CHAPTER FOUR

THE EFFECT OF FUNGICIDE SEED TREATMENTS ON GERMINATION AND VIGOUR OF UNSTORED MAIZE (*ZEA MAYS* L.) SEEDS.

Abstract

The availability of good quality seed is dependent on two very broad aspects, how healthy (disease-free) a seed is and the viability of the seed (field performance). The objective of the study was to evaluate the efficiency of fungicide seed treatments on maize (*Zea mays* L.) by comparing germination, vigour and greenhouse emergence of treated seeds. Maize seeds were treated with four fungicides: Apron® XL (metalaxyl), Thiram (thiram), Celest® XL (fludioxonil, metalaxyl) and Apron® Star (thiamethoxam, metalaxyl, difenoconazole). After seeds were treated the moisture content of the seeds were calculated according to the rules outlined by the International Seed Testing Association (ISTA). The standard germination and vigour tests were conducted according to the rules outlined by ISTA. Thereafter seeds were planted under greenhouse conditions. The control consisted of seeds that were untreated. In the standard germination test, results were expressed as percentage seedlings that have germinated at the end of the test period. All treated seed maintained germination above 75%. Seeds treated with Apron® XL had the highest germination of 83%. This higher percentage germination compared to the other treatments was maintained following the cold test and the greenhouse trial. Thiram had 82% germination which decreased following imbition.. In this study none of the treated seeds had major imbibition damage as indicated by the percentage weight increase and the low leachate conductivity (1012-1271 $\mu Scm^{-1}g^{-1}$). The results from the vigour test gave an indication of the emergence of the seedlings following the greenhouse trial.

4.1 Introduction

Fungicide seed treatments are the most economical and easiest method to protect important seeds (Anaso *et al*, 1989) and young vulnerable seedlings (Rane and Ruhl, 2002). Seeds treatments that use pesticides can protect seeds from insect damage (Chen and Burris, 1993) and those seeds that are mechanically damaged at harvesting (Kommedahl and Windels, 1986). Most of commercially produced seed of maize (Zea mays L.) is almost universally treated with a fungicide prior to sale to protect the seed from fungal infection after planting (Kommedahl and Windels, 1986; Munkvold and O' Mara, 2002).

Protection of the seeds against pathogens has a direct impact on the germination of seeds (Gange *et al*., 1992; Jonitz and Leist, 2003). Pathogens can be successfully controlled through the use of suitable seed treatments, with a corresponding increase in the number of seeds that can germinate normally (Jonitz and Leist, 2003). A study conducted by Gange *et al*., (1992) hypothesized that pesticides used on seeds could either have a phytotoxic effect of a stimulatory effect on seed germination. Of the pesticides that were tested in their study, two had few significant effects on germination (Gange *et al*., 1992). Vigour tests are designed specifically to simulate the conditions in the field (Copeland and McDonald, 2001; Noli *et al*., 2008). Results obtained with the cold test gives an indication as to how seeds will germinate under conditions of increased pathogen density (Lovato *et al*., 2005). In a study by Lovato *et al*. (2005), it was found that maize seed had a higher vigour after being incubated at 4.5ºC than at 10ºC, results showed that the cold test is a very reliable vigour test for maize (Lovato *et al*., 2005). Subjecting treated seed to vigour tests gives an indication as to how specific treatments can indirectly increase germination (Nijënstein and Kruse, 2000; Noli *et al*., 2008).

Thiram is an organic sulphur fungicide, classified under dithiocarbamate. It is an excellent protectant compound registered for a large number of important crops (Frederickson and Leuschner, 1997; Agrios, 2005). Celest® XL includes two active ingredients, namely fludioxonil and mefenoxam. This is a water-based odourless chemical (http://www.syngenta.com). The fungicide fludioxonil is used as a seed treatment providing

protection during germination and early growth stages of the development. If the seeds are protected from biotic factors such as fungal infection then the chances of those seeds being viable is increased (Errampalli, 2004; http://www.syngenta.com).

The aim of the current study was to treat maize seeds with fungicides (Apron® XL, Apron® Star 42 WS, Thiram and Celest® XL) and to assess the effect these treatments have on germination, vigour and greenhouse emergence.

4.2 Materials and Methods

4.2.1 Treatment of the seed

Untreated seeds were obtained from Syngenta Pty. Ltd (Midrand, South Africa). All the chemicals 1) Celest® XL [*fludioxonil (25 g ai/L) + mefenoxam (10 g ai/L*]; 2) Apron® Star [*thiamethoxam (20% w/w) + metalaxyl – M (20% w/w) + difenoconazole (2% w/w)*]; 3) Apron® XL [*metalaxyl – M (350 g ai/L*] and 4) Thiram [*thiram (50.0% m/m)*] were also supplied by Syngenta. Seeds were placed in a flat-bottomed bowl and the recommended amount of fungicide and water were added to the seeds. The seeds were mixed thoroughly with the fungicide until all the seeds were covered with the fungicide, which took 5-10 min. After treatment, the seeds were left on paper towels in a laminar flow cabinet to dry. Once the seeds had dried, they were divided into three batches (1: immediate use; 2: 2 and 4 day accelerated ageing and 3: 3 and 6 months storage). This chapter focuses on the batch that was processed immediately.

4.2.2 Moisture content

Prior to proceeding with the rest of the tests, the moisture content was measured for the treated seeds. Two samples of 10 g were used. Two metal containers of a diameter of more than 8 cm were weighed. The samples were ground individually, using a grinding mill, and placed into the metal containers. The resultant maize powder in the containers was then weighed (initial weight). The containers were placed in an oven at 130ºC for 4 hr. At the end of the 4 hr the samples were placed in a dessicator for 30 min to cool. The samples were then reweighed. The percentage moisture content was calculated according to the formula outlined in the International Seed Testing Association rules (ISTA, 2008).

(M2-M3) x 100/(M2-M1)

Where M1 – is the weight in grams of the containers and the cover

M2 – is the weight in grams of the container, its cover and its contents before drying

M3 – is the weight in grams of the container, cover and contents after drying

4.2.3 Standard germination test

Standard germination tests were conducted for all samples according to the between-paper (BP) method of the ISTA rules (ISTA, 2008). Two hundred maize seeds were randomly chosen from each sample and were placed on moist germination paper (containing 4 sheets of germination paper and 1 sheet of paper towel) {Anchor Paper 54x30 cm, (Agricol (Pty) Ltd, South Africa)} equidistant apart. Paper towels were rolled up and placed individually in polythene bags. These bags were sealed with an elastic band. They were incubated in an upright position at 25 ± 1°C. Four replicates of 50 seeds were used. Percentage germination was determined after seven days and ratings for normal/abnormal seedlings were done at eleven days. Seeds were visually assessed according to the ISTA rules (ISTA, 2008). Results were presented as the percentage seedlings that had germinated at the end of the test period.

4.2.4 Vigour tests

4.2.4.1 Imbibition

Seeds were subjected to slow and rapid imbibition as outlined in the ISTA (2006) rules. For rapid imbibition, seeds were weighed individually and placed in 4 ml water in a 24 well ice-cube tray. Seeds were incubated for 6, 24 and 40 hr. At the end of the incubation times, seeds were removed, left to dry and then reweighed. The percentage weight increase was calculated according to the formula:

$$\% \text{ Weight increase} = \frac{\text{weight of 6hr imbibition}}{\text{initial weight of seed}}$$

Thereafter the seeds were placed in germination paper and left to germinate as described for the standard germination test. In contrast, with slow imbibition the seeds were weighed and then placed on germination paper as described for the standard germination test. The seeds were incubated for 6, 24 and 40 hr as described for the rapid imbibition. At the end of the

incubation times, the seeds were reweighed and put back onto the germination paper. The percentage seedlings were noted as described for the standard germination test. The percentage seeds were compared with that of the results from the standard germination test.

4.2.4.2 Conductivity

With rapid imbibition, the seeds were placed in wells of the ice-cube tray for 24 hr. Thereafter the conductivity of the solution was read on an E215 Conductivity meter (Hanna Instruments). After the conductivity was read, the same seeds were used in the tetrazolium staining test. Slow imbibition consisted of seeds being placed on germination paper for 40 hr. At the end of the 40 hr, seeds were placed in the trays for 6 hr, thereafter the conductivity of the solution was read and the same seeds were used in the tetrazolium staining test. The conductivity of the fungicide solutions in the absence of seeds was also tested.

4.2.4.3 Tetrazolium test

Seeds from the conductivity test were used for the tetrazolium staining. A 1% solution of the 2,3,5-triphenyl tetrazolium chloride (TTC) (Labretoria, Pretoria) (10 g of TTC dissolved in a small quantity of hot water in a beaker) was transferred to a 1 L flask and tap water was added to make it up to 1 L The seed coats of the seeds were removed and the seeds were slit longitudinally through the embryo and ¾ of the endosperm. The slit seeds were placed in individual wells and covered with the TTC. The trays were incubated at 30ºC for 2 hr. The seeds were then removed from the stain, cut into two halves and the cut surface was examined using a stereo-microscope (Nikon/SMZ-1, Japan). The seeds were rated as 1 – totally stained seed, 2 – part of the seed was not stained and 3 – if the seed is totally unstained (e.g. hard seed). Results were expressed as the percentage of seeds containing living tissue.

4.2.4.4 Cold Test

Soil was obtained from a maize field and brought back to the Plant Pathology laboratories (University of Pretoria). The germination paper was prepared as for the standard germination test with one difference, soil was included onto the paper and seeds were planted onto the soil. Paper towels were rolled up and placed individually in polythene

bags. They were incubated in an upright position at 5°C for a week, thereafter incubated at 25°C. Four replicates of 50 seeds were used. Percentage germination was determined after 7 d following incubation at ambient temperature and rating for normal / abnormal seedlings were done at 11 d. Seeds were visually assessed according to the ISTA rules (ISTA, 2008). Results were presented as the number of seedlings that had germinated at the end of the test period.

4.2.5 Greenhouse trial

The seedling trays were cleaned using 2% sodium hypochlorite and left to dry for 24 hr before the trays were filled with pasteurised soil (Braaks, Pretoria). The trays were watered until run-off. This was done a day before the seeds of the different treatments were sown. Four replicates of 25 seeds were used per treatment. Each tray had three different treatments in a randomised block design. The temperature within the greenhouse ranged from 25-30ºC. The trays were monitored regularly and were watered daily. The trial was terminated three weeks after planting and the trial was repeated. Results were expressed as the percentage seedlings that have emerged at the end of the test period.

4.2.6 Statistical analysis

Two-way analysis of variance (ANOVA) was performed on all data and least significant differences (P= 0.05) were determined according to the student's t test.

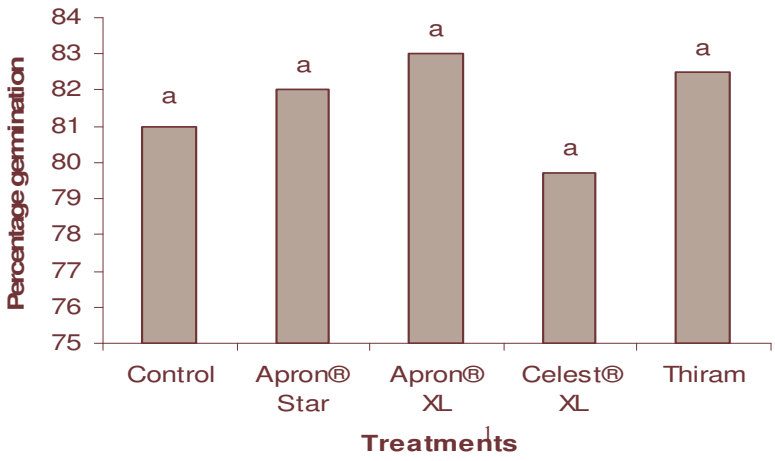
4.3 Results

4.3.1 Moisture content

The average percentage moisture content for each of the treatments and the control was between 11.0 to 11.5%. All the seed samples were within tolerance (< 0.3%) for maize as stipulated according to ISTA (ISTA, 2008).

4.3.2 Standard germination test

All the seed treatments had germination percentages above 75% and did not differ significantly from the control and each other (Fig 4.1). Seeds treated with Apron® XL had the highest percentage (83%) followed by Apron® Star and Thiram (82 and 82.5%, respectively).



[1] Each value is a mean percentage of four replicates of 50 seeds
Bars containing the same letters above them did not differ significantly (*P = 0.05*)

Fig 1: Standard germination results of treated and untreated maize seeds.

4.3.3 Vigour test

4.3.3.1 Imbibition

Results show that the different treatments had a high percentage weight increase following 40 hr rapid imbibition and did not differ significantly from each other (Table 4.1). Within the different treatments the lowest weight increase was seen following 6 hr slow imbibition (Table 4.1).

The untreated control had a lower percentage (46.7%) weight increase following slow imbibition compared to some of the treatments (Table 4.1) and this was reflected in the germination following 40 hr slow imbibition (83%). The percentage weight increase of seeds treated with Apron® Star did not differ significantly following 40 hr rapid and slow imbibition (58.5 and 57.3%). Percentage germination results for seeds treated with Apron® Star following slow imbibition was 63.6% but it did not differ significantly from the germination following rapid imbibition (56.2%)

Table 4.1: The percentage weight increase and germination following imbibition of the treated and untreated maize seeds

	Time (hr)	Treatments[1]				
		Control	Apron® Star	Apron® XL	Celest® XL	Thiram
		Weight increase (%)				
Rapid imbibition	6	34.3*a**w	34.0aw	31.5aw	34.7av	34.8ay
	24	47.7ax	51.7ay	48.1ay	52.2ay	51.4az
	40	56.4ay	58.5az	59.2az	56.0ay	58.4az
Slow imbibition	6	16.3av	18.3av	19.0av	21.0au	19.3ax
	24	35.2aw	44.2bx	39.4abx	40.0abw	40.0aby
	40	46.7ax	57.3byz	41.7ax	46.0aw	48.2az
		Germination (%)				
Rapid imbibition	40	60.4*b**x	56.2ax	59.4bx	67.7bx	52.1ax
Slow imbibition	40	83.4by	63.6ax	80.2by	64.0ax	77.1by

[1]- Each value is a mean percentage of four replicates of 24 seeds
*Means within a ROW not followed by the same letter are significantly different ($P = 0.05$)
**Means within a COLUMN not followed by the same letter are significantly different ($P = 0.05$)

Seeds treated with Apron® XL had the highest percentage weight increase following rapid imbibition however it did not differ significantly from all the other treatments (Table 4.1). This treatment had the lowest percentage weight increase (41.7%) following slow imbibition and the highest percentage germination (80.2%) of the four treatments.

Seeds treated with Celest® XL had 56.0% weight increase and was similar to the untreated control (56.4%) but did not differ significantly from the other treatments. This treatment recorded the second lowest germination (64.0%) following 40 hr slow and did not differ significantly from Apron® Star (63.6%). In contrast to the trend of the other treatments, Celest® XL had the highest percentage germination (67.7%) following 40 hr rapid imbibition.

Thiram had 48.2% weight increase after slow imbibition and did not differ significantly from the other treatments. When comparing with the other treatments, it differed from weight increase following rapid imbibition (58.4%). The percentage germination following slow imbibition was 77.1% and did not differ significantly from the untreated control (83.4%) and Apron® XL (80.2%) (Table 4.1).

4.3.3.2 Conductivity and Tetrazolium test

Compared to the water control, the conductivity of the fungicide solutions did not differ from control. Following imbibition all treatments had low leachate conductivity values with Thiram having the lowest (1012 $\mu Scm^{-1}g^{-1}$), although there were no significant differences between treatments (Table 4.2). These low conductivity values were mirrored in the percentage seeds with living tissue following the tetrazolium test. Thiram had the highest percentage seeds with living tissue (82%) and differed from all treatments (Table 4.2). Following rapid imbibition the percentage of the seeds with living tissue was lower, with Thiram having the highest (66%) and differed significantly from the other treatments (Table 4.2).

Table 4.2: The effect of seed treatments on the seed coat permeability of the maize seeds as measured by leachate conductivity

	Treatments				
	Control	**Apron® Star**	**Apron® XL**	**Celest**	**Thiram**
[1]Conductivity ($\mu Scm^{-1}g^{-1}$)	1233 *a	1271a	1053a	1242a	1012a
	Living tissue (%)				
[t]TTZ: slow imbibition	52a**y	60by	62by	74cy	82dy
TTZ: rapid imbibition	33ax	40ax	36ax	33ax	66bx

[1]- Each value is a mean percentage of four replicates of 24 seeds
*Means within a ROW not followed by the same letter are significantly different (*P = 0.05*)
**Means within a COLUMN not followed by the same letter are significantly different (*P = 0.05*)
[t] – triphenyl tetrazolium chloride test, a mean of 24 seeds expressed as percentage cotyledons with living tissue

4.3.3.3 Cold Test

The percentage seedlings that have germinated following the cold test were all below 70% (Table 4.3, Column A) and did not differ from each other and the control. Seeds treated with Apron® XL did have the highest percentage germination (66%) (Table 4.3).

Table 4.3: Percentage germination following the cold test on treated and untreated maize seeds

	Cold Test	Greenhouse emergence	
Treatments	A Germination (%)[1]	B Emergence (%)[2]	C Emergence (%)[3]
Control	60*a	67ab**x	66.7ax
Apron® Star	58a	65abx	65.3ax
Apron® XL	66a	71bcx	78.7bx
Celest® XL	61a	73bcy	65.3ax
Thiram	59a	77cx	76.0bx

[1]Each value is a mean percentage of four replicates of 25 seeds that have germinated in the cold test.
[2]Each value is a mean percentage of four replicates of 25 seeds that have emerged in the greenhouse.
[3]Each value is a mean percentage of four replicates of 25 seeds that have emerged in the greenhouse.

*Means within a COLUMN not followed by the same letter are significantly different (*P = 0.05*)
**Means within a ROW not followed by the same letter are significantly different (*P = 0.05*)

4.3.4 Greenhouse trial

The percentage seedlings that emerged following the first greenhouse trial showed that all the treatments and the untreated control had percentages emergence above 65%. Of the treatments, seeds treated with Thiram had the highest percentage emergence (77%) and differed significantly from the untreated control (67%) and seeds treated with Apron® Star (Table 4.3, Column B). Similar results were obtained in the second trial with all the treatments and the untreated control having percentage emergence above 65% (Table 4.3, Column C). Seeds treated with Apron® XL recorded the highest percentage emergence (78.8%) and did not differ from Thiram (76%).

Comparison of the two greenhouse trials, columns B and C in Table 4.3, results for the untreated control were similar in both trials. A similar trend was seen with the treatments, with the exception of seeds treated with Celest ® XL, where the percentage emergence for both trials did differ significantly from each other. With seeds treated with Celest ® XL, this treatment gave a higher emergence in trial one (73%) compared to trial two (65.3%).

4.4 Discussion

In this study none of the fungicides tested reduced the germination, vigour or caused any phytotoxic effects as reflected by the greenhouse emergence results. This is in agreement with a study conducted by Smith (1969), where the effect of fungicides tested on the germination and emergence of maize showed that all the fungicides increased germination of the maize by 5-6% (Smith, 1969). Khan (1992) reported similar results on wheat, where two systemic fungicides increased percentage germination by a small margin, germination was increased from 9.9 to 14.0% (Khan, 1992). Metalaxyl seed treatment also proved to be successful in controlling downy mildew in sorghum and increasing the yield of this crop (Anaso *et al*., 1989) and similarly increasing yield of maize (Pedersen *et al*., 2003). Seeds treated with Apron® XL, had one of the higher percentage germination and its results were consistent for most of the vigour tests, as indicated in this study.

The fungicide and pesticide treatment did not have any effect on the moisture content of the seeds and all the seeds had a moisture content below 14% and was within tolerance (<0.3%), which is the acceptable percentage range for maize (ISTA, 2008). Imbibition was not effected by the fungicide treatment; this was confirmed with the germination of the seeds. This was in agreement with methods in the study done on the uptake of Triticonazole by wheat (Quérou *et al*., 1997.). The fungicide did not affect the pathways needed for the uptake of water and the wheat seeds tested were able to germinate. The percentages germination following 40 hr rapid imbibition confirmed in this study that there was no imbibition damage.

The germination of the fungicide treated seeds did not differ significantly from the untreated control, the treated seeds were processed shortly after treatment and a major effect on the seeds would not be expected. Fungicides containing both t thiram and metalaxyl was tested against eleven fungal species including *Fusarium* and *Ulocladium* on lettuce seeds. Results showed that the fungicide treatment increased seed germination by 64.5% when seeds were incubated at 35ºC (Jin and Tylkowska, 2005). In this current study, seeds treated with Thiram and Apron® XL (metalaxyl) gave germination results that were higher that the other treatments. Seeds treated with Apron® XL gave better results than Apron® Star as indicated by standard germination and emergence percentages.

The vigour tests gave promising results in that none of the fungicides tested negatively affected the vigour of the seeds. Fungicide seed treatments do not affect vigour and viability of maize seeds (Crozier, 1890, Bradley *et al*., 2001). Studies where fungicides were used proved that if the fungicides are used at the recommended dosage then the treatment will not have an affect on the functioning of the seeds (Crozier, 1890). Other studies confirmed that maize seeds treated with thiram did not negatively effect the germination as long as it was used at the recommended dosage (Tort *et al*., 2006). In this study the dosage at which these fungicides were tested did not have an effect on the germination.

Conductivity values were relatively low for seeds that were treated and the untreated control, this was confirmed with the relatively low percentage weight increase and the high percentage seeds with living tissue Zhang and Hampton (1999) tested the effects of three systemic fungicide based products on peas and legumes. Thiram and Apron TZ were among the fungicides tested. At the recommended dosage, the conductivity of the treated seeds did not differ from the untreated control (Zhang and Hampton, 1999). Similar results were obtained when maize seeds were treated with three fungicides and two insectides (Marchi and Cicero, 2003). The fungicides contained thiram and fludioxonil, which are two of the active ingredients found in the fungicides tested in this current study. Treating the maize seeds did not affect conductivity of the seeds (Marchi and Cicero, 2003) and seeds treated with fludioxonil had increased radicle length as indicated by Munkvold and O'Mara (2002).

Germination following the cold test mirrored results of the standard germination test and gave an indication of the results for the greenhouse emergence trial. The greenhouse emergence showed that Thiram and Apron® XL were able to protect the seeds in an *in vivo* environment and allowed the seeds to germinate. Nijënstein and Kruse (2000) reported that with all the problems associated with standardising the cold test, it remains a test that has been used on maize to simulate field conditions and to predict field behaviour. This was confirmed by Noli *et al.* (2008). In their study they found that the cold test was the most accurate vigour test to predict field performance as long as the conditions for the laboratory

test were kept at a low temperature and the soil microflora was similar to that of the field as was indicated in this current study where soil from a maize field was used in the cold test.

In this study all seeds had a low degree of imbibition damage as this was indicted by the low conductivity values and the high percentage of seeds with living tissue. Results from this study conclude that treating maize seeds will not affect germination the functioning of the seed. The next chapter addresses whether the fungicide seed treatments will continue to sustain germination of the seeds when they are subjected to storage under stress conditions.

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CHAPTER FIVE

THE EFFECT OF ACCELERATED AGEING AND LONG-TERM STORAGE ON UNTREATED AND FUNGICIDE TREATED MAIZE (*ZEA MAYS* L.) SEED

Abstract

Seed aging is a natural process that occurs during storage and it is expressed as a reduction in germination and this process can be accelerated by unfavourable environmental conditions (high humidity and temperatures). The aim of the current study was to test the effect of accelerated ageing [2 and 4 days accelerated ageing (AA)] and long-term storage (3 and 6 months) on germination and vigour of treated maize seeds. Maize seeds were treated with four fungicides: Apron® XL (metalaxyl); Thiram (thiram) and Celest® XL (fludioxonil, metalaxyl) and (Apron® Star (thiamethoxam, metalaxyl, difenoconazole). The control consisted of seeds that were untreated. After treatment the moisture content of the seeds was determined according to the rules outlined by the International Seed Testing Association (ISTA). Seeds were then subjected to 2 and 4 d accelerated ageing at 45ºC and high RH (100%). Concurrently seeds were stored for 3 and 6 months at 30ºC and 75% RH. Following the accelerated ageing and storage, seeds were subjected to standard germination and vigour tests according to the rules outlined by ISTA. In the standard germination test, results were expressed as percentage seedlings that had germinated at the end of the test period. The control and the treated seeds still germinated after 2 and 4 d AA. Seeds treated with thiram had the highest germination (69%) following 2 d AA. There was a gradual decrease in germination following ageing and storage of the treated seeds and the control. Seeds treated with Apron® XL failed to germinate at 3 months. The decrease in germination was mirrored by the leachate conductivity readings. Germination following the cold test linked well with the results of the standard germination. Seed treated with thiram was the only treatment to maintain germination after 6 months, all the other treatments and the control failed to germinate.

5.1 Introduction

The accelerated ageing (AA) test was initially proposed as a method to evaluate seed storability (Copeland and McDonald, 2001; Rice and Dyer, 2001) and indirectly field emergence (Jensen, 2002). This test exposes seeds for a short period of time (2 to 4 days) to conditions of high temperature (45ºC) and high humidity (100%) (Copeland and McDonald, 2001). The seeds absorb moisture from the humid environment and the raised seed moisture content, along with the high temperature, causes rapid seed ageing (Rice and Dyer, 2001; Sowiak, 2004). High vigour seed lots will withstand these extreme stress conditions and age more slowly than low vigour seed lots (Basu *et al*., 2004).

In situations where maize seed is not sold immediately after harvest and due to delays in processing, this seed is required to be stored for up to 8-10 months under ambient conditions (Basu *et al*., 2004). Safe storage is defined as storage in which the seed quality and vigour is maintained for at least three years. (Harrington, 1958, as cited in Basu *et al*., 2004). Many changes occur in the lipid composition of most seed types during storage (Kadlag *et al.*, 1995) but one of the most devastating changes occur when conditions are favourable for mould development and that seed is no longer usable (Appert, 1987).

In a study by Lovato *et al*. (2005) a comparison was done on the standard germination, cold temperature and accelerated ageing tests of maize. Germination was high for most of the maize lots tested but this percentage decreased following the vigour tests (cold and AA tests). The findings were that the AA test was just as effective as the 10ºC cold test for assessing maize seed lot vigour as the results obtained for both tests were similar (Lovato *et al*., 2005).

Exposing seeds to conditions of high temperature, even for a short amount of time gives valuable information about the internal condition of the seed (Wang *et al*., 2005). In a study by Dreyer and van de Venter (1992), an investigation was carried out on the mitochondrial activity of the etiolated shoots of freshly harvested and aged kernels of maize. Impaired mitochondrial activity was not evident at 25ºC (favourable temperature) but was detected at 13 and 46ºC.

In the previous chapter (Chapter Four), results proved that treating maize seeds did not affect germination and vigour of unstored seeds. As was expected most seed treatments had percentages germination that did not differ from the untreated control and maintained greenhouse emergence over two seasons. In this chapter subjecting treated maize seeds to stress conditions before germination gives an indication as to which fungicide seed treatment may affect viability or vigour when treated maize seeds have to be stored for a longer period of time i.e 3-6 months. The aim of the current study, therefore, was to test the effect of accelerated ageing (2 and 4 d AA) and long-term storage (3 and 6 months) on germination and vigour of fungicide treated maize seeds.

5.2 Materials and methods

5.2.1 Treatment of the seeds

Untreated seeds were obtained from Syngenta® Pty. Ltd (Midrand, South Africa). All the chemicals: 1) Celest® XL [*fludioxonil* (*25 g ai/L*) + *mefenoxam* (*10 g ai/L*)]; 2) Apron® Star [*thiamethoxam* (*20%w/w*) + *metalaxyl – M* (*20%w/w*) + *difenoconazole* (*2%w/w*)]; 3) Apron® XL [*metalaxyl – M* (*350 g ai/L*)] and 4) Thiram [*thiram* (*50.0% m/m*)] were also supplied by Syngenta® Pty. Ltd. Seeds were treated as described in Chapter Four. After treatment, the seeds were left on paper towels in a laminar flow cabinet to dry. Once the seeds had dried, they were divided into three batches (1: immediate use (Chapter Four; 2: 2 and 4 day accelerated ageing and 3: 3 and 6 months storage). This chapter focuses on the batches that were subjected to accelerated ageing and long term storage.

5.2.2 Moisture content

Prior to proceeding with the tests, the moisture content of two samples of 10 g of the treated seeds was measured. Two metal containers of a diameter of 10 cm were weighed. The samples were ground individually, using a grinding mill, and placed into the metal containers. The resultant maize powder in the containers was then weighed (initial weight). The containers were placed in an oven at 130ºC for 4 hr and then placed in a dessicator for 30 min to cool. The samples were then reweighed. The percentage moisture content was calculated according to the formula outlined in the International Seed Testing Association (ISTA) rules (ISTA, 2008).

(M2-M3) x 100/(M2-M1)

Where M1 – is the weight in grams of the containers and its cover

M2 – is the weight in grams of the container, cover and contents before drying

M3 – is the weight in grams of the container, cover and contents after drying

5.2.3 Accelerated ageing (AA) and long term storage

The seed batch that was subjected to accelerated ageing at high relative humidity (90-100%) and temperature (45ºC) was divided in half, with one half being used for 2 d AA and the other half for 4 d AA. A humid environment was created by placing seeds on a grid above a salt solution in a sealed chamber which was incubated at 45ºC. At the end of a 2 d or 4 d incubation period the seeds were removed and subjected to the standard germination and vigour test. The third batch to be used for the storage test was also divided into two halves with one half being subjected to 3 months and the other half 6 months storage at 30ºC and 75% RH. Incubation in the humid environment was created as described for the 2 and 4 d AA. At the end of the 3 months and 6 months, seeds were removed and subjected to the standard germination and vigour tests.

5.2.4 Standard Germination

Standard germination tests were conducted for all samples according to the between-paper (BP) method of the ISTA rules (ISTA, 2008). Two hundred maize seeds were randomly chosen from each sample and were placed equidistant apart on moist germination paper (containing four sheets of germination paper and one sheet of paper towel) {Anchor Paper 54x30 cm, (Agricol (Pty) Ltd, South Africa)}. Paper towels were rolled up and placed individually in polythene bags. These bags were sealed with an elastic band. They were incubated in an upright position at 25 ± 1°C. Four replicates of 50 seeds were used. Percentage germination was determined after seven days and ratings for normal/abnormal seedlings were done at 11 d. Seeds were visually assessed according to the ISTA rules (ISTA, 2008). Results were presented as the percentage seedlings that had germinated at the end of the test period.

5.2.4 Vigour tests

5.2.4.1 Imbibition

Seeds were subjected to slow and rapid imbibition as outlined in the ISTA (2008) rules. For rapid imbibition, seeds were weighed individually and placed in 4 ml water in a 24-well ice-cube tray. Seeds were incubated for 6, 24 and 40 hr. At the end of the incubation times, seeds were removed, left to dry and then reweighed. The percentage weight increase was calculated according to the formula:

$$\% \text{ Weight increase} = \frac{\text{weight of 6hr imbibition}}{\text{initial weight of seed}}$$

Thereafter the seeds were placed on germination paper and left to germinate as described for the standard germination test. In contrast, with slow imbibition the seeds were weighed and then placed on germination paper as described for the standard germination test. The seeds were incubated for 6, 24 and 40 hr as described for the rapid imbibition. At the end of the incubation times, the seeds were reweighed and returned to the germination paper. The percentage seedlings were noted as described for the standard germination test.

5.2.4.2 Conductivity test

With rapid imbibition, the seeds were placed in wells of the ice-cube tray for 24 hr. Afterwards the conductivity of the solution was read on an E215 conductivity meter (Hanna Instruments). After the conductivity of the solution was read, the same seeds were used in the tetrazolium staining test. In slow imbibition, seeds were placed on germination paper for 40 hr and then in ice-cube trays for 6 hr. Thereafter the conductivity of the solution was read and the same seeds were used in the tetrazolium staining test.

5.2.4.3 Tetrazolium test

Seeds from the conductivity test were used for tetrazolium staining. A 1% solution of 2,3,5-triphenyl tetrazolium chloride (TTC) (Labretoria, Pretoria) (10 g of TTC dissolved in a small quantity of hot water in a beaker) was transferred to a 1 L flask and tap water was added to make up 1 L. The seed coats of the seeds were removed and the seeds were slit longitudinally through the embryo and ¾ of the endosperm. The slit seeds were placed individually in ice-cube wells and covered with the TTC. The trays were incubated at 30ºC

for 2 hr after which the seeds were removed from the stain, cut into two halves and the cut surface was examined using a stereo-microscope (Nikon/SMZ-1, Japan). The seeds were rated as 1 – totally stained seed, 2 – part of the seed was not stained and 3 – if the seed was totally unstained (e.g. hard seed). Results were expressed as the percentage of seeds containing living tissue.

5.2.4.4 Cold test

The germination paper was prepared as for the standard germination test with one difference, soil from a cultivated maize field was included onto the paper and seeds were placed equidistant on the soil. Four replicates of 50 seeds were used. Paper towels were rolled up and placed individually in polythene bags. They were incubated in an upright position at 5°C for 7 d and then at 25°C for a further 7 d. Percentage germination was then determined and rating for normal/abnormal seedlings was done at 11 d. Seeds were visually assessed according to the ISTA rules (ISTA, 2008). Results were presented as the number of seedlings that had germinated by the end of the test period.

5.2.5 Statistical analysis

Two-way analysis of variance (ANOVA) was performed on all data and least significant differences (P= 0.05) were determined according to the student's t test.

5.3 Results

5.3.1 Moisture content

The average percentage moisture content for each of the fungicide treatments and the control was between 11.7 to 12.5%. All the seed samples were within tolerance (< 0.3%) for maize as stipulated by the ISTA rules (ISTA, 2008).

5.3.2 Standard Germination

Table 5.1 reflects the effect of accelerated ageing and storage on germination of maize seeds that have been treated with fungicides. Comparing the different treatments and the untreated control within the 2 d AA column in Table 5.1 shows that seeds treated with Thiram had the highest percentage germination (69%) followed by seeds treated with Celest® XL (68.5%). The untreated control had the lowest germination (61%) and differed

from both Thiram and Celest® XL treatments. After 4 d AA seeds treated with Apron® Star had the highest percentage germination and differed significantly from the rest of the fungicide treatments and the control. Seeds treated with Apron® XL had a significant decrease in percentage germination to 9% (Table 5.1). After 3 months storage, the untreated control had the highest germination percentage (71.5%) and differed from the other fungicide/insecticide treatments. Seeds treated with Thiram had the second highest (58%) but did not differ from the Apron® Star (56%) treatment. Seeds treated with Celest® XL decreased to 15% and seeds treated with Apron® XL failed to germinate (Table 5.1). After 6 months storage, only seeds treated with Thiram had 12.5% germination with the untreated control and the other fungicide treated seeds failing to germinate.

Table 5.1: Impact of accelerated ageing and storage on germination of maize that has been untreated or treated with fungicides/insecticide

	Germination (%)			
	Accelerated ageing		Storage	
Treatment	**2 d AA**	**4 d AA**	**3 month**	**6 month**
Control	61*b**y	46.5bx	71.5ayz	0bw
Apron® Star	62.5by	49ax	56bxy	0bw
Apron® XL	65aby	9dx	0w	0bw
Celest® XL	68.5az	42by	15cx	0bw
Thiram	69ay	31.5cx	58by	12.5aw

AA = Accelerated ageing.

* Means within a column not followed by the same letter are significantly different (P = 0.05)

** Means within a row not followed by the same letter are significantly different (P = 0.05)

5.3.3 Vigour tests

5.3.3.1 Imbibition

Table 5.2 shows the percentage weight increase following rapid and slow imbibition. Following 24 hr imbibition there are major differences between the treated seeds and the untreated control. An increase in the period of ageing and storage showed an increase in percentage weight of the seeds, especially following rapid imbibition. Comparing the percentage weight increase of seeds for slow imbibition showed that at 2 d and 4 d AA

there were differences between the treatments and the untreated control but not significantly (Table 5.2).

Table 5.2: Percentage weight increase of untreated and fungicide/insecticide treated maize seeds following imbibition

			Weight increase (%)			
			Accelerated ageing		Storage	
	Time (hr)	Treatments	2 d AA	4 d AA	3 month	6 month
Rapid imbibition	**24**	Control	42.4*b**x	51.3cxy	56.0by	70.2az
		Apron® Star	45.6bx	49.7bcx	64.7cy	75.3bz
		Apron® XL	44.5bw	50.0cwx	62.8cy	74.9bz
		Celest® XL	46.0bx	45.1ax	74.4dz	76.8bz
		Thiram	45.0x	47.4bx	59.0by	70.0az
Slow imbibition	**24**	Control	33.1ax	43.5ay	46.9ay	79.0bz
		Apron® Star	38.0bw	40.8ax	50.1aby	71.6abz
		Apron® XL	33.3aw	41.7ax	59.3by	66.4az
		Celest® XL	35.7aw	48.3bx	54.6by	67.5az
		Thiram	33.7aw	40.6aw	49.2ay	65.2az

AA= Accelerated ageing

* Means within a column not followed by the same letter are significantly different (P = 0.05)

** Means within a row not followed by the same letter are significantly different (P = 0.05)

After 3 months storage the percentage weight increase reflected the standard germination results. The untreated control had the lowest percentage weight increase of the seeds (46.9%) (Table 5.2) and this is reflected in the standard germination test where the control had the highest percentage germination (71.5%) (Table 5.1). After 3 months storage seeds treated with Apron® XL and Celest® XL showed a higher percentage weight increase (59.3 and 54.6%) compared to the other treatments. There was an increase in imbibition damage following 6 months storage, and therefore increased water uptake (Table 5.2). For slow imbibition, seeds treated with Thiram had the lowest percentage weight increase (65.2%)

and this is the only fungicide treatment that maintained germination following 6 months storage.

5.3.2.2 Conductivity test

The trend shown in Table 5.3 indicates that as the period of ageing and storage of the seeds increases so does the leachate conductivity values. At 2 d AA, the untreated control had the highest (2404 $\mu Scm^{-1}g^{-1}$) conductivity reading. Seeds treated with Celest® XL had a lower conductivity value following 4 d AA and 3 months storage. After 6 months storage the trend with the leachate conductivity values mirrored that of the germination results in Table 5.1 where seeds treated with Thiram had the lowest value 1252 $\mu Scm^{-1}g^{-1}$ compared to the other fungicide treatments and the untreated control. From the standard germination results seeds treated with Thiram were the only treatment to have germinated when all the other treated seeds and the untreated control failed to germinate which is reflected by the high leachate conductivity values.

Table 5.3: The effect of accelerated ageing and storage on conductivity of untreated and fungicide/insecticide treated maize seed

	Conductivity ($\mu Scm^{-1}g^{-1}$)			
	Accelerated ageing		Storage	
	2 d AA	4 d AA	3 month	6 month
Treatments				
Control	2404*a**y	2412ay	2819ay	3455az
Apron® Star	1643cx	2049ay	2756ay	3041az
Apron® XL	1219dy	1170by	2139abz	2336bz
Celest® XL	1134dx	1571by	1464cy	2475bz
Thiram	2186abz	1796by	2556az	1252cy

AA= Accelerated ageing

* Means within a column not followed by the same letter are significantly different (P = 0.05)

** Means within a row not followed by the same letter are significantly different (P = 0.05)

5.3.2.3 Tetrazolium test

For the untreated control, there were no significant differences between the percentage seeds with living tissue following slow and rapid imbibition (Table 5.4). A similar trend shown by the leachate conductivity values was seen in the control and the treated seeds. There was a decrease in the percentage of seeds with living tissue following the ageing of the seeds and storage. After 6 month storage, seeds treated with Thiram had the highest percentage seeds with living tissue following slow (36%) and rapid (20%) imbibition.

Table 5.4: The percentage seeds with living tissue following accelerated ageing and storage of untreated and fungicide/insecticide treated maize seed

		Seeds with living tissue (%)			
		Accelerated ageing		Storage	
	Treatments	2d AA	4d AA	3 month	6 month
Slow imbibition	Control	54*cd**z	36y	20x	4aw
	Apron® Star	30bz	25byz	21bxy	17bx
	Apron® XL	64ez	40cdy	25cx	13bw
	Celest® XL	58dz	34cy	33dy	13bx
	Thiram	42bcy	42dy	25cx	36cy
Rapid imbibition	Control	52cdz	20by	8ax	4ax
	Apron® Star	21ayz	25bz	17by	4ax
	Apron® XL	36bz	20by	13aby	4ax
	Celest® XL	46cz	33cy	20bcx	4aw
	Thiram	50cz	12ax	25cy	20by

AA = Accelerated ageing

* Means within a column not followed by the same letter are significantly different (P = 0.05)

** Means within a row not followed by the same letter are significantly different (P = 0.05)

5.3.2.4 Cold test

Results from the cold test (Table 5.5) mirrored results from the standard germination test (Table 5.1). Seeds treated with Celest® XL had the highest percentage germination at 2 d AA (57%), however, it decreased to 20% at 4 d AA and failed to germinate following

storage at 3 and 6 months. Seeds treated with Apron® XL failed to germinate (Table 5.5) following 3 months storage as found in the standard germination test. After 6 months storage the results from the cold test reflected the standard germination results where seeds treated with Thiram was the only treatment that germinated (10%) (Table 5.5).

Table 5.5: The effect of accelerated ageing and storage on untreated and fungicide/insecticide treated maize seed subjected to the cold test

	Germination (%)			
	Accelerated ageing		Storage	
	2d AA	4d AA	3 month	6 month
Treatments				
Control	33*b**y	54dz	22bx	0aw
Apron® Star	40by	43cy	15bx	0aw
Apron® XL	20ay	10ax	0aw	0aw
Celest® XL	57dy	20bx	0aw	0aw
Thiram	38bz	19by	16bxy	10bx

AA= Accelerated ageing

* Means within a column not followed by the same letter are significantly different (P = 0.05)

** Means within a row not followed by the same letter are significantly different (P = 0.05)

5.4 Discussion

In contrast to findings of the previous study (Chapter Four), this study showed that ageing and storage of treated maize seeds does have an effect on their viability and vigour. In this current study the moisture content was higher for seeds that were subjected to stress conditions, where it increased from a range of 11.0-11.5% (results of Chapter Four) to a range of 11.7-12.5%. Albeit it within tolerance, higher moisture content of the seeds does influence germination. Abba and Lovato (1999) subjected maize seeds that were treated with a fungicide to accelerated ageing and storage at temperatures that ranged from 20-30ºC. Following AA moisture content of the seeds (both treated and untreated) increased from 10.5 to 17% (Abba and Lovato, 1999).

Following 3 months storage, results from this study showed that seeds treated with Apron® XL failed to germinate compared to the other seed treatments. Previous studies have confirmed that storage under stress conditions will result in decline in germination of low vigour seeds (Basu *et al*., 2004.). This is in agreement with Lugo and Leopold, (1992) and Simić *et al*. (2004). Lugo and Leopold (1992) showed that decline in maize seed vigour is closely related to the decline of content of several sugars in the embryo under accelerated conditions. In the study conducted by Simić and co-workers, the vigour test following accelerated ageing test has proven its potential for predicting seed storability.?

In a study conducted by Basu *et al*., (2004) physiologically mature maize seeds were subjected to accelerated ageing and natural ageing. Results showed that accelerated ageing was effective in predicating the influence of natural ageing over time on the maize seeds (Basu *et al*., 2004). This was seen in this current study in the case of seeds treated with Apron® XL. The higher decrease in germination following 4 d AA (standard germination and the cold test) was indicative of the extreme decrease in germination following 6 months storage. None of the other treatments and the untreated control showed that trend.

Following the harsh conditions that the seeds were stored under, the other seeds have deteriorated (visually) and failed to germinate. In contrast seeds treated with Thiram consistently proved (germination following imbibition, cold test and the leachate conductivity value) to have higher vigour than the other seed treatments. The function of Thiram functioned was to protecting the seed from loosing vigour and viability to a greater extent than the other treatments. Treated maize seeds are expected to withstand the cold test as the soil for the test is obtained from a maize field. However, as the storage of these seeds favour deterioration and proliferation of storage fungi (Qasem and Christensen, 1958), these seeds are already damaged before the onset of this test. In this study all the seeds were exposed to the same pathogen density. It is possible that as Thiram is a broad-spectrum contact fungicide (http://www.syngenta.com), it may have protected the seeds from damage by storage fungi and hence from pathogens found in the soil.

For 2d AA, the percentage germination after 40 hr following slow imbibition ranged from 53-58%. Interestingly seeds treated with thiram and the untreated control had the lowest

percentage germination. Following 3 month storage, seeds treated with thiram were the only seeds that germinated following both slow and rapid imbibition. The low germination results under the low water stress (slow imbibition) was explained by Perissé and co-workers (2002) as the threshold of water content required in the embryo as a requisite for the initiation of cell elongation and radicle emergence. The ageing process is accompanied by alterations in the mitochondrial activity of the cells, which in turn affects respiration and consequently germination (Dreyer and van de Venter, 1992).

The cold test is normally considered to be the best for predicting field emergence (Lovato and Balboni, 2003, Basu *et al*., 2004). Results from this current study showed that the cold test results corresponded to the standard germination results. Woltz *et al*. (1998) conducted standardization studies on the cold test for maize. The test was conducted in 20 laboratories where five seed lots were tested. Some of these seed samples tested consisted of Thiram treated maize and untreated seeds. Thiram was able to protect the seeds and was not affected by the range of species of soil-borne pathogens present (Woltz *et al*., 1998). Although the cold test is recommended for maize, difficulties in standardizing the test occur with differences in pathogen levels, pH, moisture etc (Nijënstein and Kruse, 2000). Suggestions from their study included conducting a standard germination test with the cold test (Nijënstein and Kruse, 2000). Results from this current study proved that that is a good comparison where cold test results linked well with the standard germination results.

In this study there was a distinct influence of AA and long-term storage on the viability and vigour of fungicide treated seeds. Wilson *et al*. (1992) showed that the accelerated ageing, leachate conductivity and other vigour tests were combined to develop a prediction of final stand in sweet corn (Wilson *et al*., 1992). Due to climatic stress high vigour lots do not necessarily give high yield (http://www.ag.ohio-state.edu) and storage for more than 4 months is not recommended according to findings of Abba and Lovato (1999). Seeds treated with Thiram, as in this study, maintained viability even after accelerated ageing and storage under sub-optimum condition. The conditions tested in this study were very harsh and even if subsistence farmers treated their seeds and stored them at sub-optimum conditions (which are less harsh than the conditions tested here), a small percentage of the seeds would still germinate and could be used for planting. The next step is to test these

seed treatments and the untreated control under greenhouse conditions. The vigour test results can then be compared to the emergence in the greenhouse. In addition, the seed treatments can be tested against a pathogen of maize under greenhouse conditions.

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CHAPTER SIX

GREENHOUSE EMERGENCE OF UNTREATED AND TREATED MAIZE SEED AND CONTROL OF *FUSARIUM GRAMINEARUM* (SCHWABE)

Abstract

Seed treatments are important in protecting seeds from diseases and insects prior to and after planting and during storage. Apart from being the casual agent of seedling blight, *Fusarium graminearum* (Schwabe) is also a serious storage fungus, producing mycotoxins, which have deleterious health implications. The objective of the study was to investigate the effect of seed treatments on maize (*Zea mays* L.) seedling emergence and against *F. graminearum* under greenhouse conditions. Maize seeds were treated with Celest® XL [fludioxonil + mefenoxam], Apron® Star [thiamethoxam + metalaxyl–M + difenoconazole], Apron® XL [metalaxyl–M] and Thiram [thiram]. The control consisted of untreated seeds. Following treatment, seeds were subjected to 2 and 4 d accelerated ageing (AA) and 3 and 6 months storage. In the un-inoculated trial following 2 d AA, seeds treated with Thiram had the highest percentage emergence (70.7%) followed by Celest® XL (68%) and the untreated control (62.7%). Subjecting the treated seeds to stress conditions resulted in a decrease in emergence. Following the 6 months storage, only the control and seeds treated with Thiram germinated and had 1.3 and 6.7% emergence, respectively. Following inoculation, a similar trend was seen for seeds treated with Thiram and the untreated control. Seeds treated with Celest® XL had among the lowest percentage diseased seedlings (1, 2 and 10%) but failed to germinate at 6 months.

6.1. Introduction

Maize (*Zea mays* L.) is important as a source of energy and protein in the human diet throughout the world (Rehman, 2006). The loss of quality of maize seed is not only visually observed by the poor condition of the seed (Hell *et al*., 2000) but also by poor emergence (Cardwell *et al*., 2000).

One of the challenges facing the resource-poor smallholder farmers who produce the bulk of Nigeria's maize is how to preserve the quality of the grains in storage. Maize can be contaminated in the field and in the store where kernels are subject to infection by a variety of toxigenic fungi (Cardwell *et al*., 2000). The most common genera are *Aspergillus*, *Penicillium* and *Fusarium* that produce the aflatoxins, fumonisins and other mycotoxins that have important economic impact on the grain industry and risks to human and animal health (Bradley *et al*., 2001). Fungi of the genus *Fusarium* colonize various host plants, including crops that are essential for human nutrition such as maize and wheat (Klix *et al*., 2007). Within the *Fusarium* complex, *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae* ([Schw.] Petch) has been reported to be the dominant species (Klix *et al*., 2007).

Fungicide seed dressings were found to reduce deterioration of seeds that are stored (Adebisi *et al*., 2004). Fungicide formulations that are not compatible with the seeds, could alter membrane function, altered membrane function can result in reduced seed and seedling performance (Chen and Burris, 1993). Fungicide seed treatments have been used with success. In a study conducted by Leishman *et al*. (2000), two climatic conditions were chosen (wet winter and summer) , treated and untreated *Medicago* seeds were buried in the test field in those conditions. Fungicide treated seeds remained viable longer in soil (Leishman *et al*., 2000). The active ingredients of the fungicides used in this study have previously been effective in improving germination. Difenoconazole has previously been shown to improve seed germination of wheat (*Triticum aestivum* L.) (Allen *et al*., 2004) and maize (Munkvold and O'Mara, 2002). Maize seeds treated with Apron® XL increases yield and vigour and therefore fewer seeds are destroyed (www.syngenta.com). With Thiram, improved emergence and high plant stand in the fungicide/insecticide mixture treatments compared to the untreated control could have resulted from control of seed rot and pre-emergence damping-off diseases reported earlier (Ahmed *et al*., 2001).

Fungicide seed treatments do not affect germination and plants develop as normal (Tort *et al*., 2006). This was confirmed in this study where the effect of fungicides were tested on unstored seeds. The objective of the current study was to investigate the effect of pesticide

seed treatments on maize (*Zea mays* L.) seed emergence and their effectiveness against *Fusarium graminearum* (Schwabe) under greenhouse conditions.

6.2. Materials and methods

6.2.1 Treatment of seeds

Untreated seed and the chemicals, Celest® XL [*fludioxonil (25 g ai/L) + mefenoxam (10 g ai/L*], Apron® Star [*thiamethoxam (20% w/w)+ metalaxyl–M (20% w/w) + difenoconazole (2% w/w)*], Apron® XL [*metalaxy–M (350 g ai/L*] and Thiram [*thiram (50.0% m/m)*] were supplied by Syngenta® Pty. Ltd (Midrand, South Africa). Seeds were treated as discussed in Chapter Four (4.2.1). After treatment, the seeds were left on paper towels in a laminar flow cabinet to dry. Once the seeds had dried, they were divided into three batches (1: immediate use; 2: 2 and 4 day accelerated ageing (AA) and 3: 3 and 6 months storage). This chapter focuses on the seeds that were aged and stored.

6.2.2 Greenhouse trial

6.2.2.1 Preparation of the pathogen

Fusarium graminearum (CAMS 1256) was obtained from the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria. This fungus was initially isolated from a maize plant. The fungus was cultured on potato dextrose agar (PDA) and incubated at 25°C for seven days (12 hr day/night cycle).

6.2.2.2 Inoculation of the pasteurised soil

The seedling trays were cleaned using 2% sodium hypochlorite and left to dry a day before they were filled with pasteurised soil (Braaks, Pretoria). The filled trays were watered until run-off the day before inoculation. A cork borer (diameter of 5 mm) was used to remove mycelial plugs from the actively growing cultures. Two mycelial plugs were inoculated per cell of the seedling tray with the mycelial plugs being placed on opposite ends of a single cell. Inoculation was done prior to planting. Maize seeds were sown the next day in the space between the two plugs. Four replicates of 25 seeds were used per treatment. Each tray had three different treatments in a random block design. The temperature within the greenhouse ranged from 25-30ºC. The trays were monitored regularly and were watered daily. The trial was terminated three weeks after planting and the results were expressed as

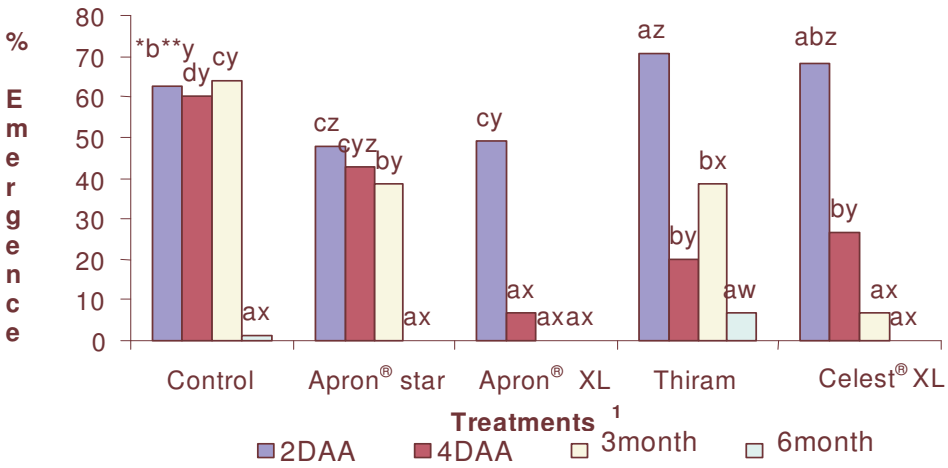
the percentage seedling emergence and percentage diseased seedlings at the end of the test period.

6.2.3 Statistical analysis

Two-way analysis of variance (ANOVA) was performed on all data and least significant differences (P= 0.05) were determined according to the student's t test.

6.4 Results

Comparing the 2 d AA, seeds treated with Thiram had the highest percentage emergence (70.7%) (Fig. 6.1). This was followed by seeds treated with Celest® XL (68%). The untreated control had the third highest emergence and did not differ significantly from Celest® XL. It did, however, differ significantly from the other treatments. For 4 d AA, seeds treated with Thiram had a 50% decrease in emergence followed by Celest® XL which had a 41.3% decrease in emergence (Figure 6.1).



[1]Each value is a mean percentage of four replicates of 25 seeds that have emerged in the greenhouse; Means above the bars not followed by the same letter are significantly different (*P = 0.05*). *Means are being compared amongst treatments. **Means are being compared within storage conditions (2 and 4d accelerated ageing or 3 and 6 months long-term storage).

Fig. 6.1: Emergence of un-inoculated untreated and treated maize seeds following accelerated ageing and long- term storage.

The untreated control had the highest (60%) emergence and differed significantly from the treatments. Apron® XL had the lowest emergence, already decreasing to 6.6% after 4 d AA. After 3 months storage, the control once again had the highest percentage emergence (64%). Seeds treated with Apron® Star and Thiram had a 38.7% emergence and did differ significantly from the other treatments and the control. Apron® XL failed to germinate (Fig. 6.1). After 6 months storage, with the exception of seeds treated with Thiram, the rest of the treatments failed to germinate and had 0% emergence. The control and seeds treated with Thiram had emergence of 1.3 and 6.7%, respectively (Fig. 6.1).

Table 6.1: Emergence of untreated and treated maize seeds following inoculation with *Fusarium graminearum*

	Emergence (%)[1]			
	2d AA	4d AA	3 month	6 month
Treatments				
Control	57.3 *bc**z	52.0 dz	44.0 dy	2.7 abx
Apron® Star	46.7 abz	34.7 cy	22.7 bx	0.0 aw
Apron® XL	40.0 ay	5.3 ax	0.0 ax	0.0 ax
Celest® XL	64.0 cz	28.0 bcy	4.0 ax	0.0 ax
Thiram	58.7 bcz	17.3 bx	33.3 cy	4.0 bw

[1]Each value is a mean percentage of four replicates of 25 seeds that have emerged in the greenhouse; *Means within a COLUMN not followed by the same letter are significantly different ($P = 0.05$); **Means within a ROW not followed by the same letter are significantly different ($P = 0.05$)

Emergence following inoculation with *.F. graminearum* showed a difference in the results. After 2 d AA, seeds treated with Celest® XL had the highest percentage emergence (64%) (Table 6.1). Seeds treated with Thiram and the control had the second and third highest percentage emergence (58.7 and 57.3%, respectively) and did not differ significantly from seeds treated with Celest® XL. Following 4 d AA, control had the highest percentage emergence and differed from all the treatments. Seeds treated with Thiram once again, had a decrease in the percentage emergence of 41.4%, although Apron® XL dropped to 5.3% emergence (Table 6.1). After 3 and 6 months, a similar trend was seen, with the control having the highest percentage emergence (44%) and seeds treated with Apron® XL failing

to germinate after 3 months. Only the control and seeds treated with Thiram had germinated after 6 months storage (Table 6.1).

In the inoculated trial following 2 d AA, the untreated control had the highest percentage diseased seedlings (17%) (Table 6.2) and there was no significant differences between the treatments. Following the 4 d AA, the control once again had the highest percentage diseased seedlings (11%). Both seeds treated with Apron® XL and Celest® XL had lower percentage diseased plants (2.0%) and only differed significantly from the control (Table 6.2). After 3 months storage the untreated control had the highest percentage diseased seedlings (10.0%), followed by Apron® Star (6.0%). Seeds treated with Celest® XL had the lowest percentage diseased seedlings (1.0%). After 3 months storage, seeds treated with Apron® XL failed to emerge. Following 6 months storage only the control and seeds treated with Thiram had seedlings that emerged. The control had 1.0% diseased seedlings, with Thiram having no diseased seedlings (0.0%).

Table 6.2: Percentage of diseased seedlings, following inoculation with *Fusarium graminearum*

	Diseased seedlings (%)[1]			
	2d AA	4d AA	3 month[2]	6 month[2]
Treatments				
Control	17.0 *by	11.0 by	10.0 by	1.0ax
Apron® Star	10.0 abx	7.0 abx	6.0 abx	-
Apron® XL	9.0 ay	2.0 ax	-	-
Celest® XL	10.0 aby	2.0 ax	1.0 ax	-
Thiram	10.0 abx	6.0 abx	4.0 abx	0.0 a

[1]Each value is a mean percentage of four replicates of 25 seeds that have emerged in the greenhouse; [2] - = no emergence. *Means within a COLUMN not followed by the same letter are significantly different (*P = 0.05*); **Means within a ROW not followed by the same letter are significantly different (*P = 0.05*).

6.5 Discussion

In this study the trend in emergence followed a similar pattern as was noted with germination in the previous study (Chapter Five). Seeds that were treated and processed immediately (Chapter Four), showed no significant differences from the control. Following accelerated ageing and storage, a decline in emergence was seen. This was in contradiction to what Adebisi *et al.* (2004) found in their study where fungicide treated soybean seeds had a significantly longer storage life than untreated seeds. The results obtained for the 4 d AA, 3 and 6 months storage could be as a consequence of the way in which the seeds were treated.

Although the control had no protection by any fungicide following inoculation with *F. graminearum,* it had the highest percentage emergence after 2 and 4 d AA and 3 months storage. After 6 months storage, seeds treated with Thiram had the highest percentage emergence. The results for the untreated control can be explained by the vigour test of the previous chapter. Following imbibition, the untreated control and the seeds treated with Thiram had a lower percentage weight increase, and thus less imbibition damage, compared to the other treatments and were able to emerge despite the stress conditions the seeds were subjected to.

Emergence of seeds treated with Apron® XL decreased following ageing, but already after 3 months storage there was no emergence. These results mirrored those of the germination tests in the previous chapter (Chapter Five). Apron® XL is known to increase yield and vigour (www.syngenta.com), which was evident in the samples that were processed immediately. Results for the inoculated trial for the aged and stored seeds showed a similar trend as the un-inoculated seed where following 3 and 6 months storage Apron® XL failed to germinate.

Thiram has proved to be an effective fungicide providing protection to vegetable crops (Maude, 1977) and was the most important alternative to captan as a seed treatment fungicide for maize (Kommedahl and Windels, 1986). In this study Thiram treated seed still germinated and emerged even after 6 months storage in comparison to the other treatments with the exception of the untreated control. The thiram treatment alo had a

lower level of disease than the inoculated untreated control after being subjected to the harsh 6 month storage period. something about controlling storage fungi. This reiterated the protective nature of Thiram (Maude, 1977, Falloon, 1982). Some fungicides are able to improve germination, not emergence (Gilbert *et al.*, 1997). In this current study Thiram improved emergence and protected the seed in the inoculated trial.

With seeds treated with Celest® XL there was an obvious decrease in emergence following ageing and storage. A similar trend was noted in the inoculated trial. The trend in the control of *F. graminearum* found in this study with Celest® XL confirmed results obtained from other studies. In a study by Broders *et al*. (2007) seed treatment fungicides azoxystrobin, trifloxystrobin, fludioxonil and captan were tested for their effectiveness against *F. graminearum* on soybean seeds and seedlings. Of the fungicides tested, it was only fludioxonil that provided sufficient inhibition of mycelial growth *in vitro* (Broders *et al*., 2007). A similar result was found against *F. graminearum* on maize (Munkvold and O' Mara, 2002).In another study, fludioxonil was found to reduce certain parameters associated with the disease in barley, including incidence, severity, and deoxynivalenol concentration, while increasing the percentage of plump kernels and yield (Jones, 2000).

In terms of disease control, fungicides that are registered for use on maize differ in their effectiveness against certain diseases, depending on their active ingredients and will protect the seeds under field conditions and allow for emergence of the seedlings (Van Dyk, 2000). In terms of fungicide performance, Thiram, a broad-spectrum fungicide, effectively controlled *F. graminearum* whilst insuring some emergence. In the greenhouse trials, Thiram was found to be the best treatment in this study. The results for the vigour tests of the previous chapter have been substantiated by the results from this chapter. .

6.6 Literature cited

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