7	0.006	0.009	0.008
	0.18	0.47	1.04	2.6	2.2	5.8	0.007	0.010	0.009
100 mg/l	0.06	0.15	0.37	2.5	2.5	6.2	0.002	0.004	0.003
	0.11	0.24	0.29	2.2	1.2	2.6	0.003	0.001	0.002
	0.12	0.16	0.60	1.3	3.8	5.0	0.001	0.007	0.005
	0.13	0.18	0.51	1.4	2.8	3.9	0.001	0.006	0.004
	0.16	0.20	0.35	1.3	1.8	2.2	0.001	0.003	0.002
	0.16	0.20	0.41	1.3	2.1	2.6	0.001	0.004	0.003
	0.20	0.20	0.58	1.0	2.9	2.9	0.000	0.006	0.004
	0.21	0.20	0.18	1.0	0.9	0.9	0.000	0.000	0.000
	0.23	0.27	0.40	1.2	1.5	1.7	0.001	0.002	0.002
	0.23	0.25	0.47	1.1	1.9	2.0	0.001	0.004	0.002

* Growth rate 0 to 6: mass week 6/ mass week 0; Growth rate 6 to 14: mass week 14/ mass week 6;
Growth rate 0 to 14: mass week 14/ mass week 0;

** week 0 to 6 = 39 days; week 6 to 14 = 59 days

Measurements of the cell wall thickness (cm) of contact-prints of maize, *Zea mays*, callus cells grown for six weeks on culture medium (Green & Phillips, 1975) containing different concentrations of fumonisin B_1, a mycotoxin of *Fusarium moniliforme* (A), and the statistical analysis of the data (B)

A

	measured (mm)	1cm = ? µm	cell wall (µm)
control			
	1.3	1.00	0.130
	1.7	2.00	0.085
	1.8	1.00	0.180
	1.0	0.83	0.120
	1.2	1.60	0.075
	1.1	1.60	0.069
	1.5	1.00	0.150
	2.0	2.00	0.100
	0.9	8.30	0.011
	1.5	1.00	0.150
0.1 mg/l			
	1.0	0.66	0.152
	2.0	0.83	0.241
	1.0	0.83	0.120
	1.9	0.83	0.229
	2.7	1.30	0.208
	0.5	0.33	0.152
	1.0	0.83	0.120
	1.1	0.83	0.133
	1.8	1.00	0.180
	2.0	0.83	0.241
1.0 mg/l			
	4.1	1.60	0.256
	1.0	1.00	0.100
	0.8	0.50	0.160
	1.0	0.33	0.303
	0.9	0.33	0.273
	1.5	0.66	0.227
	2.0	0.83	0.241
	1.1	1.00	0.110
	1.8	0.50	0.360
	3.0	1.00	0.300

	measured (mm)	1cm = ? µm	cell wall (µm)
10 mg/l			
	5.8	2.00	0.290
	1.0	0.50	0.200
	2.0	0.83	0.241
	2.1	1.00	0.210
	5.0	1.30	0.385
	9.0	1.30	0.692
	2.5	1.00	0.250
	2.0	0.83	0.241
	1.0	0.50	0.200
	3.0	0.83	0.361
100 mg/l			
	4.0	1.00	0.400
	4.0	1.00	0.400
	6.0	2.00	0.300
	4.0	1.00	0.400
	1.2	0.33	0.364
	6.0	0.83	0.723
	9.0	1.00	0.900
	4.0	0.66	0.606
	1.8	0.33	0.545
	11.3	2.00	0.565

B

STATISTICAL ANALYSIS:

	concentration				
	control	0.1	1.0	10	100
average	0.107	0.178	0.233	0.307	0.520
std.dev.*	0.047	0.046	0.081	0.142	0.177
minimum	0.011	0.120	0.100	0.200	0.300
maximum	0.180	0.241	0.360	0.692	0.900

* std.dev. = standard deviation

Statistical analysis of the height (cm) of the combined samples of seedlings of two maize, *Zea mays*, inbred lines, I137TN and F2834, injected with 0.1 ml of either a solution of 0.1 g/l, or 10 g/l fumonisin B_1, a mycotoxin of *Fusarium moniliforme*, or with deionised water at the base of the stalk at 21 days, four weeks after treatment. The dry mass (g) of the combined samples was also recorded. The statistical analysis of the individual inbred lines is presented in APPENDIX 2.10. Data in APPENDIX 2.11

		HEIGHT (cm)		DRY MASS (g)	
		rep 1	rep 2	rep 1	rep 2
control	average	54.0	59.0	0.37	0.63
	std.dev.*	7.6	7.0		
	variance	57.4	49.5		
	minimum	37.5	46.5		
	maximum	66.4	69.6		
	n =	30	17		
water	average	52.9	55.3	0.41	0.59
	std.dev.	9.6	7.0		
	variance	91.8	48.9		
	minimum	27.0	42.6		
	maximum	70.0	65.7		
	n =	30	16		

		HEIGHT (cm)		MASS (g)	
		rep 1	rep 2	rep 1	rep 2
0.1 g/l	average	39.1	40.1	0.24	0.41
	std.dev.	10.8	10.1		
	variance	116.9	101.3		
	minimum	14.7	24.3		
	maximum	60.1	58.3		
	n =	29	15		
10 g/l	average	34.4	38.6	0.27	0.34
	std.dev.	6.7	8.2		
	variance	45.6	67.1		
	minimum	23.3	30.0		
	maximum	48.7	63.7		
	n =	19	12		

* std.dev. = standard deviation

Statistical analysis of the height (cm) of seedlings of two maize, *Zea mays*, inbred lines, I137TN and F2834, injected with 0.1 ml of either a solution of 0.1 g/l, or 10 g/l fumonisin B_1, a mycotoxin of *Fusarium moniliforme*, or with deionised water at the base of the stalk at 21 days, four weeks after treatment. The dry mass (g) of the combined samples was also recorded. Data in APPENDIX 2.11

inbred line: I137TN

		HEIGHT (cm)		DRY MASS (g)	
		rep 1	rep 2	rep 1	rep 2
control	average	53.9	60.0	0.40	0.60
	std.dev.*	7.0	6.5		
	minimum	39.7	46.5		
	maximum	66.4	67.6		
	n =	15	8		
water	average	51.8	57.4	0.41	0.57
	std.dev.	5.9	5.6		
	minimum	42.9	46.2		
	maximum	63.3	63.9		
	n =	15	8		

		HEIGHT (cm)		DRY MASS (g)	
		rep 1	rep 2	rep 1	rep 2
0.1 g/l	average	36.4	41.2	0.23	0.36
	std.dev.	10.9	12.8		
	minimum	14.7	24.3		
	maximum	54.4	58.3		
	n =	15	8		
10 g/l	average	35.8	40.1	0.27	0.36
	std.dev.	7.7	10.8		
	minimum	23.3	31.1		
	maximum	48.7	63.7		
	n =	12	6		

inbred line: F2834

control	average	54.1	58.2	0.34	0.65
	std.dev.	8.2	7.4		
	minimum	37.5	48.7		
	maximum	66.0	69.6		
	n =	15	9		
water	average	54.0	53.1	0.41	0.61
	std.dev.	12.1	7.6		
	minimum	27.0	42.6		
	maximum	70.0	65.7		
	n =	15	8		

0.1 g/l	average	42.0	38.8	0.26	0.48
	std.dev.	10.0	5.2		
	minimum	24.8	31.6		
	maximum	60.1	48.5		
	n =	14	7		
10 g/l	average	31.9	37.0	0.28	0.32
	std.dev.	3.6	3.6		
	minimum	23.5	30.0		
	maximum	35.7	41.0		
	n =	7	6		

* std.dev. = standard deviation

Data of the height (cm) of seedlings of two maize, *Zea mays*, inbred lines, injected at the stalk base with 0.1 ml of a fumonisin B_1, a mycotoxin of *Fusarium moniliforme*, solution (0.1 and 10 g/l), or with deionised water. The dry mass (g) of the samples was also recorded

inbred line: I137TN

	HEIGHT (cm)				TOTAL MASS (g)	
	replication 1		replication 2		rep 1	rep 2
control	57.6	56.8	61.2			
	58.9	57.1	63.5			
	52.3	46.2	53.0			
	55.8	46.8	46.5			
	56.3	56.0	64.0			
	55.0	42.5	64.7			
	61.2	66.4	59.2			
	39.7		67.6		6.03	4.80
water	42.9	53.5	59.7			
	52.4	59.1	60.6			
	57.7	63.3	63.9			
	61.0	44.4	62.3			
	47.8	47.8	59.9			
	48.1	50.0	54.1			
	50.0	51.2	52.7			
	47.1		46.2		6.14	4.57

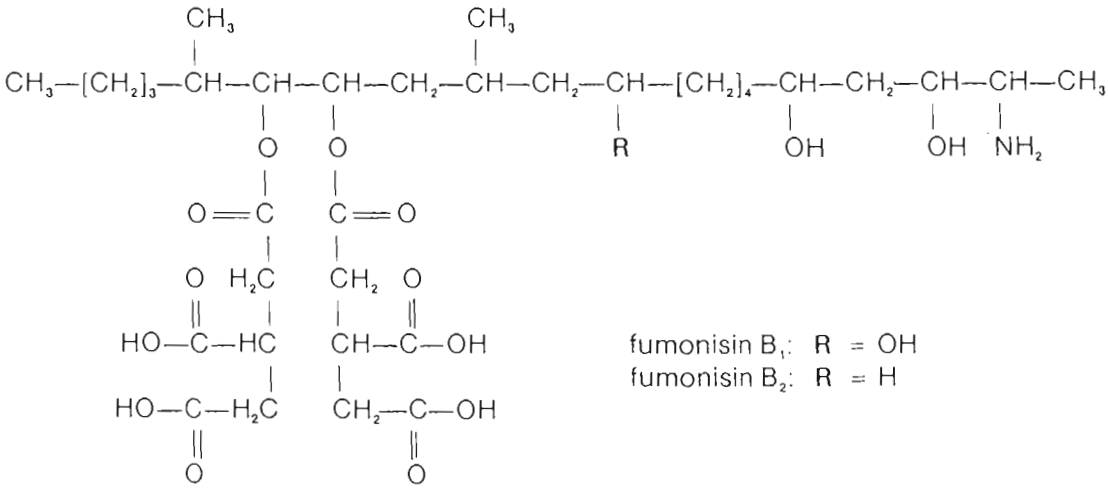
	HEIGHT (cm)				TOTAL MASS (g)	
	replication 1		replication 2		rep 1	rep 2
0.1 g/l	32.5	36.5	39.8			
	34.1	32.0	26.6			
	36.5	54.4	49.5			
	50.5	51.6	48.5			
	25.6	25.0	24.3			
	38.1	47.8	27.3			
	26.1	14.7	58.3			
	41.0		55.3		3.41	2.86
10 g/l	36.2	45.5	31.1			
	48.7	27.5	63.7			
	30.3	47.3	34.0			
	31.7	35.6	37.5			
	38.9		38.0			
	30.6		36.5			
	34.5					
	23.3				3.23	2.18

inbred line: F2834

control	54.0	63.6	50.2	69.6		
	51.3	52.3	54.3			
	40.6	66.0	64.8			
	50.9	56.8	48.7			
	60.7	58.7	60.8			
	37.5	61.9	66.3			
	51.3	44.5	49.8			
	61.4		59.5		5.17	5.88
water	69.0	50.7	49.5			
	70.0	47.0	60.0			
	63.2	48.6	55.1			
	50.0	63.4	42.6			
	68.3	40.0	57.4			
	55.7	27.0	65.7			
	52.6	40.0	42.7			
	64.5		51.8		6.14	4.88

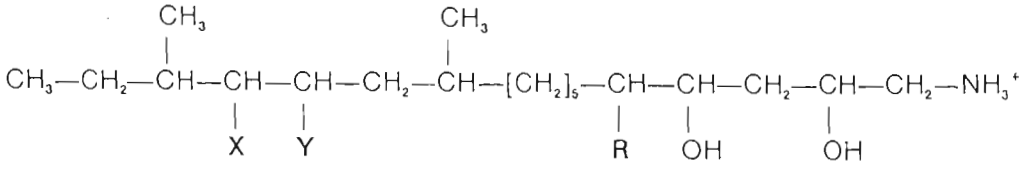
0.1 g/l	40.7	48.9	34.7			
	25.7	24.8	35.0			
	43.2	29.5	39.4			
	37.4	43.8	31.6			
	48.2	56.2	48.5			
	47.2	60.1	40.3			
	42.9		42.2			
	38.7				3.64	3.35
10 g/l	32.3		37.1			
	23.5		36.8			
	32.3		40.3			
	33.4		30.0			
	31.8		41.0			
	35.7		36.6			
	34.0					
					1.98	1.93

Structural formulas of fumonisin B, a mycotoxin of *Fusarium moniliforme* (Gelderblom *et al.*, 1988), and AL-toxin, a mycotoxin of *Alternaria alternata* f.sp. *lycopersici* (Nishimura & Kohmoto, 1983)



fumonisin B_1: R = OH
fumonisin B_2: R = H

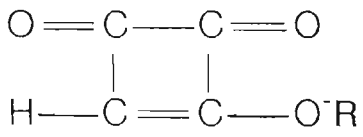
fumonisin B-toxin of *Fusarium moniliforme*

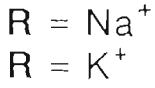


	R	X	Y
T_A a	OH	OH	$^{-}O_2C-CH_2-CH(CO_2^{-})-CH_2-CO_2$
T_A b	OH	$^{-}O_2C-CH_2-CH(CO_2^{-})-CH_2-CO_2$	OH
T_B a	H	OH	$^{-}O_2C-CH_2-CH(CO_2^{-})-CH_2-CO_2$
T_B b	H	$^{-}O_2C-CH_2-CH(CO_2^{-})-CH_2-CO_2$	OH

AL-toxin of *Alternaria alternata* f.sp. *lycopersici*

Structural formula of moniliformin, a mycotoxin of *Fusarium* spp., e.g. *F. subglutinans* (Yoshizawa, 1983)





moniliformin, a mycotoxin of *Fusarium subglutinans*

APPENDIX 3.1

Mass (g), taken at weekly intervals, of individual maize, *Zea mays*, callus pieces grown for six weeks on culture medium (Green & Phillips, 1975) containing different concentrations of moniliformin, a mycotoxin of *Fusarium subglutinans* (replication 2). Figures in a row followed by a different letter are statistically different ($P > 0.05$) in a LSD-test. No letter means that no statistical differences were found

week	concentration				
	control	0.1	1.0	10	100
1					
	0.00	0.01	0.03	0.03	0.00
	0.01	0.01	0.04	0.03	0.01
	0.02	0.02	0.04	0.04	0.03
	0.03	0.03	0.05	0.05	0.03
	0.03	0.03	0.05	0.06	0.04
	0.03	0.05	0.05	0.07	0.06
	0.07	0.05	0.05	0.07	0.06
	0.08	0.08	0.07	0.09	0.06
	0.08	0.10	0.09	0.09	0.06
	0.09	0.13	0.14	0.12	0.08
2					
	-0.01	-0.01	0.01	0.01	0.03
	0.03	0.01	0.02	0.03	0.03
	0.05	0.01	0.10	0.03	0.04
	0.08	0.03	0.10	0.04	0.06
	0.10	0.06	0.13	0.08	0.06
	0.13	0.08	0.14	0.09	0.07
	0.13	0.12	0.16	0.11	0.08
	0.15	0.13	0.17	0.12	0.09
	0.22	0.15	0.18	0.19	0.13
	0.31	0.15	0.26	0.20	0.13

week	concentration				
	control	0.1	1.0	10	100
3					
	0.05	-0.01	-0.03	0.03	-0.01
	0.11	0.00	0.01	0.05	0.00
	0.11	0.04	0.07	0.08	0.00
	0.13	0.08	0.07	0.12	0.01
	0.13	0.09	0.10	0.13	0.03
	0.14	0.18	0.11	0.14	0.07
	0.15	0.19	0.11	0.16	0.07
	0.19	0.19	0.19	0.23	0.08
	0.26	0.19	0.21	0.26	0.09
	0.30	0.29	0.34	0.26	0.11
4					
	0.02	-0.01	0.07	0.02	-0.03
	0.12	0.15	0.10	0.03	-0.01
	0.18	0.18	0.15	0.12	0.06
	0.18	0.18	0.16	0.19	0.09
	0.23	0.23	0.22	0.23	0.10
	0.30	0.26	0.22	0.25	0.11
	0.31	0.29	0.29	0.27	0.12
	0.36	0.34	0.33	0.28	0.15
	0.38	0.36	0.34	0.31	0.17
	0.39	0.38	0.35	0.40	0.22

week	concentration				
	control	0.1	1.0	10	100
5					
	0.25	0.17	0.05	0.01	0.01
	0.28	0.25	0.11	0.02	0.03
	0.30	0.30	0.30	0.03	0.09
	0.32	0.33	0.34	0.11	0.09
	0.34	0.35	0.35	0.19	0.13
	0.35	0.38	0.37	0.36	0.14
	0.39	0.41	0.46	0.39	0.15
	0.49	0.43	0.50	0.45	0.16
	0.55	0.51	0.50	0.53	0.24
	0.64	0.58	0.65	0.60	0.26

average* and standard deviation**

1 *	0.04	0.05	0.06	0.07	0.04
**	0.03	0.04	0.03	0.03	0.02
2 *	0.12	0.07	0.13	0.09	0.07
**	0.09	0.06	0.07	0.06	0.03
3 *	0.16 a	0.12 a	0.12	0.15	0.05 b
**	0.07	0.09	0.10	0.08	0.04
4 *	0.25 a	0.24 a	0.22	0.21	0.10 b
**	0.12	0.11	0.10	0.12	0.07
5 *	0.39 a	0.37 a	0.36	0.27	0.13 b
**	0.12	0.11	0.17	0.21	0.08

Statistical analysis of the average mass (g) of maize, *Zea mays*, callus pieces grown on culture medium (Green & Phillips, 1975) containing different concentrations of moniliformin, a mycotoxin of *Fusarium subglutinans*. "initial" is mass at the start of the experiment, "final" is mass after six weeks of growth. Data of the replications in APPENDIX 3.3 and 3.4

CONCENTRATION		n =	MASS (gram) initial	std.dev.*	final	std.dev.	increase**	std.dev.
0 mg/l (control)	rep 1	49	0.154	0.044	0.554	0.215	0.400	0.204
	rep 2	49	0.179	0.066	0.594	0.169	0.415	0.158
	average		0.167	0.012	0.574	0.020	0.408	0.008
	sum	98					(0.408)	
0.1 mg/l	rep 1	45	0.167	0.043	0.552	0.251	0.385	0.243
	rep 2	41	0.111	0.035	0.520	0.179	0.409	0.182
	average		0.139	0.028	0.536	0.016	0.397	0.012
	sum	86					(0.396)	
1.0 mg/l	rep 1	48	0.141	0.053	0.415	0.190	0.274	0.175
	rep 2	47	0.170	0.068	0.537	0.229	0.367	0.218
	average		0.156	0.014	0.476	0.061	0.321	0.046
	sum	95					(0.320)	
10 mg/l	rep 1	48	0.153	0.046	0.287	0.125	0.134	0.127
	rep 2	47	0.115	0.044	0.377	0.201	0.262	0.212
	average		0.134	0.019	0.332	0.045	0.198	0.064
	sum	95					(0.197)	
100 mg/l	rep 1	49	0.130	0.040	0.149	0.052	0.018	0.031
	rep 2	47	0.088	0.034	0.189	0.078	0.101	0.079
	average		0.109	0.021	0.169	0.020	0.060	0.041
	sum	96					(0.059)	

* std.dev. = standard deviation

** the figure given in brackets is the average mass increase of all the data of both rep. 1 & 2

Mass (g) of individual maize, *Zea mays*, callus pieces grown on culture medium (Green & Phillips, 1975) containing different concentrations of moniliformin, a mycotoxin of *Fusarium subglutinans* (replication 1). "initial" is mass at the start of the experiment, "final" is mass after six weeks of growth

CONCENTRATION:

	0 mg/l (control)			0.1 mg/l			1.0 mg/l			10 mg/l			100 mg/l		
	initial	final	increase	initial	final	increase	initial	final	increase	initial	final	increase	initial	final	increase
1	0.15	0.15	0.00	0.15	0.14	-0.01	0.30	0.35	0.05	0.11	0.11	0.00	0.11	0.09	-0.02
2	0.24	0.30	0.06	0.19	0.21	0.02	0.08	0.15	0.07	0.14	0.14	0.00	0.13	0.11	-0.02
3	0.17	0.25	0.08	0.15	0.18	0.03	0.12	0.19	0.07	0.19	0.19	0.00	0.10	0.09	-0.01
4	0.20	0.32	0.12	0.14	0.18	0.04	0.15	0.22	0.07	0.17	0.18	0.01	0.17	0.16	-0.01
5	0.16	0.31	0.15	0.12	0.22	0.10	0.16	0.23	0.07	0.23	0.24	0.01	0.17	0.16	-0.01
6	0.25	0.41	0.16	0.23	0.35	0.12	0.10	0.19	0.09	0.13	0.14	0.01	0.17	0.16	-0.01
7	0.14	0.31	0.17	0.20	0.35	0.15	0.15	0.25	0.10	0.08	0.10	0.02	0.22	0.21	-0.01
8	0.14	0.32	0.18	0.16	0.32	0.16	0.07	0.18	0.11	0.09	0.11	0.02	0.12	0.11	-0.01
9	0.13	0.32	0.19	0.09	0.27	0.18	0.08	0.19	0.11	0.17	0.20	0.03	0.18	0.17	-0.01
10	0.10	0.30	0.20	0.15	0.33	0.18	0.12	0.24	0.12	0.20	0.23	0.03	0.09	0.09	0.00
11	0.14	0.34	0.20	0.16	0.34	0.18	0.11	0.25	0.14	0.17	0.21	0.04	0.10	0.10	0.00
12	0.17	0.38	0.21	0.14	0.33	0.19	0.11	0.26	0.15	0.11	0.15	0.04	0.12	0.12	0.00
13	0.15	0.38	0.23	0.17	0.41	0.24	0.12	0.27	0.15	0.11	0.15	0.04	0.13	0.13	0.00
14	0.09	0.37	0.28	0.14	0.38	0.24	0.19	0.35	0.16	0.12	0.16	0.04	0.15	0.15	0.00
15	0.13	0.43	0.30	0.12	0.37	0.25	0.19	0.36	0.17	0.12	0.16	0.04	0.17	0.18	0.01
16	0.10	0.40	0.30	0.10	0.38	0.28	0.10	0.28	0.18	0.18	0.22	0.04	0.05	0.06	0.01
17	0.13	0.43	0.30	0.10	0.39	0.29	0.12	0.31	0.19	0.22	0.26	0.04	0.07	0.08	0.01
18	0.07	0.38	0.31	0.26	0.57	0.31	0.15	0.34	0.19	0.24	0.29	0.05	0.08	0.09	0.01
19	0.14	0.45	0.31	0.22	0.53	0.31	0.15	0.34	0.19	0.12	0.18	0.06	0.08	0.09	0.01
20	0.12	0.46	0.34	0.16	0.48	0.32	0.07	0.27	0.20	0.18	0.24	0.06	0.11	0.12	0.01
21	0.13	0.49	0.36	0.19	0.52	0.33	0.28	0.49	0.21	0.16	0.24	0.08	0.11	0.12	0.01
22	0.10	0.47	0.37	0.13	0.49	0.36	0.13	0.35	0.22	0.16	0.24	0.08	0.03	0.04	0.01
23	0.15	0.52	0.37	0.24	0.62	0.38	0.14	0.36	0.22	0.17	0.25	0.08	0.12	0.13	0.01
24	0.15	0.53	0.38	0.18	0.56	0.38	0.14	0.36	0.22	0.19	0.27	0.08	0.13	0.14	0.01
25	0.15	0.54	0.39	0.16	0.55	0.39	0.23	0.48	0.25	0.26	0.34	0.08	0.15	0.16	0.01
26	0.22	0.62	0.40	0.09	0.50	0.41	0.09	0.35	0.26	0.15	0.24	0.09	0.15	0.16	0.01
27	0.14	0.55	0.41	0.23	0.64	0.41	0.11	0.37	0.26	0.14	0.24	0.10	0.16	0.17	0.01
28	0.16	0.58	0.42	0.14	0.56	0.42	0.14	0.40	0.26	0.10	0.20	0.10	0.10	0.12	0.02
29	0.09	0.51	0.42	0.19	0.62	0.43	0.16	0.43	0.27	0.16	0.26	0.10	0.14	0.16	0.02
30	0.15	0.59	0.44	0.23	0.66	0.43	0.07	0.35	0.28	0.23	0.33	0.10	0.16	0.18	0.02
31	0.09	0.53	0.44	0.16	0.62	0.46	0.11	0.39	0.28	0.16	0.28	0.12	0.08	0.10	0.02
32	0.16	0.61	0.45	0.13	0.60	0.47	0.06	0.36	0.30	0.22	0.34	0.12	0.08	0.11	0.03
33	0.21	0.68	0.47	0.16	0.63	0.47	0.13	0.43	0.30	0.20	0.35	0.15	0.10	0.13	0.03
34	0.16	0.65	0.49	0.17	0.64	0.47	0.12	0.44	0.32	0.20	0.38	0.18	0.10	0.13	0.03
35	0.13	0.63	0.50	0.20	0.69	0.49	0.10	0.43	0.33	0.10	0.30	0.20	0.12	0.15	0.03
36	0.16	0.73	0.57	0.24	0.74	0.50	0.13	0.47	0.34	0.11	0.32	0.21	0.14	0.17	0.03
37	0.23	0.80	0.57	0.13	0.70	0.57	0.17	0.51	0.34	0.09	0.31	0.22	0.14	0.17	0.03
38	0.12	0.70	0.58	0.18	0.75	0.57	0.13	0.48	0.35	0.18	0.44	0.26	0.14	0.17	0.03
39	0.15	0.73	0.58	0.18	0.82	0.64	0.24	0.60	0.36	0.06	0.35	0.29	0.15	0.18	0.03
40	0.16	0.74	0.58	0.25	0.97	0.72	0.10	0.52	0.42	0.12	0.43	0.31	0.17	0.20	0.03
41	0.21	0.79	0.58	0.21	1.01	0.80	0.19	0.63	0.44	0.14	0.45	0.31	0.17	0.20	0.03
42	0.20	0.79	0.59	0.16	0.99	0.83	0.21	0.68	0.47	0.13	0.45	0.32	0.21	0.24	0.03
43	0.11	0.74	0.63	0.13	1.02	0.89	0.11	0.65	0.54	0.16	0.50	0.34	0.12	0.16	0.04
44	0.11	0.78	0.67	0.15	1.08	0.93	0.24	0.80	0.56	0.17	0.51	0.34	0.15	0.19	0.04
45	0.13	0.80	0.67	0.14	1.14	1.00	0.12	0.69	0.57	0.18	0.52	0.34	0.21	0.25	0.04
46	0.22	0.96	0.74				0.12	0.79	0.67	0.10	0.45	0.35	0.11	0.16	0.05
47	0.23	1.00	0.77				0.17	0.93	0.76	0.10	0.49	0.39	0.12	0.17	0.05
48	0.15	0.93	0.78				0.19	0.95	0.76	0.14	0.63	0.49	0.14	0.20	0.06
49	0.24	1.14	0.90										0.16	0.35	0.19

Mass (g) of individual maize, *Zea mays*, callus pieces grown on culture medium (Green & Phillips, 1975) containing different concentrations of moniliformin, a mycotoxin of *Fusarium subglutinans* (replication 2). "initial" is mass at the start of the experiment, "final" is mass after six weeks of growth

CONCENTRATION:

	0 mg/l (control)			0.1 mg/l			1.0 mg/l			10 mg/l			100 mg/l		
	initial	final	increase	initial	final	increase	initial	final	increase	initial	final	increase	initial	final	increase
1	0.21	0.21	0.00	0.11	0.08	-0.03	0.16	0.13	-0.03	0.13	0.12	-0.01	0.15	0.11	-0.04
2	0.19	0.22	0.03	0.09	0.07	-0.02	0.16	0.14	-0.02	0.16	0.15	-0.01	0.07	0.06	-0.01
3	0.22	0.32	0.10	0.08	0.10	0.02	0.22	0.21	-0.01	0.05	0.04	-0.01	0.08	0.07	-0.01
4	0.08	0.20	0.12	0.11	0.20	0.09	0.06	0.10	0.04	0.13	0.13	0.00	0.04	0.04	0.00
5	0.18	0.39	0.21	0.10	0.26	0.16	0.19	0.28	0.09	0.16	0.16	0.00	0.08	0.08	0.00
6	0.28	0.52	0.24	0.20	0.38	0.18	0.22	0.32	0.10	0.09	0.10	0.01	0.05	0.06	0.01
7	0.23	0.48	0.25	0.16	0.35	0.19	0.24	0.36	0.12	0.12	0.13	0.01	0.10	0.11	0.01
8	0.12	0.37	0.25	0.09	0.35	0.26	0.20	0.33	0.13	0.17	0.19	0.02	0.10	0.11	0.01
9	0.07	0.36	0.29	0.14	0.42	0.28	0.10	0.27	0.17	0.12	0.14	0.02	0.04	0.05	0.01
10	0.14	0.44	0.30	0.13	0.45	0.32	0.11	0.28	0.17	0.26	0.31	0.05	0.09	0.10	0.01
11	0.17	0.47	0.30	0.16	0.49	0.33	0.14	0.32	0.18	0.07	0.13	0.06	0.10	0.12	0.02
12	0.24	0.56	0.32	0.17	0.52	0.35	0.18	0.36	0.18	0.18	0.24	0.06	0.17	0.20	0.03
13	0.16	0.49	0.33	0.08	0.45	0.37	0.27	0.46	0.19	0.08	0.15	0.07	0.23	0.26	0.03
14	0.10	0.44	0.34	0.10	0.47	0.37	0.12	0.34	0.22	0.16	0.23	0.07	0.06	0.11	0.05
15	0.34	0.69	0.35	0.12	0.49	0.37	0.22	0.45	0.23	0.09	0.17	0.08	0.09	0.15	0.06
16	0.16	0.51	0.35	0.08	0.48	0.40	0.04	0.28	0.24	0.09	0.20	0.11	0.12	0.19	0.07
17	0.30	0.65	0.35	0.13	0.53	0.40	0.15	0.42	0.27	0.10	0.25	0.15	0.10	0.18	0.08
18	0.05	0.44	0.39	0.10	0.51	0.41	0.08	0.37	0.29	0.10	0.25	0.15	0.10	0.18	0.08
19	0.06	0.45	0.39	0.13	0.55	0.42	0.19	0.48	0.29	0.13	0.29	0.16	0.13	0.21	0.08
20	0.12	0.51	0.39	0.16	0.59	0.43	0.27	0.60	0.33	0.10	0.26	0.16	0.08	0.16	0.08
21	0.14	0.53	0.39	0.10	0.53	0.43	0.18	0.51	0.33	0.22	0.38	0.16	0.09	0.18	0.09
22	0.17	0.56	0.39	0.09	0.54	0.45	0.24	0.59	0.35	0.07	0.27	0.20	0.10	0.19	0.09
23	0.27	0.68	0.41	0.18	0.64	0.46	0.24	0.61	0.37	0.12	0.33	0.21	0.03	0.13	0.10
24	0.21	0.63	0.42	0.15	0.62	0.47	0.26	0.63	0.37	0.10	0.32	0.22	0.07	0.17	0.10
25	0.11	0.54	0.43	0.09	0.56	0.47	0.08	0.48	0.40	0.09	0.33	0.24	0.09	0.19	0.10
26	0.26	0.71	0.45	0.13	0.61	0.48	0.11	0.52	0.41	0.10	0.34	0.24	0.10	0.21	0.11
27	0.17	0.62	0.45	0.05	0.53	0.48	0.30	0.73	0.43	0.18	0.44	0.26	0.13	0.24	0.11
28	0.18	0.64	0.46	0.07	0.56	0.49	0.10	0.53	0.43	0.13	0.44	0.31	0.08	0.19	0.11
29	0.28	0.75	0.47	0.15	0.65	0.50	0.19	0.63	0.44	0.12	0.44	0.32	0.09	0.20	0.11
30	0.16	0.63	0.47	0.05	0.58	0.53	0.07	0.53	0.46	0.17	0.50	0.33	0.09	0.20	0.11
31	0.26	0.75	0.49	0.11	0.64	0.53	0.13	0.60	0.47	0.11	0.51	0.40	0.08	0.20	0.12
32	0.14	0.64	0.50	0.09	0.65	0.56	0.12	0.61	0.49	0.12	0.52	0.40	0.06	0.20	0.14
33	0.18	0.69	0.51	0.08	0.65	0.57	0.14	0.63	0.49	0.06	0.48	0.42	0.08	0.22	0.14
34	0.21	0.72	0.51	0.11	0.68	0.57	0.22	0.71	0.49	0.14	0.58	0.44	0.08	0.22	0.14
35	0.23	0.74	0.51	0.08	0.69	0.61	0.11	0.61	0.50	0.12	0.56	0.44	0.08	0.22	0.14
36	0.21	0.73	0.52	0.10	0.71	0.61	0.12	0.62	0.50	0.10	0.56	0.46	0.05	0.20	0.15
37	0.16	0.70	0.54	0.09	0.72	0.63	0.23	0.75	0.52	0.10	0.57	0.47	0.11	0.26	0.15
38	0.08	0.62	0.54	0.10	0.73	0.63	0.32	0.84	0.52	0.10	0.58	0.48	0.10	0.26	0.16
39	0.13	0.67	0.54	0.10	0.75	0.65	0.17	0.71	0.54	0.14	0.63	0.49	0.05	0.23	0.18
40	0.24	0.78	0.54	0.15	0.80	0.65	0.12	0.67	0.55	0.06	0.55	0.49	0.08	0.26	0.18
41	0.25	0.79	0.54	0.06	0.74	0.68	0.29	0.88	0.59	0.08	0.60	0.52	0.07	0.26	0.19
42	0.17	0.72	0.55				0.10	0.73	0.63	0.11	0.65	0.54	0.07	0.27	0.20
43	0.15	0.73	0.58				0.13	0.76	0.63	0.06	0.63	0.57	0.04	0.26	0.22
44	0.11	0.71	0.60				0.14	0.83	0.69	0.08	0.65	0.57	0.08	0.33	0.25
45	0.20	0.80	0.60				0.22	0.98	0.76	0.02	0.61	0.59	0.10	0.36	0.26
46	0.24	0.85	0.61				0.13	0.95	0.82	0.10	0.77	0.67	0.08	0.35	0.27
47	0.16	0.78	0.62				0.20	1.09	0.89	0.11	0.83	0.72	0.07	0.34	0.27
48	0.10	0.74	0.64												
49	0.18	0.95	0.77												

Statistical analysis of the average mass (g) of maize, *Zea mays*, callus pieces grown on culture medium (Green & Phillips, 1975) containing different concentrations of T-2 toxin, a mycotoxin of *Fusarium tricinctum*. "initial" is mass at the start of the experiment, "final" is mass after six weeks of growth. Data of the replications in APPENDIX 3.6, 3.7 and 3.8

CONCENTRATION		n =	MASS (gram) initial	std.dev.*	final	std.dev.	increase**	std.dev.
0 mg/l (control)	rep 1	43	0.147	0.044	0.735	0.245	0.588	0.216
	rep 2	40	0.140	0.042	0.455	0.162	0.315	0.167
	average		0.144	0.003	0.595	0.140	0.452	0.136
	sum	83					(0.456)	
0.1 mg/l	rep 1	46	0.128	0.039	0.662	0.202	0.533	0.184
	rep 2	47	0.143	0.040	0.436	0.205	0.293	0.197
	average		0.136	0.007	0.549	0.113	0.413	0.120
	sum	93						(0.412)
1.0 mg/l	rep 1	45	0.150	0.044	0.660	0.163	0.510	0.140
	rep 2	44	0.136	0.037	0.363	0.173	0.228	0.171
	average		0.143	0.007	0.512	0.148	0.369	0.141
	sum	89					(0.370)	
10 mg/l	rep 1	49	0.117	0.040	0.542	0.152	0.425	0.137
	rep 2	34	0.136	0.038	0.321	0.166	0.185	0.159
	average		0.127	0.010	0.432	0.110	0.305	0.120
	sum	83					(0.327)	
100 mg/l	rep 1	48	0.108	0.037	0.391	0.112	0.284	0.101
	rep 2	35	0.139	0.041	0.270	0.151	0.131	0.152
	average		0.123	0.016	0.331	0.061	0.207	0.076
	sum	83					(0.219)	
ethanol 2 ml/l	rep 1	36	0.137	0.050	0.578	0.169	0.441	0.168
	rep 2	37	0.154	0.033	0.483	0.188	0.329	0.188
	average		0.146	0.008	0.531	0.047	0.385	0.056
	sum	73					(0.384)	

* std.dev.= standard deviation

** the figure given in brackets is the average mass increase of all the data of both rep. 1 & 2

Mass (g) of individual maize, *Zea mays*, callus pieces grown on culture medium (Green & Phillips, 1975) containing different concentrations of T-2 toxin, a mycotoxin of *Fusarium tricinctum* (replication 1). "initial" is mass at the start of the experiment, "final" is mass after six weeks of growth

CONCENTRATION:

	0 mg/l (control)			0.1 mg/l			1.0 mg/l			10 mg/l			100 mg/l		
	initial	final	increase	initial	final	increase	initial	final	increase	initial	final	increase	initial	final	increase
1	0.09	0.27	0.18	0.07	0.27	0.20	0.07	0.26	0.19	0.10	0.38	0.28	0.09	0.09	0.00
2	0.12	0.31	0.19	0.11	0.33	0.22	0.15	0.39	0.24	0.16	0.46	0.30	0.15	0.23	0.08
3	0.18	0.37	0.19	0.07	0.30	0.23	0.11	0.39	0.28	0.12	0.43	0.31	0.16	0.27	0.11
4	0.20	0.41	0.21	0.06	0.30	0.24	0.11	0.41	0.30	0.12	0.46	0.34	0.06	0.19	0.13
5	0.06	0.27	0.21	0.08	0.41	0.33	0.11	0.45	0.34	0.16	0.50	0.34	0.11	0.25	0.14
6	0.10	0.32	0.22	0.20	0.57	0.37	0.07	0.43	0.36	0.14	0.49	0.35	0.14	0.30	0.16
7	0.09	0.33	0.24	0.07	0.47	0.40	0.13	0.50	0.37	0.09	0.44	0.35	0.17	0.34	0.17
8	0.05	0.32	0.27	0.10	0.50	0.40	0.11	0.51	0.40	0.09	0.44	0.35	0.09	0.27	0.18
9	0.09	0.38	0.29	0.08	0.49	0.41	0.12	0.52	0.40	0.12	0.49	0.37	0.06	0.25	0.19
10	0.10	0.39	0.29	0.12	0.53	0.41	0.10	0.51	0.41	0.12	0.51	0.39	0.11	0.31	0.20
11	0.13	0.43	0.30	0.08	0.50	0.42	0.24	0.65	0.41	0.10	0.51	0.41	0.08	0.29	0.21
12	0.10	0.40	0.30	0.17	0.59	0.42	0.14	0.57	0.43	0.18	0.60	0.42	0.07	0.30	0.23
13	0.07	0.39	0.32	0.18	0.60	0.42	0.22	0.65	0.43	0.10	0.53	0.43	0.13	0.36	0.23
14	0.09	0.42	0.33	0.11	0.54	0.43	0.13	0.58	0.45	0.11	0.54	0.43	0.08	0.31	0.23
15	0.08	0.45	0.37	0.12	0.55	0.43	0.16	0.61	0.45	0.13	0.56	0.43	0.14	0.38	0.24
16	0.08	0.47	0.39	0.16	0.60	0.44	0.17	0.62	0.45	0.12	0.56	0.44	0.08	0.32	0.24
17	0.07	0.46	0.39	0.10	0.54	0.44	0.09	0.54	0.45	0.12	0.62	0.50	0.08	0.33	0.25
18	0.11	0.50	0.39	0.19	0.64	0.45	0.16	0.62	0.46	0.16	0.66	0.50	0.09	0.34	0.25
19	0.14	0.53	0.39	0.24	0.70	0.46	0.19	0.65	0.46	0.10	0.65	0.55	0.11	0.37	0.26
20	0.11	0.51	0.40	0.15	0.61	0.46	0.12	0.59	0.47	0.17	0.73	0.56	0.13	0.40	0.27
21	0.11	0.51	0.40	0.14	0.61	0.47	0.09	0.58	0.49	0.19	0.75	0.56	0.16	0.43	0.27
22	0.08	0.49	0.41	0.09	0.56	0.47	0.15	0.65	0.50	0.12	0.69	0.57	0.10	0.39	0.29
23	0.17	0.58	0.41	0.12	0.63	0.51	0.17	0.68	0.51	0.14	0.73	0.59	0.06	0.36	0.30
24	0.11	0.52	0.41	0.13	0.64	0.51	0.17	0.70	0.53	0.12	0.73	0.61	0.07	0.37	0.30
25	0.13	0.55	0.42	0.17	0.69	0.52	0.17	0.71	0.54	0.11	0.73	0.62	0.08	0.38	0.30
26	0.15	0.58	0.43	0.14	0.67	0.53	0.08	0.62	0.54	0.09	0.72	0.63	0.11	0.41	0.30
27	0.09	0.52	0.43	0.11	0.66	0.55	0.11	0.65	0.54	0.11	0.74	0.63	0.15	0.46	0.31
28	0.10	0.54	0.44	0.13	0.68	0.55	0.19	0.73	0.54	0.21	0.85	0.64	0.06	0.38	0.32
29	0.16	0.62	0.46	0.16	0.72	0.56	0.29	0.83	0.54	0.16	0.84	0.68	0.06	0.38	0.32
30	0.18	0.64	0.46	0.09	0.65	0.56	0.15	0.70	0.55	0.19	0.87	0.68	0.12	0.44	0.32
31	0.11	0.58	0.47	0.12	0.68	0.56	0.14	0.70	0.56	0.18	0.88	0.70	0.05	0.38	0.33
32	0.14	0.61	0.47	0.12	0.69	0.57	0.15	0.71	0.56	0.11	0.82	0.71	0.07	0.40	0.33
33	0.10	0.58	0.48	0.10	0.67	0.57	0.15	0.73	0.58	0.15	0.86	0.71	0.12	0.45	0.33
34	0.17	0.66	0.49	0.09	0.67	0.58	0.17	0.75	0.58	0.13	0.86	0.73	0.10	0.44	0.34
35	0.10	0.60	0.50	0.17	0.81	0.64	0.23	0.81	0.58	0.12	0.86	0.74	0.16	0.50	0.34
36	0.05	0.56	0.51	0.10	0.75	0.65	0.13	0.73	0.60	0.23	1.00	0.77	0.12	0.47	0.35
37	0.16	0.67	0.51	0.12	0.80	0.68	0.14	0.74	0.60	0.26	1.04	0.78	0.13	0.49	0.36
38	0.09	0.61	0.52	0.16	0.85	0.69	0.17	0.82	0.65	0.14	1.01	0.87	0.10	0.46	0.36
39	0.14	0.68	0.54	0.13	0.86	0.73	0.17	0.86	0.69	0.20	1.07	0.87	0.11	0.48	0.37
40	0.09	0.65	0.56	0.16	0.93	0.77	0.20	0.92	0.72	0.20	1.07	0.87	0.07	0.45	0.38
41	0.13	0.70	0.57	0.12	0.90	0.78	0.16	0.90	0.74	0.18	1.22	1.04	0.07	0.45	0.38
42	0.11	0.69	0.58	0.14	0.94	0.80	0.16	0.91	0.75	0.21	1.26	1.05	0.08	0.46	0.38
43	0.08	0.68	0.60	0.14	1.02	0.88	0.19	0.94	0.75	0.25	1.43	1.18	0.10	0.48	0.38
44	0.05	0.66	0.61	0.14	1.06	0.92	0.14	0.92	0.78				0.17	0.55	0.38
45	0.19	0.81	0.62	0.18	1.13	0.95	0.17	0.97	0.80				0.12	0.52	0.40
46	0.16	0.80	0.64	0.18	1.14	0.96							0.11	0.52	0.41
47	0.20	0.85	0.65										0.19	0.62	0.43
48	0.18	0.85	0.67										0.19	0.75	0.56
49	0.15	0.85	0.70												

Mass (g) of individual maize, *Zea mays*, callus pieces grown on culture medium (Green & Phillips, 1975) containing different concentrations of T-2 toxin, a mycotoxin of *Fusarium tricinctum* (replication 2). "initial" is mass at the start of the experiment, "final" is mass after six weeks of growth

CONCENTRATION:

	0 mg/l (control)			0.1 mg/l			1.0 mg/l			10 mg/l			100 mg/l		
	initial	final	increase	initial	final	increase	initial	final	increase	initial	final	increase	initial	final	increase
1	0.19	0.17	-0.02	0.16	0.13	-0.03	0.15	0.15	0.00	0.14	0.14	0.00	0.19	0.15	-0.04
2	0.12	0.12	0.00	0.10	0.09	-0.01	0.15	0.16	0.01	0.05	0.06	0.01	0.10	0.09	-0.01
3	0.27	0.27	0.00	0.11	0.12	0.01	0.14	0.16	0.02	0.16	0.17	0.01	0.16	0.15	-0.01
4	0.14	0.18	0.04	0.17	0.21	0.04	0.06	0.08	0.02	0.12	0.15	0.03	0.09	0.08	-0.01
5	0.16	0.23	0.07	0.09	0.13	0.04	0.19	0.23	0.04	0.16	0.19	0.03	0.08	0.08	0.00
6	0.11	0.19	0.08	0.09	0.16	0.07	0.13	0.18	0.05	0.09	0.13	0.04	0.12	0.12	0.00
7	0.07	0.22	0.15	0.12	0.19	0.07	0.17	0.22	0.05	0.19	0.23	0.04	0.13	0.13	0.00
8	0.13	0.28	0.15	0.12	0.19	0.07	0.12	0.17	0.05	0.13	0.18	0.05	0.18	0.18	0.00
9	0.11	0.28	0.17	0.15	0.22	0.07	0.18	0.23	0.05	0.12	0.17	0.05	0.22	0.22	0.00
10	0.09	0.29	0.20	0.15	0.22	0.07	0.10	0.16	0.06	0.15	0.20	0.05	0.10	0.11	0.01
11	0.17	0.38	0.21	0.13	0.21	0.08	0.08	0.15	0.07	0.10	0.16	0.06	0.15	0.16	0.01
12	0.22	0.46	0.24	0.11	0.19	0.08	0.25	0.32	0.07	0.16	0.22	0.06	0.19	0.21	0.02
13	0.10	0.36	0.26	0.20	0.28	0.08	0.12	0.20	0.08	0.17	0.26	0.09	0.15	0.18	0.03
14	0.10	0.38	0.28	0.11	0.21	0.10	0.08	0.17	0.09	0.11	0.22	0.11	0.15	0.19	0.04
15	0.16	0.44	0.28	0.17	0.28	0.11	0.19	0.28	0.09	0.08	0.20	0.12	0.15	0.19	0.04
16	0.16	0.44	0.28	0.12	0.25	0.13	0.17	0.28	0.11	0.15	0.27	0.12	0.14	0.19	0.05
17	0.17	0.45	0.28	0.25	0.38	0.13	0.17	0.29	0.12	0.15	0.29	0.14	0.16	0.21	0.05
18	0.17	0.45	0.28	0.15	0.35	0.20	0.13	0.25	0.12	0.21	0.37	0.16	0.19	0.26	0.07
19	0.13	0.42	0.29	0.12	0.32	0.20	0.11	0.24	0.13	0.23	0.40	0.17	0.14	0.22	0.08
20	0.09	0.41	0.32	0.13	0.37	0.24	0.13	0.29	0.16	0.09	0.28	0.19	0.11	0.20	0.09
21	0.12	0.44	0.32	0.18	0.42	0.24	0.11	0.28	0.17	0.16	0.36	0.20	0.08	0.20	0.12
22	0.19	0.52	0.33	0.13	0.42	0.29	0.15	0.33	0.18	0.14	0.34	0.20	0.27	0.40	0.13
23	0.14	0.51	0.37	0.21	0.51	0.30	0.18	0.38	0.20	0.11	0.32	0.21	0.08	0.25	0.17
24	0.16	0.54	0.38	0.11	0.44	0.33	0.14	0.37	0.23	0.15	0.37	0.22	0.10	0.28	0.18
25	0.19	0.58	0.39	0.12	0.45	0.33	0.15	0.38	0.23	0.13	0.37	0.24	0.13	0.32	0.19
26	0.12	0.51	0.39	0.15	0.51	0.36	0.10	0.38	0.28	0.06	0.33	0.27	0.13	0.33	0.20
27	0.17	0.57	0.40	0.19	0.55	0.36	0.13	0.42	0.29	0.12	0.40	0.28	0.10	0.32	0.22
28	0.11	0.54	0.43	0.13	0.50	0.37	0.09	0.38	0.29	0.15	0.48	0.33	0.11	0.35	0.24
29	0.14	0.58	0.44	0.14	0.53	0.39	0.20	0.50	0.30	0.10	0.48	0.38	0.15	0.41	0.26
30	0.12	0.57	0.45	0.14	0.54	0.40	0.10	0.40	0.30	0.12	0.50	0.38	0.15	0.45	0.30
31	0.17	0.62	0.45	0.20	0.61	0.41	0.08	0.39	0.31	0.13	0.61	0.48	0.17	0.49	0.32
32	0.11	0.56	0.45	0.15	0.57	0.42	0.11	0.43	0.32	0.18	0.67	0.49	0.15	0.56	0.41
33	0.15	0.62	0.47	0.09	0.51	0.42	0.09	0.43	0.34	0.15	0.67	0.52	0.12	0.54	0.42
34	0.12	0.61	0.49	0.10	0.53	0.43	0.11	0.49	0.38	0.15	0.72	0.57	0.10	0.54	0.44
35	0.11	0.62	0.51	0.17	0.63	0.46	0.13	0.51	0.38				0.13	0.68	0.55
36	0.11	0.64	0.53	0.08	0.55	0.47	0.11	0.52	0.41						
37	0.21	0.74	0.53	0.16	0.66	0.50	0.15	0.60	0.45						
38	0.09	0.63	0.54	0.19	0.71	0.52	0.15	0.60	0.45						
39	0.08	0.65	0.57	0.17	0.70	0.53	0.13	0.62	0.49						
40	0.11	0.72	0.61	0.09	0.62	0.53	0.14	0.63	0.49						
41				0.14	0.67	0.53	0.15	0.65	0.50						
42				0.16	0.70	0.54	0.16	0.70	0.54						
43				0.18	0.72	0.54	0.14	0.68	0.54						
44				0.25	0.82	0.57	0.16	0.71	0.55						
45				0.12	0.70	0.58									
46				0.10	0.68	0.58									
47				0.12	0.72	0.60									
48															
49															

Mass (g) of individual maize, *Zea mays*, callus pieces grown on culture medium (Green & Phillips, 1975) containing 2 ml ethanol per litre. "initial" is mass at the start of the experiment, "final" is mass after six weeks of growth

	REPLICATION 1: 2 ml/l			REPLICATION 2: 2 ml/l		
	initial	final	increase	initial	final	increase
1	0.09	0.28	0.19	0.21	0.20	-0.01
2	0.13	0.69	0.56	0.18	0.19	0.01
3	0.09	0.48	0.39	0.13	0.15	0.02
4	0.16	0.78	0.62	0.14	0.17	0.03
5	0.21	0.33	0.12	0.13	0.18	0.05
6	0.19	0.55	0.36	0.10	0.16	0.06
7	0.12	0.61	0.49	0.17	0.30	0.13
8	0.14	0.51	0.37	0.14	0.27	0.13
9	0.17	0.39	0.22	0.15	0.31	0.16
10	0.09	0.68	0.59	0.17	0.34	0.17
11	0.16	0.45	0.29	0.19	0.39	0.20
12	0.17	0.55	0.38	0.23	0.44	0.21
13	0.18	0.88	0.70	0.18	0.43	0.25
14	0.11	0.51	0.40	0.18	0.47	0.29
15	0.15	0.30	0.15	0.14	0.45	0.31
16	0.23	0.49	0.26	0.13	0.47	0.34
17	0.20	0.80	0.60	0.12	0.46	0.34
18	0.19	0.40	0.21	0.18	0.54	0.36
19	0.23	0.75	0.52	0.18	0.55	0.37
20	0.26	0.57	0.31	0.13	0.51	0.38
21	0.06	0.55	0.49	0.15	0.54	0.39
22	0.12	0.88	0.76	0.16	0.55	0.39
23	0.13	0.56	0.43	0.12	0.54	0.42
24	0.14	0.95	0.81	0.14	0.56	0.42
25	0.04	0.26	0.22	0.12	0.55	0.43
26	0.09	0.62	0.53	0.12	0.55	0.43
27	0.13	0.76	0.63	0.10	0.57	0.47
28	0.09	0.56	0.47	0.21	0.68	0.47
29	0.08	0.46	0.38	0.13	0.62	0.49
30	0.10	0.45	0.35	0.19	0.69	0.50
31	0.10	0.60	0.50	0.11	0.61	0.50
32	0.10	0.69	0.59	0.12	0.62	0.50
33	0.13	0.66	0.53	0.18	0.71	0.53
34	0.12	0.62	0.50	0.12	0.67	0.55
35	0.15	0.73	0.58	0.19	0.77	0.58
36	0.08	0.47	0.39	0.19	0.77	0.58
37				0.15	0.88	0.73
38						
39						
40						
41						
42						
43						
44						
45						
46						
47						
48						
49						

APPENDIX 3.9

Mass (g), taken at weekly intervals, of individual maize, *Zea mays*, callus pieces grown for six weeks on culture medium (Green & Phillips, 1975) containing different concentrations of HT-toxin, a pathotoxin extract of *Exserohilum turcicum* (replication 1). Figures in a row followed by a different letter are statistically different ($P > 0.05$) in a LSD-test. No letter means that no statistical differences were found

week	concentration				
	control	0.1	1.0	10	100
1					
	0.02	0.02	0.04	0.03	0.00
	0.02	0.04	0.04	0.04	0.02
	0.03	0.05	0.04	0.05	0.03
	0.03	0.05	0.05	0.05	0.04
	0.04	0.05	0.05	0.05	0.04
	0.04	0.06	0.06	0.06	0.05
	0.05	0.07	0.08	0.07	0.05
	0.07	0.08	0.09	0.08	0.05
	0.08	0.10	0.09	0.09	0.08
	0.09	0.12	0.11	0.13	0.09
2					
	0.00	0.05	0.01	0.03	0.03
	0.09	0.06	0.08	0.08	0.06
	0.09	0.09	0.10	0.09	0.08
	0.12	0.13	0.12	0.11	0.09
	0.15	0.14	0.13	0.11	0.09
	0.17	0.16	0.14	0.12	0.12
	0.18	0.18	0.16	0.13	0.13
	0.19	0.18	0.17	0.16	0.14
	0.22	0.21	0.18	0.19	0.16
	0.24	0.25	0.26	0.20	0.19

week	concentration				
	control	0.1	1.0	10	100
3					
	0.06	0.05	0.08	0.08	0.00
	0.14	0.09	0.08	0.12	0.06
	0.14	0.12	0.12	0.14	0.07
	0.15	0.13	0.14	0.14	0.08
	0.18	0.15	0.16	0.16	0.12
	0.18	0.17	0.19	0.19	0.13
	0.21	0.23	0.23	0.23	0.15
	0.24	0.25	0.24	0.23	0.18
	0.34	0.27	0.27	0.26	0.19
	0.36	0.31	0.36	0.26	0.25
4					
	-0.01	0.02	0.02	0.05	0.02
	0.06	0.18	0.09	0.09	0.06
	0.08	0.19	0.10	0.10	0.07
	0.14	0.24	0.11	0.10	0.08
	0.17	0.28	0.27	0.15	0.10
	0.17	0.32	0.28	0.24	0.10
	0.23	0.34	0.29	0.25	0.12
	0.26	0.40	0.35	0.28	0.12
	0.35	0.43	0.36	0.34	0.45
	0.92	0.53	0.43	0.63	0.49

week	concentration				
	control	0.1	1.0	10	100
5					
	0.17	0.01	0.03	0.02	0.10
	0.35	0.05	0.18	0.16	0.19
	0.36	0.29	0.21	0.16	0.21
	0.37	0.41	0.33	0.24	0.26
	0.48	0.53	0.50	0.25	0.27
	0.53	0.61	0.51	0.34	0.32
	0.53	0.76	0.56	0.37	0.46
	0.77	0.77	0.72	0.43	0.47
	0.79	0.81	0.78	0.60	0.51
	0.82	0.90	0.92	0.78	0.64

average* and standard deviation**

1 *	0.05	0.06	0.07	0.07	0.05
**	0.02	0.03	0.02	0.03	0.03
2 *	0.15	0.15	0.14	0.12	0.11
**	0.07	0.06	0.06	0.05	0.05
3 *	0.20 a	0.18	0.19	0.18	0.12 b
**	0.09	0.08	0.08	0.06	0.07
4 *	0.24	0.29	0.23	0.22	0.16
**	0.25	0.14	0.13	0.16	0.16
5 *	0.52	0.51	0.47	0.34	0.34
**	0.21	0.30	0.27	0.21	0.16

Statistical analysis of the average mass (g) of maize, *Zea mays*, callus pieces grown on culture medium (Green & Phillips, 1975) containing different concentrations of HT-toxin, a pathotoxin extract of *Exserohilum turcicum*. "initial" is mass at the start of the experiment, "final" is mass after six weeks of growth. Data of the replications in APPENDIX 3.11 and 3.12

CONCENTRATION		n =	MASS (gram) initial	std.dev.*	final	std.dev.	increase**	std.dev.
0 ml/l (control)	rep 1	47	0.161	0.064	0.740	0.277	0.578	0.255
	rep 2	49	0.159	0.042	0.791	0.172	0.632	0.166
	average		0.160	0.001	0.765	0.026	0.605	0.027
	sum	96					(0.606)	
0.1 ml/l	rep 1	45	0.132	0.059	0.669	0.294	0.537	0.281
	rep 2	46	0.124	0.028	0.693	0.240	0.569	0.234
	average		0.128	0.004	0.681	0.012	0.553	0.016
	sum	91					(0.553)	
1.0 ml/l	rep 1	49	0.158	0.051	0.621	0.143	0.463	0.128
	rep 2	47	0.096	0.065	0.643	0.276	0.547	0.259
	average		0.127	0.031	0.632	0.011	0.505	0.042
	sum	96					(0.504)	
10 ml/l	rep 1	47	0.149	0.059	0.540	0.315	0.391	0.304
	rep 2	48	0.152	0.044	0.645	0.146	0.494	0.115
	average		0.150	0.001	0.593	0.053	0.442	0.051
	sum	95					(0.443)	
100 ml/l	rep 1	48	0.172	0.054	0.550	0.233	0.378	0.233
	rep 2	43	0.160	0.045	0.632	0.134	0.472	0.141
	average		0.166	0.006	0.591	0.041	0.425	0.047
	sum	91					(0.422)	

* std.dev. = standard deviation

** the figure given in brackets is the average mass increase of all the data of both rep. 1 & 2

Mass (g) of individual maize, *Zea mays*, callus pieces grown on culture medium (Green & Phillips, 1975) containing different concentrations of HT-toxin, a pathotoxin extract of *Exserohilum turcicum* (replication 1). "initial" is mass at the start of the experiment, "final" is mass after six weeks of growth

CONCENTRATION:	0 ml/l (control)			0.1 ml/l			1.0 ml/l			10 ml/l			100 ml/l		
	initial	final	increase	initial	final	increase	initial	final	increase	initial	final	increase	initial	final	increase
1	0.12	0.09	-0.03	0.09	0.09	0.00	0.19	0.19	0.00	0.20	0.16	-0.04	0.27	0.22	-0.05
2	0.06	0.06	0.00	0.12	0.12	0.00	0.10	0.33	0.23	0.10	0.09	-0.01	0.15	0.19	0.04
3	0.09	0.25	0.16	0.19	0.21	0.02	0.15	0.41	0.26	0.10	0.12	0.02	0.28	0.33	0.05
4	0.15	0.38	0.23	0.12	0.16	0.04	0.18	0.48	0.30	0.12	0.14	0.02	0.08	0.16	0.08
5	0.15	0.38	0.23	0.02	0.08	0.06	0.15	0.46	0.31	0.13	0.16	0.03	0.10	0.19	0.09
6	0.11	0.41	0.30	0.17	0.34	0.17	0.14	0.46	0.32	0.13	0.22	0.09	0.11	0.23	0.12
7	0.23	0.53	0.30	0.11	0.31	0.20	0.15	0.48	0.33	0.05	0.14	0.09	0.28	0.42	0.14
8	0.23	0.55	0.32	0.08	0.29	0.21	0.20	0.53	0.33	0.15	0.25	0.10	0.23	0.37	0.14
9	0.17	0.54	0.37	0.15	0.42	0.27	0.14	0.50	0.36	0.16	0.29	0.13	0.14	0.29	0.15
10	0.13	0.51	0.38	0.18	0.49	0.31	0.14	0.50	0.36	0.11	0.24	0.13	0.19	0.34	0.15
11	0.19	0.58	0.39	0.17	0.51	0.34	0.21	0.57	0.36	0.13	0.26	0.13	0.13	0.33	0.20
12	0.17	0.56	0.39	0.16	0.57	0.41	0.11	0.49	0.38	0.30	0.44	0.14	0.21	0.42	0.21
13	0.19	0.59	0.40	0.17	0.61	0.44	0.21	0.59	0.38	0.15	0.30	0.15	0.19	0.41	0.22
14	0.12	0.55	0.43	0.16	0.61	0.45	0.18	0.56	0.38	0.21	0.38	0.17	0.20	0.42	0.22
15	0.11	0.57	0.46	0.10	0.55	0.45	0.12	0.57	0.45	0.09	0.28	0.19	0.09	0.31	0.22
16	0.27	0.74	0.47	0.03	0.49	0.46	0.12	0.57	0.45	0.09	0.28	0.19	0.12	0.41	0.29
17	0.13	0.61	0.48	0.24	0.71	0.47	0.17	0.62	0.45	0.18	0.38	0.20	0.22	0.51	0.29
18	0.06	0.54	0.48	0.15	0.63	0.48	0.11	0.56	0.45	0.10	0.32	0.22	0.23	0.52	0.29
19	0.16	0.65	0.49	0.08	0.57	0.49	0.13	0.59	0.46	0.23	0.45	0.22	0.14	0.44	0.30
20	0.27	0.77	0.50	0.08	0.57	0.49	0.15	0.61	0.46	0.19	0.42	0.23	0.14	0.45	0.31
21	0.06	0.59	0.53	0.02	0.51	0.49	0.17	0.63	0.46	0.10	0.36	0.26	0.18	0.49	0.31
22	0.07	0.60	0.53	0.15	0.64	0.49	0.20	0.66	0.46	0.16	0.42	0.26	0.19	0.51	0.32
23	0.14	0.69	0.55	0.14	0.70	0.56	0.11	0.58	0.47	0.23	0.49	0.26	0.23	0.55	0.32
24	0.23	0.80	0.57	0.14	0.71	0.57	0.16	0.63	0.47	0.12	0.41	0.29	0.14	0.47	0.33
25	0.16	0.75	0.59	0.09	0.66	0.57	0.18	0.66	0.48	0.19	0.50	0.31	0.17	0.50	0.33
26	0.17	0.78	0.61	0.23	0.81	0.58	0.15	0.64	0.49	0.21	0.55	0.34	0.26	0.60	0.34
27	0.11	0.74	0.63	0.06	0.68	0.62	0.16	0.65	0.49	0.13	0.48	0.35	0.12	0.46	0.34
28	0.21	0.84	0.63	0.15	0.79	0.64	0.10	0.60	0.50	0.13	0.52	0.39	0.17	0.52	0.35
29	0.08	0.73	0.65	0.06	0.72	0.66	0.15	0.65	0.50	0.12	0.56	0.44	0.15	0.53	0.38
30	0.11	0.80	0.69	0.10	0.77	0.67	0.15	0.65	0.50	0.21	0.67	0.46	0.06	0.45	0.39
31	0.31	1.02	0.71	0.09	0.77	0.68	0.11	0.62	0.51	0.09	0.58	0.49	0.14	0.55	0.41
32	0.18	0.92	0.74	0.10	0.80	0.70	0.12	0.63	0.51	0.08	0.62	0.54	0.15	0.56	0.41
33	0.09	0.84	0.75	0.26	0.97	0.71	0.17	0.68	0.51	0.15	0.71	0.56	0.24	0.66	0.42
34	0.15	0.92	0.77	0.19	0.95	0.76	0.17	0.69	0.52	0.09	0.69	0.60	0.25	0.67	0.42
35	0.20	0.99	0.79	0.11	0.89	0.78	0.13	0.65	0.52	0.09	0.69	0.60	0.20	0.72	0.52
36	0.26	1.05	0.79	0.21	0.99	0.78	0.16	0.68	0.52	0.08	0.71	0.63	0.15	0.68	0.53
37	0.21	1.02	0.81	0.15	0.95	0.80	0.22	0.74	0.52	0.16	0.81	0.65	0.15	0.71	0.56
38	0.11	0.94	0.83	0.30	1.13	0.83	0.12	0.66	0.54	0.08	0.74	0.66	0.16	0.73	0.57
39	0.16	0.99	0.83	0.08	0.91	0.83	0.19	0.73	0.54	0.15	0.81	0.66	0.22	0.80	0.58
40	0.17	1.00	0.83	0.10	0.94	0.84	0.17	0.72	0.55	0.34	1.00	0.66	0.08	0.70	0.62
41	0.25	1.08	0.83	0.11	0.96	0.85	0.13	0.69	0.56	0.09	0.88	0.79	0.14	0.78	0.64
42	0.24	1.10	0.86	0.10	0.98	0.88	0.15	0.71	0.56	0.21	1.09	0.88	0.14	0.84	0.70
43	0.07	1.01	0.94	0.15	1.07	0.92	0.12	0.68	0.56	0.19	1.09	0.90	0.20	0.96	0.76
44	0.19	1.14	0.95	0.13	1.17	1.04	0.17	0.74	0.57	0.20	1.17	0.97	0.21	1.00	0.79
45	0.08	1.05	0.97	0.13	1.30	1.17	0.10	0.72	0.62	0.14	1.11	0.97	0.16	0.98	0.82
46	0.23	1.24	1.01				0.19	0.81	0.62	0.20	1.23	1.03	0.21	1.03	0.82
47	0.23	1.27	1.04				0.10	0.77	0.67	0.14	1.19	1.05	0.10	0.93	0.83
48							0.43	1.12	0.69				0.18	1.06	0.88
49							0.19	0.97	0.78						

Mass (g) of individual maize, *Zea mays*, callus pieces grown on culture medium (Green & Phillips, 1975) containing different concentrations of HT-toxin, a pathotoxin extract of *Exserohilum turcicum* (replication 2). "initial" is mass at the start of the experiment, "final" is mass after six weeks of growth

CONCENTRATION:	0 ml/l (control)			0.1 ml/l			1.0 ml/l			10 ml/l			100 ml/l		
	initial	final	increase	initial	final	increase	initial	final	increase	initial	final	increase	initial	final	increase
1	0.19	0.35	0.16	0.13	0.25	0.12	0.02	0.04	0.02	0.09	0.36	0.27	0.24	0.31	0.07
2	0.11	0.43	0.32	0.09	0.26	0.17	0.10	0.14	0.04	0.16	0.46	0.30	0.14	0.33	0.19
3	0.10	0.50	0.40	0.11	0.28	0.17	0.14	0.25	0.11	0.09	0.41	0.32	0.25	0.45	0.20
4	0.09	0.50	0.41	0.13	0.34	0.21	0.02	0.16	0.14	0.09	0.44	0.35	0.24	0.48	0.24
5	0.14	0.58	0.44	0.07	0.39	0.32	0.02	0.18	0.16	0.15	0.50	0.35	0.25	0.49	0.24
6	0.24	0.69	0.45	0.09	0.51	0.42	0.06	0.26	0.20	0.16	0.52	0.36	0.13	0.39	0.26
7	0.14	0.60	0.46	0.10	0.45	0.35	0.02	0.25	0.23	0.13	0.51	0.38	0.11	0.46	0.35
8	0.17	0.63	0.46	0.14	0.54	0.40	0.02	0.29	0.27	0.10	0.49	0.39	0.17	0.57	0.40
9	0.13	0.61	0.48	0.11	0.58	0.47	0.13	0.41	0.28	0.17	0.57	0.40	0.13	0.54	0.41
10	0.14	0.62	0.48	0.10	0.47	0.37	0.08	0.37	0.29	0.10	0.51	0.41	0.16	0.58	0.42
11	0.15	0.65	0.50	0.10	0.54	0.44	0.19	0.49	0.30	0.15	0.56	0.41	0.10	0.52	0.42
12	0.17	0.68	0.51	0.14	0.44	0.30	0.02	0.33	0.31	0.15	0.57	0.42	0.12	0.54	0.42
13	0.14	0.66	0.52	0.16	0.46	0.30	0.18	0.52	0.34	0.17	0.59	0.42	0.23	0.65	0.42
14	0.22	0.74	0.52	0.16	0.57	0.41	0.08	0.44	0.36	0.09	0.52	0.43	0.11	0.54	0.43
15	0.28	0.82	0.54	0.12	0.47	0.35	0.15	0.52	0.37	0.11	0.54	0.43	0.20	0.64	0.44
16	0.17	0.73	0.56	0.08	0.48	0.40	0.22	0.61	0.39	0.12	0.55	0.43	0.12	0.56	0.44
17	0.15	0.72	0.57	0.17	0.49	0.32	0.04	0.46	0.42	0.18	0.62	0.44	0.10	0.57	0.47
18	0.15	0.72	0.57	0.13	0.56	0.43	0.20	0.65	0.45	0.12	0.57	0.45	0.12	0.59	0.47
19	0.16	0.74	0.58	0.11	0.55	0.44	0.07	0.57	0.50	0.12	0.58	0.46	0.28	0.75	0.47
20	0.09	0.69	0.60	0.11	0.66	0.55	0.08	0.58	0.50	0.16	0.62	0.46	0.15	0.62	0.47
21	0.11	0.71	0.60	0.12	0.56	0.44	0.18	0.75	0.57	0.10	0.56	0.46	0.13	0.62	0.49
22	0.13	0.73	0.60	0.12	0.85	0.73	0.02	0.60	0.58	0.21	0.67	0.46	0.19	0.68	0.49
23	0.15	0.76	0.61	0.18	1.04	0.86	0.05	0.65	0.60	0.16	0.63	0.47	0.14	0.64	0.50
24	0.28	0.89	0.61	0.11	0.94	0.83	0.11	0.71	0.60	0.17	0.65	0.48	0.12	0.63	0.51
25	0.20	0.81	0.61	0.12	1.01	0.89	0.07	0.67	0.60	0.18	0.66	0.48	0.15	0.66	0.51
26	0.14	0.77	0.63	0.12	0.61	0.49	0.02	0.63	0.61	0.14	0.63	0.49	0.15	0.66	0.51
27	0.20	0.83	0.63	0.09	0.71	0.62	0.09	0.71	0.62	0.17	0.66	0.49	0.17	0.68	0.51
28	0.14	0.78	0.64	0.12	0.66	0.54	0.16	0.78	0.62	0.13	0.64	0.51	0.16	0.68	0.52
29	0.16	0.83	0.67	0.15	0.85	0.70	0.13	0.78	0.65	0.14	0.65	0.51	0.16	0.68	0.52
30	0.16	0.85	0.69	0.11	0.91	0.80	0.09	0.76	0.67	0.11	0.63	0.52	0.19	0.71	0.52
31	0.13	0.83	0.70	0.18	0.87	0.69	0.02	0.71	0.69	0.14	0.66	0.52	0.19	0.73	0.54
32	0.22	0.93	0.71	0.12	0.86	0.74	0.21	0.96	0.75	0.20	0.73	0.53	0.17	0.72	0.55
33	0.15	0.87	0.72	0.16	1.12	0.96	0.09	0.85	0.76	0.15	0.69	0.54	0.11	0.66	0.55
34	0.16	0.89	0.73	0.13	0.94	0.81	0.17	0.93	0.76	0.17	0.71	0.54	0.19	0.74	0.55
35	0.17	0.90	0.73	0.15	1.02	0.87	0.09	0.86	0.77	0.19	0.73	0.54	0.15	0.72	0.57
36	0.19	0.92	0.73	0.11	0.78	0.67	0.05	0.83	0.78	0.14	0.69	0.55	0.14	0.73	0.59
37	0.19	0.92	0.73	0.10	0.80	0.70	0.08	0.86	0.78	0.10	0.65	0.55	0.16	0.75	0.59
38	0.19	0.93	0.74	0.15	0.93	0.78	0.02	0.82	0.80	0.11	0.66	0.55	0.17	0.77	0.60
39	0.14	0.91	0.77	0.13	0.59	0.46	0.11	0.91	0.80	0.18	0.73	0.55	0.16	0.77	0.61
40	0.15	0.92	0.77	0.11	0.84	0.73	0.13	0.93	0.80	0.12	0.69	0.57	0.10	0.72	0.62
41	0.11	0.89	0.78	0.10	0.79	0.69	0.16	0.97	0.81	0.10	0.68	0.58	0.09	0.73	0.64
42	0.15	0.93	0.78	0.18	0.86	0.68	0.24	1.10	0.86	0.20	0.78	0.58	0.18	0.95	0.77
43	0.15	0.95	0.80	0.11	0.92	0.81	0.02	0.89	0.87	0.22	0.89	0.67	0.17	0.96	0.79
44	0.08	0.89	0.81	0.15	0.94	0.79	0.07	0.94	0.87	0.27	0.97	0.70			
45	0.11	0.96	0.85	0.07	1.09	1.02	0.02	0.92	0.90	0.20	0.91	0.71			
46	0.21	1.10	0.89	0.15	1.08	0.93	0.20	1.13	0.93	0.21	0.92	0.71			
47	0.15	1.05	0.90				0.05	1.05	1.00	0.20	0.97	0.77			
48	0.17	1.16	0.99							0.26	1.05	0.79			
49	0.17	1.18	1.01												

Statistical analysis of the average mass (g) of maize, *Zea mays*, callus pieces grown on culture medium (Green & Phillips, 1975) containing different concentrations of SM-toxin, a pathotoxin extract of *Stenocarpella macrospora*, or ethyl acetate. "initial" is at the start of the experiment, "final" is after six weeks of growth. Data of the replications in APPENDIX 4.2, 4.3, 4.4 and 4.5

CONCENTRATION		n =	MASS (gram) initial	std.dev.*	final	std.dev.	increase**	std.dev.
0 ml/l (control)	rep 1	45	0.171	0.066	0.442	0.290	0.271	0.275
	rep 2	49	0.133	0.041	0.590	0.201	0.457	0.174
	rep 3	49	0.085	0.031	0.457	0.290	0.372	0.288
	average		0.130	0.035	0.496	0.067	0.367	0.076
	sum	143					(0.369)	
0.01 ml/l	rep 1	42	0.163	0.063	0.263	0.212	0.099	0.176
	rep 2	49	0.129	0.034	0.526	0.132	0.397	0.116
	rep 3	49	0.080	0.026	0.451	0.296	0.371	0.293
	average		0.124	0.034	0.413	0.111	0.289	0.135
	sum	140					(0.299)	
0.1 ml/l	rep 1	48	0.140	0.049	0.280	0.200	0.140	0.198
	rep 2	48	0.116	0.034	0.620	0.174	0.504	0.169
	rep 3	49	0.080	0.027	0.470	0.315	0.389	0.313
	average		0.112	0.024	0.457	0.139	0.344	0.152
	sum	145					(0.345)	
1.0 ml/l	rep 1	48	0.075	0.029	0.295	0.191	0.219	0.177
	rep 2	33	0.118	0.044	0.784	0.221	0.665	0.211
	rep 3	49	0.084	0.030	0.712	0.306	0.628	0.297
	average		0.093	0.019	0.597	0.216	0.504	0.202
	sum	130					(0.487)	
10.0 ml/l	rep 1	47	0.136	0.059	0.143	0.065	0.007	0.038
	rep 2	28	0.128	0.035	0.446	0.263	0.318	0.248
	rep 3	49	0.076	0.027	0.196	0.242	0.120	0.240
	average		0.113	0.027	0.262	0.132	0.148	0.129
	sum	124					(0.122)	

* std.dev. = standard deviation

** the figure given in brackets is the average mass increase of all the data of both rep. 1, 2 & 3

Statistical analysis of the average mass (g) of maize, *Zea mays*, callus pieces grown on culture medium (Green & Phillips, 1975) containing different concentrations of SM-toxin, a pathotoxin extract of *Stenocarpella macrospora*, or ethyl acetate. "initial" is at the start of the experiment, "final" is after six weeks of growth. Data of the replications in APPENDIX 4.2, 4.3, 4.4 and 4.5

CONCENTRATION	n =	MASS (gram) initial	std.dev.*	final	std.dev.	increase**	std.dev.
control + ethyl acetate							
2 ml/l	45	0.197	0.086	0.473	0.237	0.276	0.212
20 ml/l	49	0.124	0.033	0.119	0.033	-0.005	0.013
10 ml/l	49	0.077	0.025	0.243	0.217	0.166	0.213
average		0.133	0.049	0.278	0.147	0.146	0.116
sum	143					(0.142)	

* std.dev. = standard deviation

** the figure given in brackets is the average mass increase of all the data of both rep. 1, 2 & 3

Mass (g) of individual maize, *Zea mays*, callus pieces grown on culture medium (Green & Phillips, 1975) containing different concentrations of SM-toxin, a pathotoxin extract of *Stenocarpella macrospora* (replication 1). "initial" is mass at the start of the experiment, "final" is mass after six weeks of growth

CONCENTRATION:	0 ml/l (control)			0.01 ml/l			0.1 ml/l			1.0 ml/l			10.0 ml/l		
	initial	final	increase	initial	final	increase	initial	final	increase	initial	final	increase	initial	final	increase
1	0.20	0.16	-0.04	0.20	0.16	-0.04	0.20	0.12	-0.08	0.11	0.10	-0.01	0.17	0.11	-0.06
2	0.22	0.18	-0.04	0.21	0.17	-0.04	0.21	0.14	-0.07	0.04	0.04	0.00	0.25	0.20	-0.05
3	0.26	0.22	-0.04	0.11	0.08	-0.03	0.17	0.11	-0.06	0.04	0.05	0.01	0.14	0.10	-0.04
4	0.19	0.16	-0.03	0.13	0.10	-0.03	0.17	0.12	-0.05	0.07	0.09	0.02	0.16	0.12	-0.04
5	0.17	0.16	-0.01	0.20	0.18	-0.02	0.15	0.10	-0.05	0.02	0.04	0.02	0.27	0.23	-0.04
6	0.29	0.28	-0.01	0.08	0.06	-0.02	0.13	0.10	-0.03	0.06	0.09	0.03	0.09	0.06	-0.03
7	0.05	0.05	0.00	0.13	0.11	-0.02	0.20	0.18	-0.02	0.09	0.12	0.03	0.13	0.10	-0.03
8	0.05	0.05	0.00	0.16	0.14	-0.02	0.09	0.07	-0.02	0.07	0.12	0.05	0.16	0.13	-0.03
9	0.08	0.08	0.00	0.07	0.06	-0.01	0.14	0.13	-0.01	0.05	0.12	0.07	0.14	0.12	-0.02
10	0.16	0.16	0.00	0.13	0.12	-0.01	0.20	0.19	-0.01	0.11	0.18	0.07	0.14	0.12	-0.02
11	0.18	0.18	0.00	0.17	0.16	-0.01	0.20	0.19	-0.01	0.06	0.13	0.07	0.14	0.12	-0.02
12	0.15	0.16	0.01	0.24	0.23	-0.01	0.11	0.10	-0.01	0.04	0.12	0.08	0.12	0.10	-0.02
13	0.31	0.32	0.01	0.07	0.07	0.00	0.12	0.11	-0.01	0.05	0.13	0.08	0.12	0.10	-0.02
14	0.18	0.22	0.04	0.10	0.10	0.00	0.08	0.08	0.00	0.05	0.15	0.10	0.16	0.15	-0.01
15	0.06	0.12	0.06	0.10	0.10	0.00	0.19	0.19	0.00	0.07	0.18	0.11	0.17	0.16	-0.01
16	0.20	0.26	0.06	0.16	0.16	0.00	0.20	0.20	0.00	0.07	0.18	0.11	0.19	0.18	-0.01
17	0.12	0.20	0.08	0.17	0.17	0.00	0.24	0.24	0.00	0.09	0.20	0.11	0.28	0.27	-0.01
18	0.17	0.27	0.10	0.07	0.08	0.01	0.09	0.10	0.01	0.04	0.16	0.12	0.08	0.07	-0.01
19	0.12	0.25	0.13	0.11	0.12	0.01	0.08	0.10	0.02	0.07	0.20	0.13	0.09	0.08	-0.01
20	0.15	0.33	0.18	0.15	0.16	0.01	0.08	0.11	0.03	0.10	0.23	0.13	0.11	0.10	-0.01
21	0.08	0.27	0.19	0.18	0.19	0.01	0.10	0.13	0.03	0.11	0.25	0.14	0.12	0.11	-0.01
22	0.20	0.42	0.22	0.16	0.18	0.02	0.11	0.15	0.04	0.02	0.18	0.16	0.04	0.04	0.00
23	0.15	0.37	0.22	0.13	0.16	0.03	0.14	0.20	0.06	0.05	0.21	0.16	0.05	0.05	0.00
24	0.15	0.38	0.23	0.21	0.25	0.04	0.16	0.22	0.06	0.02	0.20	0.18	0.08	0.08	0.00
25	0.05	0.35	0.30	0.19	0.24	0.05	0.10	0.18	0.08	0.08	0.26	0.18	0.10	0.10	0.00
26	0.18	0.50	0.32	0.12	0.17	0.05	0.12	0.20	0.08	0.08	0.26	0.18	0.10	0.10	0.00
27	0.25	0.60	0.35	0.12	0.17	0.05	0.16	0.25	0.09	0.08	0.26	0.18	0.13	0.13	0.00
28	0.26	0.61	0.35	0.07	0.13	0.06	0.06	0.17	0.11	0.05	0.24	0.19	0.14	0.14	0.00
29	0.21	0.56	0.35	0.20	0.27	0.07	0.08	0.22	0.14	0.12	0.31	0.19	0.17	0.17	0.00
30	0.14	0.52	0.38	0.15	0.26	0.11	0.11	0.25	0.14	0.12	0.33	0.21	0.09	0.10	0.01
31	0.12	0.51	0.39	0.15	0.26	0.11	0.21	0.37	0.16	0.09	0.34	0.25	0.18	0.19	0.01
32	0.18	0.59	0.41	0.16	0.30	0.14	0.12	0.30	0.18	0.05	0.32	0.27	0.13	0.15	0.02
33	0.20	0.61	0.41	0.14	0.30	0.16	0.22	0.43	0.21	0.06	0.33	0.27	0.16	0.18	0.02
34	0.23	0.64	0.41	0.35	0.57	0.22	0.15	0.38	0.23	0.09	0.36	0.27	0.08	0.10	0.02
35	0.09	0.51	0.42	0.32	0.58	0.26	0.07	0.31	0.24	0.10	0.42	0.32	0.06	0.09	0.03
36	0.13	0.55	0.42	0.20	0.47	0.27	0.08	0.33	0.25	0.12	0.45	0.33	0.15	0.18	0.03
37	0.22	0.70	0.48	0.23	0.50	0.27	0.11	0.36	0.25	0.06	0.40	0.34	0.04	0.07	0.03
38	0.18	0.68	0.50	0.12	0.42	0.30	0.03	0.28	0.25	0.07	0.43	0.36	0.12	0.16	0.04
39	0.27	0.83	0.56	0.17	0.52	0.35	0.14	0.44	0.30	0.07	0.44	0.37	0.12	0.16	0.04
40	0.18	0.80	0.62	0.27	0.76	0.49	0.18	0.48	0.30	0.08	0.47	0.39	0.22	0.26	0.04
41	0.07	0.73	0.66	0.20	0.79	0.59	0.19	0.50	0.31	0.07	0.49	0.42	0.08	0.13	0.05
42	0.27	1.05	0.78	0.23	0.97	0.74	0.15	0.47	0.32	0.11	0.54	0.43	0.22	0.27	0.05
43	0.15	1.00	0.85				0.17	0.50	0.33	0.06	0.50	0.44	0.11	0.18	0.07
44	0.24	1.09	0.85				0.17	0.54	0.37	0.12	0.64	0.52	0.29	0.36	0.07
45	0.18	1.21	1.03				0.08	0.57	0.49	0.08	0.61	0.53	0.06	0.14	0.08
46							0.15	0.67	0.52	0.11	0.70	0.59	0.15	0.23	0.08
47							0.14	0.89	0.75	0.15	0.78	0.63	0.11	0.24	0.13
48							0.16	0.95	0.79	0.09	0.79	0.70			
49															

Mass (g) of individual maize, *Zea mays*, callus pieces grown on culture medium (Green & Phillips, 1975) containing different concentrations of SM-toxin, a pathotoxin extract of *Stenocarpella macrospora* (replication 2). "initial" is mass at the start of the experiment, "final" is mass after six weeks of growth

CONCENTRATION:	0 ml/l (control)			0.01 ml/l			0.1 ml/l			1.0 ml/l			10.0 ml/l		
	initial	final	increase	initial	final	increase	initial	final	increase	initial	final	increase	initial	final	increase
1	0.07	0.21	0.14	0.17	0.34	0.17	0.07	0.23	0.16	0.09	0.34	0.25	0.08	0.14	0.06
2	0.10	0.25	0.15	0.12	0.30	0.18	0.07	0.28	0.21	0.07	0.36	0.29	0.08	0.15	0.07
3	0.13	0.30	0.17	0.10	0.30	0.20	0.14	0.37	0.23	0.16	0.49	0.33	0.12	0.21	0.09
4	0.14	0.36	0.22	0.11	0.34	0.23	0.09	0.34	0.25	0.08	0.47	0.39	0.14	0.23	0.09
5	0.12	0.35	0.23	0.17	0.42	0.25	0.09	0.36	0.27	0.12	0.53	0.41	0.16	0.25	0.09
6	0.09	0.32	0.23	0.13	0.38	0.25	0.12	0.39	0.27	0.13	0.55	0.42	0.11	0.20	0.09
7	0.08	0.32	0.24	0.09	0.35	0.26	0.15	0.42	0.27	0.23	0.69	0.46	0.12	0.22	0.10
8	0.10	0.34	0.24	0.06	0.33	0.27	0.13	0.42	0.29	0.14	0.62	0.48	0.08	0.25	0.17
9	0.07	0.33	0.26	0.10	0.37	0.27	0.13	0.49	0.36	0.08	0.60	0.52	0.08	0.25	0.17
10	0.10	0.36	0.26	0.09	0.37	0.28	0.11	0.49	0.38	0.10	0.66	0.56	0.11	0.28	0.17
11	0.17	0.43	0.26	0.09	0.37	0.28	0.13	0.53	0.40	0.12	0.69	0.57	0.11	0.29	0.18
12	0.14	0.44	0.30	0.08	0.38	0.30	0.09	0.50	0.41	0.07	0.67	0.60	0.12	0.31	0.19
13	0.13	0.45	0.32	0.16	0.47	0.31	0.10	0.51	0.41	0.10	0.72	0.62	0.11	0.31	0.20
14	0.09	0.42	0.33	0.10	0.42	0.32	0.16	0.58	0.42	0.08	0.71	0.63	0.10	0.31	0.21
15	0.15	0.50	0.35	0.14	0.46	0.32	0.12	0.54	0.42	0.11	0.75	0.64	0.19	0.42	0.23
16	0.08	0.44	0.36	0.14	0.46	0.32	0.12	0.54	0.42	0.07	0.72	0.65	0.15	0.38	0.23
17	0.09	0.46	0.37	0.13	0.48	0.35	0.13	0.56	0.43	0.07	0.75	0.68	0.17	0.43	0.26
18	0.13	0.52	0.39	0.15	0.50	0.35	0.14	0.58	0.44	0.08	0.78	0.70	0.18	0.45	0.27
19	0.14	0.53	0.39	0.10	0.45	0.35	0.05	0.49	0.44	0.17	0.89	0.72	0.13	0.46	0.33
20	0.10	0.50	0.40	0.11	0.49	0.38	0.17	0.63	0.46	0.20	0.92	0.72	0.08	0.45	0.37
21	0.12	0.54	0.42	0.11	0.50	0.39	0.15	0.64	0.49	0.12	0.87	0.75	0.10	0.51	0.41
22	0.13	0.55	0.42	0.08	0.48	0.40	0.18	0.67	0.49	0.16	0.93	0.77	0.15	0.62	0.47
23	0.11	0.57	0.46	0.16	0.56	0.40	0.18	0.67	0.49	0.10	0.88	0.78	0.18	0.72	0.54
24	0.15	0.62	0.47	0.12	0.53	0.41	0.08	0.58	0.50	0.10	0.89	0.79	0.13	0.80	0.67
25	0.13	0.61	0.48	0.14	0.55	0.41	0.04	0.54	0.50	0.14	0.93	0.79	0.15	0.88	0.73
26	0.13	0.62	0.49	0.19	0.61	0.42	0.08	0.59	0.51	0.07	0.89	0.82	0.20	1.00	0.80
27	0.16	0.66	0.50	0.11	0.53	0.42	0.16	0.69	0.53	0.14	0.97	0.83	0.15	0.97	0.82
28	0.06	0.58	0.52	0.11	0.53	0.42	0.10	0.63	0.53	0.07	0.92	0.85	0.11	1.00	0.89
29	0.12	0.64	0.52	0.14	0.56	0.42	0.06	0.61	0.55	0.22	1.10	0.88			
30	0.14	0.66	0.52	0.09	0.52	0.43	0.14	0.70	0.56	0.10	1.02	0.92			
31	0.16	0.69	0.53	0.12	0.55	0.43	0.10	0.66	0.56	0.13	1.11	0.98			
32	0.12	0.65	0.53	0.13	0.56	0.43	0.14	0.71	0.57	0.18	1.23	1.05			
33	0.20	0.74	0.54	0.14	0.58	0.44	0.15	0.72	0.57	0.10	1.21	1.11			
34	0.14	0.70	0.56	0.10	0.54	0.44	0.08	0.65	0.57						
35	0.10	0.66	0.56	0.20	0.65	0.45	0.10	0.67	0.57						
36	0.19	0.75	0.56	0.13	0.59	0.46	0.12	0.70	0.58						
37	0.14	0.73	0.59	0.10	0.57	0.47	0.09	0.71	0.62						
38	0.14	0.73	0.59	0.17	0.64	0.47	0.08	0.73	0.65						
39	0.13	0.73	0.60	0.18	0.65	0.47	0.12	0.77	0.65						
40	0.08	0.68	0.60	0.11	0.59	0.48	0.13	0.78	0.65						
41	0.18	0.80	0.62	0.12	0.60	0.48	0.14	0.80	0.66						
42	0.18	0.80	0.62	0.19	0.71	0.52	0.14	0.82	0.68						
43	0.19	0.84	0.65	0.12	0.67	0.55	0.18	0.88	0.70						
44	0.13	0.81	0.68	0.15	0.71	0.56	0.11	0.82	0.71						
45	0.25	0.96	0.71	0.14	0.72	0.58	0.10	0.84	0.74						
46	0.21	0.92	0.71	0.11	0.70	0.59	0.11	0.89	0.78						
47	0.14	0.86	0.72	0.20	0.82	0.62	0.11	0.95	0.84						
48	0.15	0.89	0.74	0.18	0.80	0.62	0.10	1.08	0.98						
49	0.23	1.10	0.87	0.13	0.76	0.63									

Mass (g) of individual maize, *Zea mays*, callus pieces grown on culture medium (Green & Phillips, 1975) containing different concentrations of SM-toxin, a pathotoxin extract of *Stenocarpella macrospora* (replication 3). "initial" is mass at the start of the experiment, "final" is mass after six weeks of growth

CONCENTRATION:	0 ml/l (control)			0.01 ml/l			0.1 ml/l			1.0 ml/l			10.0 ml/l		
	initial	final	increase	initial	final	increase	initial	final	increase	initial	final	increase	initial	final	increase
1	0.07	0.06	-0.01	0.08	0.06	-0.02	0.06	0.04	-0.02	0.05	0.05	0.00	0.13	0.09	-0.04
2	0.10	0.09	-0.01	0.14	0.13	-0.01	0.15	0.13	-0.02	0.06	0.06	0.00	0.10	0.07	-0.03
3	0.11	0.10	-0.01	0.08	0.07	-0.01	0.10	0.09	-0.01	0.08	0.11	0.03	0.08	0.06	-0.02
4	0.05	0.05	0.00	0.11	0.10	-0.01	0.08	0.07	-0.01	0.06	0.21	0.15	0.10	0.09	-0.01
5	0.07	0.07	0.00	0.04	0.04	0.00	0.09	0.08	-0.01	0.09	0.31	0.22	0.10	0.09	-0.01
6	0.07	0.07	0.00	0.05	0.05	0.00	0.05	0.05	0.00	0.04	0.31	0.27	0.10	0.09	-0.01
7	0.11	0.11	0.00	0.06	0.06	0.00	0.06	0.06	0.00	0.07	0.41	0.34	0.05	0.04	-0.01
8	0.08	0.09	0.01	0.09	0.09	0.00	0.07	0.07	0.00	0.03	0.39	0.36	0.06	0.05	-0.01
9	0.08	0.09	0.01	0.10	0.10	0.00	0.08	0.08	0.00	0.08	0.47	0.39	0.09	0.08	-0.01
10	0.10	0.11	0.01	0.12	0.12	0.00	0.11	0.11	0.00	0.06	0.49	0.43	0.12	0.11	-0.01
11	0.10	0.11	0.01	0.05	0.06	0.01	0.07	0.08	0.01	0.07	0.50	0.43	0.03	0.03	0.00
12	0.06	0.08	0.02	0.05	0.06	0.01	0.07	0.08	0.01	0.09	0.52	0.43	0.03	0.03	0.00
13	0.09	0.11	0.02	0.12	0.13	0.01	0.06	0.07	0.01	0.05	0.49	0.44	0.05	0.05	0.00
14	0.11	0.13	0.02	0.05	0.07	0.02	0.05	0.07	0.02	0.10	0.54	0.44	0.05	0.05	0.00
15	0.13	0.23	0.10	0.06	0.08	0.02	0.09	0.11	0.02	0.09	0.54	0.45	0.06	0.06	0.00
16	0.11	0.35	0.24	0.09	0.11	0.02	0.09	0.12	0.03	0.04	0.51	0.47	0.06	0.06	0.00
17	0.04	0.30	0.26	0.10	0.41	0.31	0.10	0.13	0.03	0.10	0.58	0.48	0.07	0.07	0.00
18	0.06	0.36	0.30	0.08	0.42	0.34	0.04	0.07	0.03	0.04	0.52	0.48	0.07	0.07	0.00
19	0.13	0.44	0.31	0.08	0.44	0.36	0.03	0.44	0.41	0.12	0.61	0.49	0.07	0.07	0.00
20	0.14	0.49	0.35	0.06	0.45	0.39	0.05	0.48	0.43	0.09	0.63	0.54	0.07	0.07	0.00
21	0.08	0.46	0.38	0.10	0.51	0.41	0.08	0.51	0.43	0.14	0.69	0.55	0.07	0.07	0.00
22	0.07	0.46	0.39	0.08	0.50	0.42	0.08	0.53	0.45	0.20	0.75	0.55	0.08	0.08	0.00
23	0.10	0.49	0.39	0.09	0.51	0.42	0.09	0.54	0.45	0.07	0.64	0.57	0.08	0.08	0.00
24	0.05	0.45	0.40	0.10	0.52	0.42	0.14	0.62	0.48	0.09	0.70	0.61	0.09	0.09	0.00
25	0.08	0.49	0.41	0.04	0.48	0.44	0.05	0.53	0.48	0.07	0.70	0.63	0.11	0.11	0.00
26	0.05	0.46	0.41	0.07	0.51	0.44	0.08	0.56	0.48	0.09	0.72	0.63	0.12	0.12	0.00
27	0.05	0.48	0.43	0.07	0.52	0.45	0.07	0.57	0.50	0.08	0.72	0.64	0.05	0.06	0.01
28	0.04	0.48	0.44	0.07	0.52	0.45	0.10	0.63	0.53	0.08	0.73	0.65	0.07	0.08	0.01
29	0.07	0.53	0.46	0.09	0.54	0.45	0.12	0.65	0.53	0.09	0.75	0.66	0.11	0.12	0.01
30	0.06	0.53	0.47	0.10	0.57	0.47	0.03	0.57	0.54	0.09	0.79	0.70	0.04	0.05	0.01
31	0.06	0.54	0.48	0.09	0.56	0.47	0.08	0.63	0.55	0.14	0.86	0.72	0.06	0.07	0.01
32	0.06	0.55	0.49	0.07	0.56	0.49	0.07	0.63	0.56	0.10	0.83	0.73	0.06	0.07	0.01
33	0.10	0.61	0.51	0.04	0.55	0.51	0.10	0.68	0.58	0.08	0.87	0.79	0.09	0.10	0.01
34	0.04	0.56	0.52	0.10	0.61	0.51	0.06	0.65	0.59	0.09	0.91	0.82	0.09	0.10	0.01
35	0.07	0.61	0.54	0.06	0.58	0.52	0.10	0.70	0.60	0.11	0.93	0.82	0.05	0.07	0.02
36	0.10	0.65	0.55	0.07	0.59	0.52	0.10	0.73	0.63	0.08	0.91	0.83	0.08	0.10	0.02
37	0.06	0.62	0.56	0.07	0.60	0.53	0.10	0.73	0.63	0.06	0.92	0.86	0.09	0.12	0.03
38	0.08	0.64	0.56	0.06	0.62	0.56	0.13	0.77	0.64	0.06	0.96	0.90	0.02	0.05	0.03
39	0.09	0.66	0.57	0.10	0.66	0.56	0.06	0.73	0.67	0.07	0.98	0.91	0.07	0.32	0.25
40	0.15	0.73	0.58	0.13	0.74	0.61	0.08	0.75	0.67	0.06	0.98	0.92	0.09	0.38	0.29
41	0.06	0.65	0.59	0.05	0.69	0.64	0.10	0.77	0.67	0.03	0.98	0.95	0.04	0.40	0.36
42	0.08	0.69	0.61	0.06	0.72	0.66	0.12	0.82	0.70	0.10	1.07	0.97	0.13	0.57	0.44
43	0.14	0.86	0.72	0.08	0.77	0.69	0.04	0.78	0.74	0.11	1.08	0.97	0.06	0.55	0.49
44	0.19	0.91	0.72	0.07	0.77	0.70	0.07	0.85	0.78	0.13	1.14	1.01	0.11	0.61	0.50
45	0.09	0.82	0.73	0.11	0.89	0.78	0.08	0.88	0.80	0.08	1.10	1.02	0.09	0.68	0.59
46	0.08	0.82	0.74	0.07	0.86	0.79	0.06	0.87	0.81	0.09	1.16	1.07	0.08	0.68	0.60
47	0.08	0.94	0.86	0.04	0.86	0.82	0.04	0.86	0.82	0.11	1.18	1.07	0.02	0.70	0.68
48	0.08	1.11	1.03	0.12	1.12	1.00	0.09	0.99	0.90	0.10	1.27	1.17	0.07	0.87	0.80
49	0.08	1.13	1.05	0.13	1.14	1.01	0.09	1.00	0.91	0.10	1.30	1.20	0.09	0.96	0.87

Mass (g) of individual maize, *Zea mays*, callus pieces grown on culture medium (Green & Phillips, 1975) containing different concentrations of ethyl acetate. "initial" is at the start of the experiment, "final" is after seven weeks of growth

	REPLICATION 1: 2 ml/l			REPLICATION 2: 20 ml/l			REPLICATION 3: 10 ml/l		
	initial	final	increase	initial	final	increase	initial	final	increase
1	0.20	0.19	-0.01	0.17	0.13	-0.04	0.12	0.10	-0.02
2	0.12	0.12	0.00	0.18	0.15	-0.03	0.09	0.08	-0.01
3	0.17	0.21	0.04	0.07	0.05	-0.02	0.05	0.05	0.00
4	0.21	0.29	0.08	0.11	0.09	-0.02	0.06	0.06	0.00
5	0.30	0.39	0.09	0.12	0.10	-0.02	0.08	0.08	0.00
6	0.30	0.43	0.13	0.12	0.10	-0.02	0.08	0.08	0.00
7	0.15	0.28	0.13	0.15	0.13	-0.02	0.08	0.08	0.00
8	0.03	0.20	0.17	0.16	0.14	-0.02	0.09	0.09	0.00
9	0.21	0.51	0.30	0.07	0.06	-0.01	0.10	0.10	0.00
10	0.22	0.53	0.31	0.10	0.09	-0.01	0.05	0.06	0.01
11	0.25	0.58	0.33	0.13	0.12	-0.01	0.05	0.06	0.01
12	0.11	0.52	0.41	0.13	0.12	-0.01	0.07	0.08	0.01
13	0.11	0.52	0.41	0.13	0.12	-0.01	0.08	0.09	0.01
14	0.22	0.63	0.41	0.14	0.13	-0.01	0.08	0.09	0.01
15	0.24	0.67	0.43	0.14	0.13	-0.01	0.08	0.09	0.01
16	0.35	0.78	0.43	0.14	0.13	-0.01	0.10	0.11	0.01
17	0.17	0.62	0.45	0.14	0.13	-0.01	0.04	0.05	0.01
18	0.15	0.65	0.50	0.16	0.15	-0.01	0.06	0.07	0.01
19	0.17	0.67	0.50	0.09	0.08	-0.01	0.06	0.07	0.01
20	0.15	0.68	0.53	0.11	0.10	-0.01	0.06	0.07	0.01
21	0.24	0.85	0.61	0.11	0.10	-0.01	0.09	0.10	0.01
22	0.16	0.81	0.65	0.12	0.11	-0.01	0.07	0.09	0.02
23	0.45	1.23	0.78	0.12	0.11	-0.01	0.05	0.07	0.02
24	0.15	0.12	-0.03	0.12	0.11	-0.01	0.06	0.08	0.02
25	0.19	0.24	0.05	0.12	0.11	-0.01	0.06	0.09	0.03
26	0.24	0.29	0.05	0.15	0.14	-0.01	0.08	0.10	0.02
27	0.31	0.36	0.05	0.18	0.17	-0.01	0.09	0.11	0.02
28	0.07	0.13	0.06	0.09	0.09	0.00	0.09	0.11	0.02
29	0.24	0.30	0.06	0.09	0.09	0.00	0.11	0.13	0.02
30	0.26	0.32	0.06	0.09	0.09	0.00	0.05	0.08	0.03
31	0.07	0.14	0.07	0.10	0.10	0.00	0.06	0.09	0.03
32	0.33	0.45	0.12	0.10	0.10	0.00	0.05	0.32	0.27
33	0.20	0.33	0.13	0.11	0.11	0.00	0.10	0.41	0.31
34	0.07	0.22	0.15	0.11	0.11	0.00	0.05	0.37	0.32
35	0.29	0.47	0.18	0.12	0.12	0.00	0.07	0.43	0.36
36	0.07	0.26	0.19	0.12	0.12	0.00	0.08	0.47	0.39
37	0.12	0.32	0.20	0.12	0.12	0.00	0.08	0.47	0.39
38	0.25	0.46	0.21	0.15	0.15	0.00	0.08	0.48	0.40
39	0.29	0.57	0.28	0.22	0.22	0.00	0.06	0.46	0.40
40	0.30	0.63	0.33	0.20	0.21	0.01	0.18	0.59	0.41
41	0.12	0.46	0.34	0.10	0.11	0.01	0.12	0.55	0.43
42	0.18	0.63	0.45	0.04	0.05	0.01	0.05	0.49	0.44
43	0.19	0.72	0.53	0.09	0.10	0.01	0.10	0.58	0.48
44	0.14	0.72	0.58	0.13	0.14	0.01	0.06	0.54	0.48
45	0.12	0.80	0.68	0.15	0.16	0.01	0.08	0.56	0.48
46				0.14	0.16	0.02	0.05	0.59	0.54
47				0.09	0.11	0.02	0.10	0.64	0.54
48				0.12	0.14	0.02	0.08	0.64	0.56
49				0.10	0.13	0.03	0.07	0.71	0.64

APPENDIX 4.6

Mass (g), taken at weekly intervals, of individual maize, *Zea mays*, callus pieces grown for six weeks on culture medium (Green & Phillips, 1975) containing different concentrations of SM-toxin, a pathotoxin extract of *Stenocarpella macrospora* (replication 1)

week	concentration				
	control	0.01	0.1	1.0	10
1	0.00	0.00	-0.02	0.00	-0.04
	0.00	0.00	-0.01	0.01	-0.02
	0.01	0.00	0.00	0.02	-0.01
	0.01	0.01	0.00	0.02	0.00
	0.02	0.01	0.01	0.02	0.01
	0.02	0.01	0.01	0.02	0.01
	0.03	0.02	0.01	0.03	0.03
	0.03	0.03	0.02	0.03	0.03
	0.07	0.05	0.03	0.04	0.04
	0.07	0.05	0.05	0.05	0.04
2	-0.01	-0.06	0.01	0.02	-0.02
	0.01	0.01	0.01	0.02	0.00
	0.02	0.02	0.03	0.02	0.00
	0.02	0.03	0.04	0.03	0.01
	0.03	0.04	0.04	0.03	0.03
	0.06	0.06	0.05	0.04	0.03
	0.08	0.08	0.06	0.04	0.04
	0.10	0.13	0.15	0.04	0.05
	0.15	0.20	0.20	0.05	0.08
	0.18	0.21	0.21	0.06	0.10

week	concentration				
	control	0.01	0.1	1.0	10
3	-0.01	-0.02	-0.06	0.01	-0.03
	0.00	-0.01	-0.04	0.01	-0.02
	0.01	-0.01	-0.03	0.03	-0.01
	0.02	0.00	-0.03	0.03	0.00
	0.03	0.01	0.01	0.04	0.00
	0.05	0.03	0.08	0.05	0.00
	0.06	0.04	0.18	0.06	0.01
	0.15	0.06	0.23	0.06	0.02
	0.16	0.18	0.23	0.08	0.03
	0.23	0.21	0.26	0.11	0.03
4	-0.02	0.01	-0.05	0.00	-0.03
	-0.01	0.01	-0.03	0.02	-0.03
	0.02	0.02	-0.03	0.04	-0.01
	0.02	0.02	0.00	0.05	0.00
	0.03	0.06	0.02	0.08	0.01
	0.03	0.09	0.04	0.09	0.02
	0.03	0.14	0.12	0.15	0.03
	0.13	0.14	0.18	0.16	0.04
	0.13	0.15	0.25	0.17	0.07
	0.53	0.33	0.29	0.49	0.09

week	concentration				
	control	0.01	0.1	1.0	10
5	-0.04	-0.03	-0.02	0.04	-0.04
	0.01	-0.01	-0.01	0.10	-0.03
	0.02	-0.01	0.00	0.11	0.00
	0.10	0.00	0.01	0.12	0.00
	0.12	0.01	0.03	0.12	0.01
	0.17	0.04	0.09	0.13	0.02
	0.19	0.06	0.09	0.14	0.02
	0.27	0.08	0.17	0.15	0.04
	0.30	0.12	0.20	0.25	0.11
	0.39	0.56	0.33	0.36	0.14

average* and standard deviation**

1 *	0.03	0.02	0.01	0.02	0.01
**	0.02	0.02	0.02	0.01	0.03
2 *	0.06	0.07	0.08	0.04	0.03
**	0.06	0.08	0.07	0.01	0.04
3 *	0.07	0.05	0.08	0.05	0.00
**	0.08	0.08	0.12	0.03	0.02
4 *	0.09	0.10	0.08	0.13	0.02
**	0.15	0.09	0.12	0.13	0.04
5 *	0.15	0.08	0.09	0.15	0.03
**	0.13	0.17	0.11	0.09	0.05

Statistical analysis of the average mass increase (g) of maize, *Zea mays*, callus pieces grown for six weeks on culture medium (Green & Phillips, 1975) containing different concentrations of SM-toxin, a pathotoxin extract of *Stenocarpella macrospora* (week 0 to 7), and the mass increase (g) when the callus was subsequently transferred to toxin-free culture medium (week 7 to 14). Data in APPENDIX 4.8. (std.dev. = standard deviation)

	MASS (gram)			GROWTH RATE			MASS INCREASE PER DAY (gram)		
	week 0	week 7	week 14	0 to 7	7 to 14	0 to 14	0 to 7	7 to 14	0 to 14
0 ml/l control average	0.09	0.44	0.75	5.65	1.39	9.79	0.008	0.006	0.007
std.dev.	0.04	0.37	0.68	5.20	0.44	9.80	0.008	0.006	0.007
minimum	0.04	0.09	0.08	0.9	0.9	0.8	0.000	0.000	0.000
maximum	0.19	1.11	1.76	14.0	2.0	26.8	0.023	0.013	0.017
0.01 ml/l average	0.09	0.42	0.78	5.05	1.57	9.79	0.007	0.007	0.007
std.dev.	0.03	0.33	0.65	4.04	0.53	9.58	0.007	0.006	0.007
minimum	0.04	0.04	0.05	0.9	0.7	0.6	0.000	0.000	0.000
maximum	0.13	1.14	1.87	13.8	2.4	32.8	0.022	0.018	0.018
0.1 ml/l average	0.08	0.47	1.02	6.98	1.75	15.92	0.009	0.010	0.010
std.dev.	0.03	0.33	0.77	5.91	0.69	16.21	0.007	0.009	0.008
minimum	0.03	0.05	0.04	0.9	0.8	0.8	0.000	0.000	0.000
maximum	0.13	0.85	1.85	19.0	2.9	54.7	0.017	0.020	0.018
1.0 ml/l average	0.09	0.71	1.48	8.68	2.09	17.98	0.014	0.015	0.014
std.dev.	0.02	0.19	0.38	4.02	0.15	8.13	0.004	0.004	0.004
minimum	0.06	0.41	0.83	4.9	1.9	10.6	0.008	0.008	0.008
maximum	0.14	0.98	2.11	16.3	2.4	35.2	0.020	0.021	0.021
10 ml/l average	0.09	0.18	0.22	1.86	1.15	2.14	0.002	0.001	0.001
std.dev.	0.03	0.21	0.41	1.58	0.56	3.01	0.004	0.006	0.004
minimum	0.04	0.05	0.05	0.9	0.1	0.7	0.000	-0.010	0.000
maximum	0.13	0.61	1.45	5.5	2.5	11.2	0.011	0.017	0.013
ethyl acetate 10 ml/l average	0.07	0.29	0.56	4.54	1.55	9.46	0.006	0.005	0.005
std.dev.	0.01	0.21	0.58	3.69	0.71	11.32	0.006	0.007	0.006
minimum	0.05	0.07	0.06	1.0	0.9	0.9	0.000	0.000	0.000
maximum	0.09	0.59	1.96	11.8	3.3	39.2	0.014	0.023	0.019

Data of the average mass increase (g) of maize, *Zea mays*, callus pieces grown for six weeks on culture medium (Green & Phillips, 1975) containing different concentrations of SM-toxin, a pathotoxin extract of *Stenocarpella macrospora* (week 0 to 7), and when the callus was subsequently transferred to toxin-free culture medium (week 7 to 14)

	MASS (gram)			GROWTH RATE*			MASS INCREASE PER DAY (gram)**		
	week 0	week 7	week 14	0 to 7	7 to 14	0 to 14	0 to 7	7 to 14	0 to 14
0 ml/l control	0.04	0.56	1.07	14.0	1.9	26.8	0.012	0.010	0.011
	0.06	0.62	1.22	10.3	2.0	20.3	0.012	0.011	0.012
	0.08	1.11	1.76	13.9	1.6	22.0	0.023	0.012	0.017
	0.08	0.09	0.09	1.1	1.0	1.1	0.000	0.000	0.000
	0.08	0.09	0.10	1.1	1.1	1.3	0.000	0.000	0.000
	0.09	0.73	1.37	8.1	1.9	15.2	0.014	0.012	0.013
	0.10	0.09	0.08	0.9	0.9	0.8	0.000	0.000	0.000
	0.10	0.11	0.10	1.1	0.9	1.0	0.000	0.000	0.000
	0.11	0.13	0.12	1.2	0.9	1.1	0.000	0.000	0.000
	0.19	0.91	1.59	4.8	1.7	8.4	0.016	0.013	0.014
0.01 ml/l	0.04	0.04	0.05	1.0	1.3	1.3	0.000	0.000	0.000
	0.05	0.69	1.64	13.8	2.4	32.8	0.014	0.018	0.016
	0.07	0.52	1.20	7.4	2.3	17.1	0.010	0.013	0.012
	0.08	0.42	0.79	5.3	1.9	9.9	0.008	0.007	0.007
	0.08	0.07	0.05	0.9	0.7	0.6	0.000	0.000	0.000
	0.10	0.52	0.81	5.2	1.6	8.1	0.009	0.005	0.007
	0.10	0.10	0.11	1.0	1.1	1.1	0.000	0.000	0.000
	0.10	0.61	1.15	6.1	1.9	11.5	0.011	0.010	0.011
	0.12	0.13	0.13	1.1	1.0	1.1	0.000	0.000	0.000
	0.13	1.14	1.87	8.8	1.6	14.4	0.022	0.014	0.018
0.1 ml/l	0.03	0.57	1.64	19.0	2.9	54.7	0.012	0.020	0.016
	0.05	0.05	0.04	1.0	0.8	0.8	0.000	0.000	0.000
	0.06	0.73	1.55	12.2	2.1	25.8	0.015	0.015	0.015
	0.07	0.85	1.85	12.1	2.2	26.4	0.017	0.019	0.018
	0.08	0.08	0.08	1.0	1.0	1.0	0.000	0.000	0.000
	0.08	0.75	1.52	9.4	2.0	19.0	0.015	0.015	0.015
	0.10	0.09	0.09	0.9	1.0	0.9	0.000	0.000	0.000
	0.10	0.73	1.66	7.3	2.3	16.6	0.014	0.018	0.016
	0.11	0.11	0.11	1.0	1.0	1.0	0.000	0.000	0.000
	0.13	0.77	1.68	5.9	2.2	12.9	0.014	0.017	0.016

* Growth rate 0 to 7: mass week 7/ mass week 0; Growth rate 7 to 14: mass week 14/ mass week 7;
Growth rate 0 to 14: mass week 14/ mass week 0;

** week 0 to 7 = 45 days; week 7 to 14 = 53 days

Data of the average mass increase (g) of maize, *Zea mays*, callus pieces grown for six weeks on culture medium (Green & Phillips, 1975) containing different concentrations of SM-toxin, a pathotoxin extract of *Stenocarpella macrospora* (week 0 to 7), and when the callus was subsequently transferred to toxin-free culture medium (week 7 to 14)

	MASS (gram)			GROWTH RATE*			MASS INCREASE PER DAY (gram)**		
	week 0	week 7	week 14	0 to 7	7 to 14	0 to 14	0 to 7	7 to 14	0 to 14
1.0 ml/l	0.06	0.98	2.11	16.3	2.2	35.2	0.020	0.021	0.021
	0.06	0.92	1.74	15.3	1.9	29.0	0.019	0.015	0.017
	0.07	0.41	0.83	5.9	2.0	11.9	0.008	0.008	0.008
	0.08	0.47	0.98	5.9	2.1	12.3	0.009	0.010	0.009
	0.08	0.87	1.95	10.9	2.2	24.4	0.018	0.020	0.019
	0.09	0.79	1.50	8.8	1.9	16.7	0.016	0.013	0.014
	0.10	0.54	1.19	5.4	2.2	11.9	0.010	0.012	0.011
	0.10	0.83	1.59	8.3	1.9	15.9	0.016	0.014	0.015
	0.12	0.61	1.45	5.1	2.4	12.1	0.011	0.016	0.014
	0.14	0.69	1.48	4.9	2.1	10.6	0.012	0.015	0.014
10 ml/l	0.04	0.05	0.05	1.3	1.0	1.3	0.000	0.000	0.000
	0.05	0.06	0.08	1.2	1.3	1.6	0.000	0.000	0.000
	0.05	0.07	0.06	1.4	0.9	1.2	0.000	0.000	0.000
	0.07	0.07	0.09	1.0	1.3	1.3	0.000	0.000	0.000
	0.07	0.07	0.07	1.0	1.0	1.0	0.000	0.000	0.000
	0.10	0.09	0.09	0.9	1.0	0.9	0.000	0.000	0.000
	0.11	0.61	0.08	5.5	0.1	0.7	0.011	-0.010	0.000
	0.12	0.12	0.14	1.0	1.2	1.2	0.000	0.000	0.000
	0.12	0.11	0.13	0.9	1.2	1.1	0.000	0.000	0.000
	0.13	0.57	1.45	4.4	2.5	11.2	0.010	0.017	0.013
ethyl acetate 10 ml/l	0.05	0.37	0.76	7.4	2.1	15.2	0.008	0.007	0.007
	0.05	0.59	1.96	11.8	3.3	39.2	0.014	0.023	0.019
	0.06	0.46	0.82	7.7	1.8	13.7	0.010	0.006	0.008
	0.06	0.07	0.06	1.2	0.9	1.0	0.000	0.000	0.000
	0.07	0.09	0.12	1.3	1.3	1.7	0.001	0.001	0.001
	0.08	0.08	0.07	1.0	0.9	0.9	0.000	0.000	0.000
	0.08	0.47	0.83	5.9	1.8	10.4	0.010	0.006	0.008
	0.08	0.10	0.09	1.3	0.9	1.1	0.001	0.000	0.000
	0.08	0.56	0.83	7.0	1.5	10.4	0.012	0.005	0.008
	0.09	0.09	0.10	1.0	1.1	1.1	0.000	0.000	0.000

* Growth rate 0 to 7: mass week 7/ mass week 0; Growth rate 7 to 14: mass week 14/ mass week 7;
Growth rate 0 to 14: mass week 14/ mass week 0;

** week 0 to 7 = 45 days; week 7 to 14 = 53 days

Statistical analysis of the data of the height (cm) and dry mass of the above-ground parts of seedlings of maize, *Zea mays*, inbred line I137TN, injected at the stalk base with 0.1 ml of solution of a phytotoxin extract of *Stenocarpella macrospora* (10 ml/l), or with deionised water. Data in APPENDIX 4.10

	HEIGHT (cm)				MASS (g)			
	rep 1	rep 2	rep 3	mean	rep 1	rep 2	rep 3	mean
control								
average	55.8	43.5	42.0	47.1	0.62	0.42	0.40	0.48
std.dev.*	7.7	3.0	5.8	6.2				0.10
variance	58.8	9.2	33.4	38.4				0.01
minimum	43.0	39.3	36.0	42.0				0.40
maximum	67.8	48.1	54.0	55.8				0.62
n =	11	8	7	3				3
50 ml/l ethyl acetate								
average	50.9	32.9	36.2	40.0	0.43	0.38	0.38	0.39
std.dev.	8.1	6.5	7.8	7.8				0.02
variance	66.2	42.7	60.6	61.3				0.00
minimum	34.7	25.6	23.1	32.9				0.38
maximum	58.7	41.1	50.5	50.9				0.43
n =	10	7	8	3				3
50 ml/l SM-toxin								
average	49.1	37.0	29.9	38.7	0.42	0.30	0.26	0.33
std.dev.	10.1	3.7	6.7	8.0				0.07
variance	102.6	13.8	45.0	63.2				0.00
minimum	32.4	31.1	22.0	29.9				0.26
maximum	64.2	42.0	43.5	49.1				0.42
n =	9	10	7	3				3

* std.dev. = standard deviation

Data of the height (cm) of seedlings of maize, *Zea mays*, inbred line I137TN, injected at the stalk base with 0.1 ml of solution of a phytotoxin extract of *Stenocarpella macrospora* (10 ml/l), or with deionised water. The dry mass (g) of the above-ground parts of the plants was also recorded

	HEIGHT (cm)						TOTAL MASS (g)		
	replication 1		replication 2		replication 3		rep 1	rep 2	rep 3
control	51.0	51.7	41.7		39.2				
	52.5	55.6	39.3		38.6				
	65.9	58.1	46.2		36.0				
	67.8		41.2		54.0				
	51.9		48.1		39.0				
	67.1		46.2		40.5				
	43.0		40.4		46.8				
	49.6		45.0				6.78	3.33	2.79
50 ml/l ethyl acetate									
	58.7	57.8	27.5		29.8				
	58.5	34.7	40.8		35.6				
	54.5		27.5		34.0				
	42.7		39.3		23.1				
	58.5		28.8		39.7				
	41.2		25.6		42.9				
	50.7		41.1		50.5				
	52.0				33.9		4.25	2.65	3.03
50 ml/l SM-toxin									
	64.2	53.7	31.4	40.5	29.2				
	53.9		37.0	38.1	28.0				
	44.6		42.0		32.3				
	35.3		37.2		43.5				
	61.6		41.3		22.5				
	49.7		38.0		31.7				
	46.9		33.3		22.0				
	32.4		31.1				3.75	2.99	1.85

Statistical analysis of the data of the height (cm) and dry mass of the above-ground parts of seedlings of maize, *Zea mays*, inbred line F2834, injected at the stalk base with 0.1 ml of solution of a phytotoxin extract of *Stenocarpella macrospora* (10 ml/l), or with deionised water. Data in APPENDIX 4.12

	HEIGHT (cm)				MASS (g)			
	rep 1	rep 2	rep 3	mean	rep 1	rep 2	rep 3	mean
control								
average	57.2	31.3	47.3	45.3	0.63	0.23	0.65	0.50
std.dev.*	5.9	6.5	2.6	10.7				0.19
variance	34.9	41.8	7.0	114.5				0.04
minimum	46.1	21.8	40.2	31.3				0.23
maximum	66.5	42.0	50.8	57.2				0.65
n =	12	12	12	3				3
50 ml/l ethyl ace								
average	56.8	33.2	46.9	45.6	0.52	0.36	0.66	0.51
std.dev.	5.8	5.6	5.8	9.7				0.12
variance	33.1	31.3	33.5	93.7				0.02
minimum	45.5	24.9	36.8	33.2				0.36
maximum	62.2	41.8	54.6	56.8				0.66
n =	9	10	12	3				3
50 ml/l SM-toxin								
average	57.6	33.1	32.0	40.9	0.54	0.35	0.37	0.42
std.dev.	4.4	5.2	6.8	11.8				0.09
variance	19.2	27.5	46.0	139.7				0.01
minimum	51.5	23.2	20.4	32.0				0.35
maximum	64.6	42.1	41.8	57.6				0.54
n =	8	12	12	3				3

* std.dev. = standard deviation

Data of the height (cm) of seedlings of maize, *Zea mays*, inbred line F2834, injected at the stalk base with 0.1 ml of solution of a phytotoxin extract of *Stenocarpella macrospora* (10 ml/l), or with deionised water. The dry mass (g) of the above-ground parts of the plants was also recorded

	HEIGHT (cm)						TOTAL MASS (g)		
	replication 1		replication 2		replication 3		rep 1	rep 2	rep 3
control	46.1	56.3	41.2	27.1	46.5	47.2			
	54.7	55.6	42.0	21.8	49.0	44.8			
	54.2	63.7	39.8	24.4	49.5	40.2			
	58.4	66.5	25.4	27.8	50.8	49.2			
	48.5		33.5		46.6				
	56.6		29.1		48.1				
	62.2		33.0		48.6				
	64.0		30.0		47.0		7.58	2.73	7.75
50 ml/l ethyl acet									
	56.5	45.5	28.2	30.8	41.7	50.7			
	47.3		39.0	41.8	49.1	41.8			
	59.5		37.0		50.0	36.8			
	62.2		24.9		37.0	47.5			
	59.2		28.1		52.1				
	61.1		37.5		54.6				
	60.2		27.5		51.0				
	59.6		37.0		50.9		4.69	3.59	7.93
50 ml/l SM-toxin									
	63.8		33.2	23.2	41.8	24.8			
	64.6		42.1	23.5	38.3	34.6			
	54.6		34.3	34.6	23.2	25.3			
	55.7		34.0	33.1	20.4	35.2			
	51.5		37.0		39.1				
	59.8		35.6		38.0				
	54.6		35.5		30.2				
	56.0		30.2		32.7		4.34	4.14	4.48