The influence of chemical seed treatment on germination, seedling survival and yield of canola

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Declaration

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

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Abstract

The influence of chemical seed treatments on the germination, seedling survival and yield of canola (cv. Varola 44) was investigated in a series of incubation studies, glasshouse experiments, as well as field trials in the canola producing areas in the Western Cape Province.

Incubation experiments were conducted to compare germination and seedling growth of untreated (control) seed with that of seed treated at different application rates (0.5, 1.0 and 2.0 times the recommended) of Cruiser® and SA-combination (which consists of Thiulin® at 0.5g a.i.; Apron® at 0.0815 g a.i.; Gaucho® at 0.6125 g a.i. and Rovral® at 0.9975 g a.i.). The results indicated that seed treatment (all rates of SA-combination and highest rate of Cruiser) delayed germination and seedling growth, especially if the seed was subjected to the Accelerated Ageing Test.

Glasshouse studies with pasteurised soil at different water contents, seed sources (storage periods) and planting depths confirmed the phytotoxic effects of the chemical seed treatments in the absence of soil borne pathogens. From the results it became clear that extreme water conditions (very wet or dry) increased the suppressing effect on germination and seedling growth, but that no phytotoxic reactions occurred in moist (favourable soil water conditions) soil, regardless of application rate of the chemicals used, planting depth and seed source. In a second glasshouse experiment conducted in moist soil (kept at 50% of field water capacity to prevent any toxic effects) from seven different localities that were naturally infested with

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pathogens, both chemicals proved to be effective where soil borne pathogens (*Rhizoctonia solani* and *Pythium* spp.) occurred. No clear trend could however be found due to either chemical or application rates used.

Finally, field trials were conducted to study the effect of chemical seed treatments on the plant populations and yield of canola planted in different row widths (17 and 34 cm) and seeding rates (3, 5 and 7 kg.ha⁻¹). Results showed that treated seeds produced more plants.m⁻² and yielded more than untreated seeds at Roodebloem Experimental Farm, while the highest seeding rate produced significantly more plants.m⁻² (Roodebloem and Langgewens Experimental Farms), but not significantly higher yields than the lowest seeding rate at the same locality. Although row width did not have an effect on plant population, yield (Roodebloem 2003) was significantly less at the wider (34 cm) rows. As in earlier experiments, no consistent differences between the two chemicals used were found.

These results clearly illustrated both the negative (in the absence of pathogens) and positive (where soil borne pathogens do occur) effects that chemical seed treatments may have on the germination, seedling growth and even yield of canola under local environmental and soil conditions. Because no significant differences were found between the chemicals used, both chemicals should be regarded as efficient. More research, especially under field conditions and with more cultivars, is needed before the registration of a chemical for seed treatment could be considered.

Uittreksel

Die invloed van chemiese saadbehandeling op die ontkieming, saailing oorlewing en opbrengs van canola (cv. Varola 44) is ondersoek in 'n reeks inkubasie studies, glashuis eksperimente en veldproewe in die canola-produserende gebiede in die Wes Kaap Provinsie.

Inkubasie eksperimente is uitgevoer om die ontkieming en saailing groei van onbehandelde (kontrole) saad te vergelyk met dié van saad wat behandel is teen verskillende dosisse (0.5, 1.0 en 2.0 keer die aanbevole) van Cruiser® en SA-kombinasie (wat bestaan uit Thiulin® teen 0.5g a.i.; Apron® teen 0.0815 g a.i.; Gaucho® teen 0.6125 g a.i. en Rovral® teen 0.9975 g a.i.). Die resultate het aangedui dat saadbehandeling (vir alle dosisse van SAkombinasie en die hoogste dosis van Cruiser) ontkieming en saailing groei vertraag, veral wanneer die saad onderwerp was aan die Versnelde Verouderings Toets. Glashuis studies met gepasteuriseerde grond by verskillende waterinhoude, saad bronne (stoor periodes) en plantdieptes, het die fitotoksiese effekte van die chemiese saadbehandelings bevestig in die afwesigheid van grondgedraagde patogene. Vanuit die resultate het dit duidelik geword dat ekstreme water toestande (baie nat of droog) die onderdrukkende effek op ontkieming en saailinggroei verhoog het, maar dat geen fitotoksiese reaksies plaasgevind het in klam (gunstige grondwater toestande) grond nie, ongeag die dosisse of chemikalieë gebruik, plantdiepte en saad bron. In 'n tweede glashuis eksperiment uitgevoer in klam grond (gehou by 50% van veldwaterkapasiteit om toksiese effekte te voorkom) van

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sewe lokaliteite wat natuurlik besmet was met patogene, was beide chemikalië effektief waar grondgedraagde patogene (*Rhizoctonia solani* en *Pythium* spp.) voorgekom het. Geen duidelike tendens is egter waargeneem vir enige van die chemikalieë of dosisse nie.

Laastens is veldproewe uitgevoer om die effek van chemiese saadbehandelings op plant populasies en opbrengs te bepaal van canola geplant in verskillende rywydtes (17 en 34 cm) en saaidigthede (3, 5 en 7 kg.ha-1). Resultate het aangedui dat behandelde saad meer plante.m-2 produseer en 'n groter opbrengs lewer as onbehandelde saad by Roodebloem Eksperimentele Plaas, terwyl die hoogste saaidigtheid betekenisvol meer plante.m-2 (Roodebloem en Langgewens Eksperimentele Plase), maar nie betekenisvol hoër opbrengste gelewer het as die laagste saaidigtheid by dieselfde lokaliteit nie. Al het rywydte nie 'n effek op plant populasie gehad nie, was opbrengs (Roodebloem 2003) betekenisvol minder by die wyer (34 cm) rye. Soos in vroeëre eksperimente is geen konsekwente verskille tussen die twee chemikalieë gevind nie.

Hierdie resultate illustreer duidelik beide negatiewe (in die afwesigheid van grondgedraagde patogene) en positiewe (in die aanwesigheid van grondgedraagde patogene) effekte wat chemiese saadbehandelings op ontkieming, saailing groei en selfs opbrengs van canola onder plaaslike omgewings en grondtoestande kan hê. Omdat geen betekenisvolle verskille tussen die chemikalieë gevind is nie, moet beide chemikalieë as doeltreffend aanvaar word. Meer navorsing, veral onder veldtoestande en met meer kultivars, is egter nodig voordat die registrasie van 'n chemiese middel vir saadbehandeling oorweeg kan word.

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Language and style used in this thesis are in accordance with the requirements of the South African Journal of Plant and Soil. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some redundancy between chapters has, therefore, been unavoidable.

Chapter 1

Introduction

Canola oil is a healthy choice for human nutrition and also a good source of protein in animal feed. Canola oil contains three nutrients *viz.* oleic acid, alphalinolenic acid (an essential omega-3 fatty acid) and vitamin E which makes it crucial in the prevention of coronary heart disease and stroke (McDonald *et al.*, 1989).

Canola oil is a soft oil (oil that is liquid at room temperature) and the market is typically bottled oil for household use, soft margarine, mayonnaise, salad oil and various industrial uses (Hunter, 1990). A huge market for this soft oil and the good competitive price of canola (as a result of the large quantities of imported oils, the international oilseed prices largely determine the local prices of canola and due to the fact that local demand for canola exceeds the local supply, with expected favourable prices due to this trend (National Department of Agriculture, 2003)), makes it a very popular choice as rotation crop.

Apart from high economic value, canola also have agronomic benefits, and is therefore a crucial part of crop rotation. The inclusion of canola as a break-crop in cereal rotation may become an integral part of crop production in the winter rainfall area of the Republic of South Africa. As a break-crop, canola reduces the risk associated with continuous grain cropping because of the build up of disease organisms (Bailey *et al.*, 2000) and insect populations which may reduce yields with 30-50% (Zhou *et al.*, 1999). These benefits arise largely from reduced

disease, specifically take-all (Smith, Kirkegaard & Howe, 2004), which declines in the absence of cereal hosts. Due to similar requirements as wheat, canola cultivation provides optimal usage of soil, machinery and labour. Results of crop rotation studies in the Great Plains revealed that where oilseeds are adapted, their inclusion in rotation with cereals could increase net returns and reduce risk through improved production stability (Lafond *et al.*, 1993). In addition, the yield of wheat was increased when following oilseeds in rotation, confirming that monoculture systems are the least effective means of optimising wheat production (Lafond, Loeppky & Derksen, 1992). In Southern Alberta winter wheat yields were increased by 25% when grown on canola vs. winter wheat stubble (Larney & Lindwall, 1994).

In spite of these advantages, the area under canola in the Western- and Southern Cape is very limited (44 200 ha canola plantings in the 2003/04 season versus 325 000 ha wheat plantings in the 2003/04 season (National Department of Agriculture, 2003)). One reason for this is the poor yields achieved with canola in these areas (average of 0.89 t/ha over the last five seasons (National Department of Agriculture, 2003)), which may cause producers to refrain from planting canola on larger scale.

To sustain the production of canola in the Western Cape, it has become necessary to improve yields achieved by canola. One of the main reasons identified for the poor yields is uneven and patchy canola stands. An even establishment is necessary so that seedlings emerge uniformly to ensure uniform ripening with minimum seed losses (Loof, 1972). There are a few factors

contributing to/causing these uneven and patchy canola stands, which can be due to seed quality, environmental conditions, production techniques and biological factors.

Seed quality measured in seed and seedling vigour can decrease due to the wrong application of chemical seed treatments (Ashley *et al.*, 2003), or the use of seed containing seed borne pathogens. Delayed emergence due to poor seed quality can further be enhanced due to unfavourable environmental conditions (Day, 2000; Johnston *et al.*, 2002) and biological effects (Oerke & Dehne, 2004).

Production techniques like depth of sowing, row width and seeding rate are some controllable aspects which can lead to an uneven and patchy stand if not implemented correctly. An uneven depth of sowing can result in non-uniform emergence and ripening of canola thus limiting yields. Low seeding rates and wide rows cause low plant density and increased weed infestation with consequent decreased canola yields (Pouzet, 1995). Environmental conditions affecting emergence comprise factors such as temperature and water (Mendham & Salisbury, 1995). Soil temperature is closely connected to the soil water levels. High soil water results in lower temperature, delaying germination and seedling emergence while favouring certain disease-causing organisms like *Pythium* (Agrios, 1978). The opposite can also be true as dry soils can result in slightly higher soil temperatures, again delaying germination and seedling emergence while favouring certain disease-causing organisms like *Fusarium* (Klaasen *et al.*, 1991). Dry conditions can also promote the formation of a surface crust, thereby preventing seedlings from emerging (Pouzet, 1995).

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Environmental conditions, seed quality and production techniques are not always a direct yield limiting factor in the Western and Southern Cape of South Africa. Environmental conditions and seed quality rather has an indirect effect through the favouring of disease-causing organisms which directly cause yield losses. Therefore, for the purpose of this study, seedling diseases are regarded as the most important factor causing uneven and patchy canola stands, which consequently cause yield reductions. Pre- and post emergence damping-off, seedling blight, and brown girdling root rot (BGRR) are important diseases of canola in South Africa and other countries (Gugel et al., 1987), and can cause annual yield losses of greater than 20% (Sippel et al., 1985; Yang & Verma, 1992). This seedling disease complex is associated with various fungi also pathogenic to other crops, viz. Fusarium spp. (Calman, Tewari & Mugala, 1986), Pythium spp. (Gugel et al., 1987; Bartlett & Martin, 1995) and Rhizoctonia spp. (Kataria & Verma, 1992; Khangura, Barbetti & Sweetingham, 1999) while the leaf feeding insects which attacks the newly emerged seedlings includes the red legged earth mite (Halotydeus destructor), the blue oat mite (Penthaleus major) (Robinson & Hoffman, 2000) and the flea beetle (*Phyllotreta* spp.) (Lamb, 1984).

A possible solution for these seedling losses is the application of a chemical seed treatment to protect the seed and seedling against pathogens and insects in the early growth stages of the seedling. Kataria & Verma (1993) and Kataria, Verma & Rakow (1993) proved that damping-off and seedling root rot caused by *Rhizoctonia solani*, could almost completely be controlled by seed treatments with fungicides and also fungicide and insecticide combinations. Chemical seed

treatments also proved effective against diseases of other crops such as narrow-

leafed lupine (Sweetingham, 1999) and snap bean (Keinath et al., 2000). Seed

treatment is further more environmentally friendly and in this respect rather

preferred to foliar sprays (Marley & Adeoti, 1995).

The implementation of this solution can be of great importance to the oil

industry in South Africa by reducing their dependency on imported plant oils (524

000 tons in 2001/02) and oil-cake (738 000 tons in 2002/03) (National

Department of Agriculture). The greater use of locally produced oil products can

have a stabilizing effect on the local prices, because of the exclusion of price

fluctuation due to the changing exchange rate.

Objectives of this study

To evaluate the use of chemical seed treatments, at different rates,

environmental- and soil conditions as well as different production techniques, to

possibly register an effective chemical seed treatment as a means of achieving

even canola stands and thereby prevented yield losses in the Western Cape

Province.

The main objectives of this study were:

To determine and compare the phytotoxic effects of two different chemical

seed treatments, at different application rates, on the germination and seedling

vigour of canola under favourable as well as unfavourable environmental

germination conditions in incubation studies.

- To evaluate two different chemical seed treatments (in the absence of disease), at different application rates, soil water contents, planting depths and times of chemical application, for phytotoxic effects in glasshouse studies at optimal temperature conditions.
- To evaluate the effectivity and possible phytotoxicity of two different chemical seed treatments, at different application rates and in unsterilised soils from different localities, when planted in optimal (controlled) conditions (according to above-mentioned objective) in a glasshouse.
- To determine the effectivity of two different chemical seed treatments to control disease and ensure even seedling emergence when planted at different row widths and seeding rates under natural environmental conditions in field trials.

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Chapter 2

Effect of chemical seed treatment on the germination and seedling vigour of canola

Abstract

Incubation studies were conducted to determine phytotoxic effects of chemical seed treatments on the germination and seedling vigour of canola (cv. Varola 44) seed. The incubation studies comprised germination of untreated (control) and treated seeds under favourable temperature and water conditions as well as germination (seed vigour) and growth (seedling vigour) of untreated and treated seeds after being subjected to high humidity and temperature during the accelerated ageing test. The seeds were treated with Cruiser (thiamethoxam, fludioxonil and metalaxyl) and a combination of chemicals (referred to as SAcombination) which consists of Thiulin® (thiram), Apron® (metalaxyl), Gaucho® (imidachloprid) and Rovral® (iprodione). Germination percentages of the treated seeds were not significantly reduced compared to untreated seeds (control) under favourable temperature and water conditions. After exposure to high temperature (41°C) and humidity to accelerate ageing of the seed, germination and seedling vigour were reduced by both seed treatments. In contrast to differences in germination which decreased with time of incubation, the effect of both chemical treatments on seedling vigour increased as the incubation period increased. As expected, phytotoxic effects increased with increasing application rates, but significant differences in germination percentage and seedling growth

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between similar rates of the two chemical treatments indicated that the SA combination treatment was more phytotoxic than Cruiser.

Introduction

Canola (*Brassica napus* var. *oleifera*) as a catch crop may become an integral part of crop rotation systems with wheat in the winter rainfall area of the Republic of South Africa. The most important advantages of wheat rotation with canola are the control of grass weeds in the canola season as well as a reduction in disease infections of both crops (Smith, Kirkegaard & Howe, 2004). This will contribute to more sustainable and environmentally friendlier production systems.

Although canola is currently highly valued due to its value as plant oil and protein source for both human and livestock consumption, low yields of about 0.86 tons per hectare on average (National Department of Agriculture – 2003 yield estimates) may not be economically viable and may cause producers to refrain from planting canola. Low mean yields are partly due to the thin and patchy plant populations of canola, which may be the result of seedling diseases and leaf feeding insects during early growth stages.

Seedling death of canola in South Africa and other countries is associated with various fungi also pathogenic to other crops, viz. *Fusarium* spp. (Calman, Tewari & Mugala, 1986; Šrobárová & Eged, 1992), *Pythium* spp. (Gugel *et al.*, 1987; Pemberton *et al.*, 1990; Huang *et al.*, 1992; Bartlett & Martin, 1995) and *Rhizoctonia* spp. (Yitbarek *et al.*, 1988; Kataria & Verma, 1992; Khangura, Barbetti & Sweetingham, 1999; Auret, Janse van Rensburg & Lamprecht,

unpublished report to Protein Research Foundation, 2002). These pathogens can cause yield losses greater than 20% (Sippel, Davidson & Sadasivaiah, 1985; Yang & Verma, 1992; Mikkelson et al., 2003). Leaf feeding insects like the redlegged earth mite (*Halotydeus destructor*) and the blue oat mite (*Penthaleus major*) (Robinson & Hoffman, 2000) as well as the flea beetle (*Phyllotreta* spp.) (Lamb, 1984) attack newly emerged canola seedlings by chewing pits in the cotyledons, leaves and stems. This reduces the leaf area available for photosynthesis and disrupts transpiration, which can lead to wilting and death of the seedlings especially under dry conditions (Dosdall et al., 1999). The growth and development of the seedling is delayed and the growing season extended resulting in limited rainfall and yield is affected negatively (Brennan & Grimm, 1992). Less severe damage can result in yield loss, reduction of seed quality by delayed plant maturation, and reduced plant height (Weiss et al., 1991).

The establishment problem (thin and patchy plant populations) in canola may possibly be partly solved by chemical seed treatments which contain or eliminate these insect pests and seedling diseases as found with wheat (Marley & Adeoti, 1995). Contact or systemic fungicides can be used to protect seedlings against pathogenic fungi. Contact fungicides destroy fungal propagules carried on the seed surface, while systemic fungicides are active against pathogens carried inside the seed and offer post-emergence protection. The soil borne fungi Fusarium and Rhizoctonia can be contained by the long-established contact fungicides, dithiocarbamate, captan, thiram and pentachloronitrobenzene, all of which are used on a wide range of crops (Powell & Matthews, 1988). The

systemic fungicide carboxin is used for treatment against *Rhizoctonia*, while metalaxyl is active against the Phycomycetes such as *Pythium* (McGee, 1995). Benomyl (Benlate®) is also used as a seed treatment against seedling diseases (Buntin, 1997). Imidachloprid (Gaucho 75ST®) is a registered seed treatment in the USA for flea beetles and mites on canola (Buntin, 1997).

Fungicide treatments must be applied to each seed uniformly at the recommended rate to provide early protection from seed- and soil borne diseases. Application of seed treatments above labelled rates can injure germinating seeds and plant stands of crops like wheat, possibly reducing yields or producing yields no higher than those produced by untreated seeds (Ashley et al., 2003).

Phytotoxic effects together with adverse temperature and water conditions increase the rate of seed deterioration and ageing. The symptoms of ageing include reduced rates of germination and emergence, decreased tolerance to sub-optimal conditions, and inferior seedling growth (Powell, Matthews & Oliveira, 1984). The accelerated ageing test (Delouche & Baskin, 1973) exposes seeds for short periods to the two environmental variables which cause rapid seed deterioration; high temperature and high relative humidity. High vigour seed lots will withstand these extreme stress conditions and deteriorate at a slower rate than low vigour seed lots (Hampton & TeKrony, 1995).

The aim of this investigation was to determine whether seed treatments impede seed and seedling vigour of emerging seedlings. Currently there is no chemical seed treatment product registered in South Africa for the control of

insects and diseases affecting canola. The result of this study may also

contribute to the registration of such a product.

Materials and methods

2001), and

Three incubation experiments were conducted under temperature-controlled conditions to determine the influence of different chemical seed treatments on the seed- and seedling vigour of canola (cv Varola 44).

Two chemical products were used, namely:

(i) Cruiser® (3.75 ml 250 g⁻¹ of canola seed) which is registered as a seed treatment for canola in Europe and Brazil (also known as Helix® in the USA and Canada) to control *Fusarium* spp., *Pythium* spp. and *Rhizoctonia solani*, as well as flea beetles and aphids (Paulsrud *et al.*,

(ii) A combination product (referred to as SA-combination) which

consists of Thiulin® (0.5 g a.i.), Apron® (0.0815 g a.i.), Gaucho® (0.6125

g a.i.) and Rovral® (0.9975 g a.i.). Thiulin protects canola against seed-

and soil borne fungi (Rhizoctonia solani and Fusarium spp.) which cause

seed decay, damping-off and seedling blight (Munkvold et al., 1999).

Apron is a systemic fungicide for control of Pythium spp. (Petch, Maude &

White, 1991; Paulsrud et al., 2001) and Rovral is a contact fungicide for

the control of Rhizoctonia solani (Kataria & Verma, 1992) and Fusarium

spp. Gaucho provides effective protection against damaging insects such

as leafhoppers, (Nault *et al.*, 2004) flea beetles, aphids and thrips (Ahmed *et al.*, 2001; Paulsrud *et al.*, 2001; Ester, De Putter & Bilsen, 2003).

After rinsing the seed with cold water and drying at room temperature, 250 g of the canola seed was moistened with 2.5 ml water in a closed container, before the chemical product(s), Cruiser or SA-combination, were added (individual components of SA-combination were added in no particular order) and the container shaken until all the powder was spread evenly over the seeds. The treatments were Cruiser (at labelled rate; 1.0X) at 3.75 ml 250 g⁻¹ of canola seed and SA-combination (1.0X) which consists of Apron at 0.25 g (0.0815 g a.i.), Thiulin at 1.0 g (0.5 g a.i.), Rovral at 3.92 ml (0.9975 g a.i.) and Gaucho at 0.875 g (0.6125 g a.i.) per 250 g of canola seed.

Experiment 1

The aim of this experiment was to determine the effect of the above-mentioned seed treatments under favourable water (moist) and temperature (20°C) regimes on the germination of the canola seed. After the chemical treatments were applied, 50 seeds per treatment were placed in filter paper lined Petri dishes (90 mm in diameter). To ensure moist conditions, the filter paper was moistened by 1.5 ml distilled water and the Petri dishes sealed in plastic bags before entering incubation in the dark.

The experiment was layed out as a randomised complete block design with each of the three treatments replicated seven times. The germination percentage was determined every 24 hours for seven days by expressing the cumulative

number of germinated seeds as a percentage of the total (50 seeds). Seeds counted as germinated were those of which the radicle appeared or the testa burst.

Experiment 2

In this experiment the same seed treatments were used as in Experiment 1, but the seed was subjected to the Accelerated Ageing Test (Delouche & Baskin, 1973) before germination and seedling vigour was determined. The incubator temperature for canola seed was 41°C for a period of 72 hours (Hampton & TeKrony, 1995). The rolled towel method (Loeffler, Meier & Burris, 1985) was used to complete the experiment. Blotting paper was used instead of paper towels for extra support. Each rolled paper represented a replicate and contained 50 seeds. Three batches containing seven replicates of 50 seeds of each treatment (Cruiser, SA-combination and control) were incubated at 20°C for 48, 96 and 144 hours respectively. At the end of the respective incubation periods the germination- and seedling vigour were determined. The germination percentage reflects the seed vigour and was determined as described in Experiment 1. The seedling vigour was determined by measuring the difference in plant length between the treated and untreated seeds. The plant length was determined by measuring the distance (in mm) from the cotyledons to the tip of the radicle. Where cotyledons had not yet developed, the plant length was taken as the length of the radicle. The differences in average plant length between the

treated and untreated seeds were used as an indication of the differences in seedling vigour.

Experiment 3

In this experiment the influence of different application rates of Cruiser and SA-combination on the vigour of the canola seed was tested. Each chemical was applied at 50 (0.5X), 100 (1.0X) and 200 % (2.0X) of the above-mentioned rates and a control treatment with untreated seeds was included. After being subjected once again to the Accelerated Ageing Test (Delouche & Baskin, 1973), four replicates of each treatment were incubated for 48 and 96 hours respectively. Thereafter the germination and seedling vigour were determined as described in Experiments 1 and 2.

Statistical analysis

Analysis of variance (ANOVA) was done on the results of the different treatments. Student's *t* least significant difference (LSD) values were calculated at the 5% probability level, using SAS software (SAS Institute Inc., 2000), to facilitate comparison between treatment means.

Results and discussion

Experiment 1

Both treated and untreated seeds started to germinate after only one day of incubation with almost 100% germination by day three (Figure 1). Both treated and untreated seeds were therefore found to be highly vigorous under the optimal germination conditions (temperature and moisture) created in the Petri dishes. Germination percentages of the treated seeds showed slight deviations from those of the untreated seeds (control) on day one and day two. Although not significant these results indicated that the seed treatment might influence seed vigour under unfavourable growth conditions.

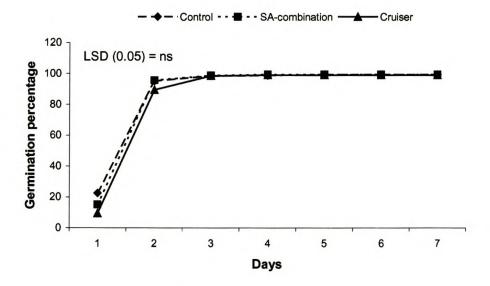


Figure 1 Germination percentages of treated and untreated canola seed after incubation in Petri dishes

Experiment 2

After seed from the same batch as used in experiment 1 was exposed to high temperature (41°C) and humid conditions to accelerate ageing of the seed, both the germination and seedling vigour were significantly reduced by the chemical treatments (Figures 2 & 3).

After 48 hours of incubation germination was reduced by 48 % and 30.3 % respectively due to the use of Cruiser and SA-combination as seed treatments (Figure 2). Although the effect of both chemical treatments decreased with an increase in time, only approximately 80 % of the treated seeds as apposed to 90 % of the untreated seeds (control) germinated after 144 hours. Initial significant differences between Cruiser- and SA-combination-treated seeds also disappeared as the incubation period increased.

In contrast to germination percentage, differences in seedling vigour did not decrease as the incubation period increased (Figure 3). After 48 hours of incubation Cruiser and SA-combination treatments reduced the average plant length of seedlings by 4.94 mm and 3.89 mm respectively (Figure 3). The effect of treatment increased as the incubation period increased to 144 hours with reductions in plant lengths of 11.04 mm and 28.33 mm for Cruiser- and SA-combination-treated seeds respectively. These results not only indicated a phytotoxic effect due to the chemical seed treatment, but also showed that the effect may be different for different chemicals.

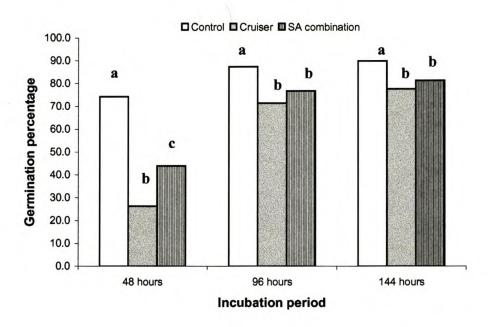


Figure 2 Germination percentages of untreated and treated canola seeds after different incubation periods. LSD(0.05) = 7.3628 (48 h); 8.5128 (96 h); 6.1766 (144 h). Bars denoted by the same letter are not significantly different at P = 0.05 according to Student's t test.

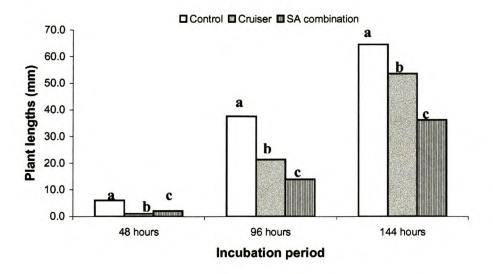


Figure 3 Plant lengths of untreated and treated canola seeds after different incubation periods. LSD(0.05) = 0.4754 (48 h); 4.4926 (96 h); 4.5292 (144 h). Bars denoted by the same letter are not significantly different at P = 0.05 according to Student's t test.

Experiment 3

After exposure to high temperature (41°C) and humid conditions to accelerate ageing of the seed, both the germination- and seedling vigour were significantly reduced in response to increasing application rates of the chemical treatments used (Figures 4 & 5).

After 48 hours of incubation, no significant differences in germination were found between the untreated seed (control) and seeds treated with Cruiser at 50% (0.5X) of the labelled rate. All other seed treatments caused a reduction in percentage germination, which increased with increased application rates. At this stage Cruiser- and SA-combination-treated seed at the 0.5X rate did not differ significantly. At application rates of 100 (1.0X) and 200 % (2.0X) of Cruiser's labelled rate, germination percentages were 84 % and 46,5 % respectively, compared to 54.5 and 6.0% of SA-combination-treated seed at the same application rates. After 96 hours, germination was not significantly affected by seed treatment with Cruiser at both the 0.5X and 1.0X rates and the SA-combination at 0.5X. At higher application rates both chemicals reduced germination, but the SA-combination was clearly more toxic than Cruiser.

Similar trends, as seen with germination percentages at different application rates, were found for plant lengths (Figure 5). After 48 hours all the chemical seed treatments reduced plant lengths, with a distinct decrease in plant length as the concentration of the chemical seed treatment increased. After 96 hours the average plant lengths of the untreated (control) seeds were not reduced by the

0.5X and 1.0X Cruiser treatment. All application rates of the SA-combination treatment reduced plant lengths. Significant differences detected between similar rates of the two chemical treatments, supported the germination percentages which indicated that the SA-combination treatment at 0.5X, 1.0X and 2.0X was more phytotoxic than Cruiser at similar rates.

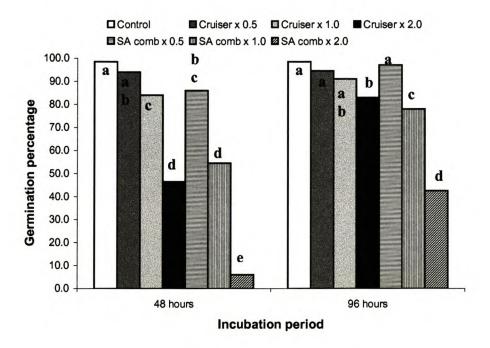


Figure 4 Germination percentage of canola seed treated with Cruiser and SA-combination at different application rates and after different incubation periods. LSD(0.05) = 8.466 (48 h); 8.8027 (96 h). Bars denoted by the same letter are not significantly different at P = 0.05 according to Student's t test.

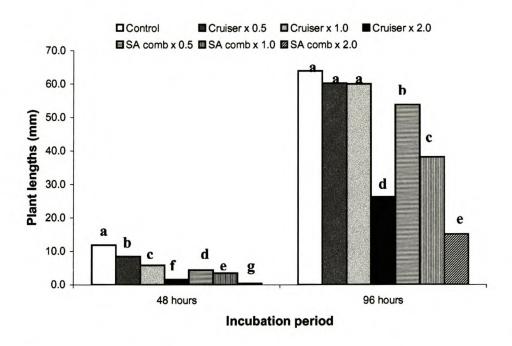


Figure 5 Plant lengths of seed treated with Cruiser and SA-combination at different application rates and after different incubation periods. LSD(0.05) = 0.8058 (48 h); 4.8176 (96 h). Bars denoted by the same letter are not significantly different at P = 0.05 according to Student's t test.

Conclusions

Differences in the germination between treated and untreated (control) seeds found in Petri dishes were enhanced when the seeds were exposed to high temperatures and high humidity's to accelerate seed ageing. Although both germination and plant (cotyledon/radicle) growth were initially reduced, the results showed that 0.5X and 1.0X treatments with Cruiser did not affect these parameters after an incubation period of 96 hours. The treatment with the SA-combination, however, appeared to be more phytotoxic.

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Chapter 3

Effect of chemical seed treatment on the germination and seedling growth of canola under different soil conditions

Abstract

Studies in a temperature controlled greenhouse were conducted to determine if phytotoxic effects of chemical seed treatments found in incubation studies also have an effect on the germination- and seedling vigour of canola (cv Varola 44) seed planted in simulated field conditions and secondly to determine the efficiency of seed treatments in soils from different localities. In the first experiment untreated (control) and seeds treated with 0.5X, 1.0X and 2.0X of the recommended rates of Cruiser® and a local mixture (SA-Combination) were planted at 5 and 10 mm depths in dry (10% of field water capacity), moist (50% of field water capacity) and wet (70% of field water capacity) pasteurized soil to ensure that only the phytotoxic effects of the seed treatments were measured. In general, results showed that some treatments (application rates) of both chemicals used delayed seed germination and therefore reduced the number of seedlings emerged after 9 days, especially when planted at a 5 mm depth in pasteurized dry soil, but not in moist soil (50% FWC). In soils naturally infested with especially Rhizoctonia and Pythium seedling emergence and survival, plant height of treated seeds were significantly higher compared to that where untreated seed was planted. Because no clear significant differences were found

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between chemicals used, both chemicals should be regarded as efficient, but more research is needed to test their efficiency against specific pathogens.

Introduction

Canola has the potential to produce yields of more than four tonnes per hectare in high-rainfall areas if adequate attention is given to disease control and nutrition (Kirkegaard & Robertson, 2004), which makes it very profitable in Australia. The production of canola in rotation with wheat may also increase wheat yields (Johnston *et al.*, 2002). Despite these beneficial effects, the production of canola in the western and southern production areas of the Western Cape Province are hampered due to uneven plant populations that result in low yields. Producers therefore may refrain from planting canola. These uneven and patchy stands may be due to soil and/or climatic conditions in combination with seedling diseases and insect pests (Richards, 1978).

If environmental conditions become sub-optimal for germination, seedlings of various crops are more vulnerable to attacks from soil borne pathogens (Smiley & Uddin, 1993), such as *Fusarium* spp. (Klaasen *et al.*, 1991; Wong, Mead & Croft, 2002), *Pythium* spp. (Pemberton *et al.*, 1990) and *Rhizoctonia* spp. (Kaminski & Verma, 1985; Kataria & Verma, 1992), whose occurrence and pathogenicity also vary for different soil- and climatic conditions (Gugel *et al.*, 1987; Klaasen *et al.*, 1991). It can be expected that this will also be true for canola.

Seed treatments can provide protection from seed- and soil borne diseases for the critical three to four weeks after planting because canola become less susceptible to the seedling disease complex with age (Yang & Verma, 1992). A disadvantage of seed treatments is that if applied above labelled rates it may injure germinating seeds and therefore possibly reduce yields (Ashley *et al.*, 2003) due to a delay in germination and reduction in emergence with an increase in fungicide application rate (Petch, Maude & White, 1991). This harmful effect of seed treatment was confirmed in incubation studies (Chapter 2) which showed that chemical seed treatment might delay germination of canola seeds and reduce the seedling vigour, especially if applied above recommended rates and to seeds subjected to conditions which reduce their germination capacity and seedling vigour (Accelerated Ageing Test).

The decision to use seed treatments must be made well in advance of planting (Nault et al., 2004) unknowing what the disease occurrence and environmental conditions will be. Therefore chemical seed treatments should not put strain on germination and early growth of seedlings in the absence of pathogens and insect pests. No chemical seed treatment for canola is currently registered in South Africa, and little is known with regard to these possible phytotoxic effects under different soil and climatic conditions.

The objective of this research was to determine the effect of different seed treatments and application rates (doses) on the germination and seedling growth of canola (cv. Varola 44) under different soil water conditions and soil types from different localities in the Western Cape Province.

Materials and methods

Two pot experiments were conducted in a temperature controlled (22/17°C day/night) glasshouse to compare the emergence and seedling growth of untreated canola (cv Varola 44) seed to that of seed treated with two different chemicals at 0.5X, 1.0X and 2.0X of the recommended rate.

Two chemical products were used, namely:

- (i) Cruiser® (3.75 ml 250 g⁻¹ of canola seed) which is registered as a seed treatment for canola in Europe and Brazil (also known as Helix® in the USA and Canada) to control *Fusarium* spp., *Pythium* spp. and *Rhizoctonia solani*, as well as flea beetles and aphids (Paulsrud *et al.*, 2001), and
- (ii) A combination product (referred to as SA-combination) which consists of Thiulin® (0.5 g a.i.), Apron® (0.0815 g a.i.), Gaucho® (0.6125 g a.i.) and Rovral® (0.9975 g a.i.). Thiulin protects canola against seedand soil borne fungi (*Rhizoctonia solani* and *Fusarium* spp.) which cause seed decay, damping-off and seedling blight (Munkvold, Sweets & Wintersteen, 1999). Apron is a systemic fungicide for control of *Pythium* spp. (Petch *et al.*, 1991; Paulsrud *et al.*, 2001) and Rovral is a contact fungicide for the control of *Rhizoctonia solani* (Kataria & Verma, 1992) and *Fusarium* spp. Gaucho provides effective protection against damaging insects such as leafhoppers, (Nault *et al.*, 2004) flea beetles, aphids and

thrips (Ahmed et al., 2001; Paulsrud et al., 2001; Ester, De Putter & Bilsen 2003).

Procedures as used for the different treatments are described in Chapter 2.

In Experiment 1, treated and untreated (control) seed, respectively were planted at 5 and 10 mm depths in dry (10% of field water capacity), moist (50% of field water capacity) and wet (70% of field water capacity) pasteurised sandy loam soil (30 minutes at 83°C) from the Welgevallen Experimental Farm to ensure that only the phytotoxic effects of the seed treatments were measured. Field water capacity (FWC) was determined as a mass ratio by determining its moist and dry weight (Hillel, 1982). In an effort to create larger differences in seedling vigour, freshly treated seed as well as seed from the same batch treated 15 months earlier were used as seed sources. Three replicates were used and ten seeds were planted per pot. To ensure that the specified soil water levels were maintained as long as possible, pots were sealed off with a plastic cover to prevent the loss of water from planting until the first seedlings emerged at 3 to 4 days after planting. After seedling emergence the required water levels were maintained by daily weighing and consequent re-watering of the pots to the original pot weight (water level). Because this method inevitably causes soil from especially the lower water levels to dry out from the bottom of the pots, thereby disrupting the uniform distribution of soil water levels in the pots, and to prevent competition between seedlings the experiment was terminated 9 days after planting. During this period emergence of seedlings were counted regularly to

determine the time to 50% as well as maximum emergence, while dry mass per pot and dry mass per plant were measured at the end of the experiment.

In experiment 2 the same seed treatments were evaluated in seven soils (not pasteurized) from different localities (Table 1) to evaluate the efficiency of different chemicals in different soil types. Based on results from Experiment 1 soil water levels were kept at 50% of field water capacity to minimize phytotoxic effects. Each seed treatment comprised four replicate pots (10 seeds per pot). Plants were again counted regularly to determine the percentage emergence, plant lengths and dry mass per pot at the end of the experiment (30 days after planting). Samples of diseased plants were used to isolate and identify pathogenic organisms in the soil from different localities.

Table 1 Characteristics of the soils from different localities used in Experiment 2.

Soil	EB	TH	NA	MRB	LD	КВ	HF
pH (KCI)	5.3	4.9	5.9	4.5	4.7	4.3	5.4
Resistance (Ohms)	1460	760	1240	430	520	990	4200
Texture	SCL	CL	SCL	SL	SCL	SCL	LS
Acidity (cmol(+)/kg)	0.43	0.85	-	0.69	0.59	0.64	0.23
Calcium (cmol(+)/kg)	2.32	4.69	5.40	1.62	2.21	1.17	0.85
Magnesium (cmol(+)/kg)	0.47	1.35	1.40	0.47	1.23	0.36	0.38
Potassium (mg/kg)	72	198	236	283	87	109	60
Sodium (mg/kg)	18	25	45	16	42	11	6
P (citric acid) (mg/kg)	47	51	39	134	67	53	35
Total cations (cmol(+)/kg)	3.48	7.51	7.61	3.57	4.44	2.50	1.64
Carbon (%)	0.71	1.51	1.34	0.98	0.91	0.94	0.21
Sand (%)	66	42	54	70	66	66	82
Clay (%)	26	32	24	20	24	24	14
Silt (%)	8	26	22	10	10	10	4

Abbreviations used: EB = Elsenburg; TH = Tygerhoek; NA = Napier; MRB = Moorreesburg; LD = Leliedam; KB =

Koringberg; HF = Hopefield; S = Sand; C = Clay; L = Loam

Statistical analysis

Analysis of variance (ANOVA) was done on the results of the different treatments. Student's t least significant difference (LSD) values were calculated

at the 5% probability level, using SAS software (SAS Institute Inc., 2000), to

compare treatment means.

Results

Experiment 1

From Table 2 it is clear that both the seedling emergence (days to 50 % and total %) and plant growth were significantly affected by chemical treatments (T), soil water content (M) and to a lesser extent planting depth (D). Seed source (S = freshly treated vs. 15 months after treatment) on the other hand only affected germination. Significant interactions between seed source (S) and other main factors did occur, but were mostly confined to seedling emergence and not seedling growth. Both seedling emergence and growth were affected by interactions between chemical seed treatments, soil water content and planting depth.

Table 2 Significance levels (Pr>F) of main effects namely soil water content (M), planting depth (D), seed source (S) and seed treatment (T) as well as interactions with regard to the emergence and dry mass production of canola seedlings (cv. Varola 44).

	Days to 50%	%	Dry mass per	Dry mass
	emergence	Emergence	pot	per plant
Soil water content	<.0001	<.0001	<.0001	<.0001
Depth	0.0060	ns	0.0090	ns
MxD	0.0087	<.0001	<.0001	ns
Seed source	<.0001	<.0001	ns	ns
MxS	ns	0.0463	0.0086	ns
DxS	ns	ns	ns	ns
MxDxS	ns	ns	ns	ns
Treatment	<.0001	0.0050	<.0001	0.0131
MxT	<.0001	<.0001	<.0001	0.0359
DxT	0.0143	ns	ns	0.0432
MxDxT	<.0001	0.0370	0.0020	ns
SxT	<.0001	ns	ns	ns
MxSxT	<.0001	0.0405	ns	ns
DxSxT	<.0001	0.0294	ns	ns
MxDxSxT	0.0003	ns	ns	ns

Germination rate

In dry soil (10% FWC) the control (untreated seed) on average reached 50% germination after 4-5 days, while treated seed needed between 6.5 and 9 days, irrespective of planting depth (Figure 1A & 1B) or seed source (Table 3). Differences between chemicals and rates (0.5X, 1.0X and 2.0X) used were found to be very inconsistent and not significant (P0.05). In moist soil (50% FWC) germination rate for the untreated seed was not faster than that of treated seed and 50% germination for all seed was achieved within 3-5 days, again irrespective of planting depth and seed source.

In wet (70% FWC) soil, 50 % germination was reached at approximately 8-9 days without any significant differences between untreated and treated seed at a planting depth of 5 mm (Figure1A). At a planting depth of 10 mm, germination rate was retarded to approximately 9 days (at which point the experiment was stopped for reasons already explained) for both control and treated seed (Figure 1B). Due to this dominant effect of most probably a lack of oxygen in wet soil, germination was suppressed to such an extend that possible effects due to the different seed treatments or seed source were disguised. Although germination rates for freshly treated seed were, surprisingly, found to be on average slower than for seed treated 15 months earlier, trends due to chemicals used, planting depth and soil water content were similar (Table 3). This is because chemical treatment of seed, in general, caused a slower rate of germination at 10 % FWC levels, but not at 50 % FWC levels. At 70 % FWC levels germination rates for all treatment combinations were very slow due to the dominating (negative) effect of

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the high soil water content on germination. The difference due to seed source (freshly vs. 15 months) is difficult to explain and needs further investigation, as earlier results (Petch *et al.*, 1991) showed the opposite trend.

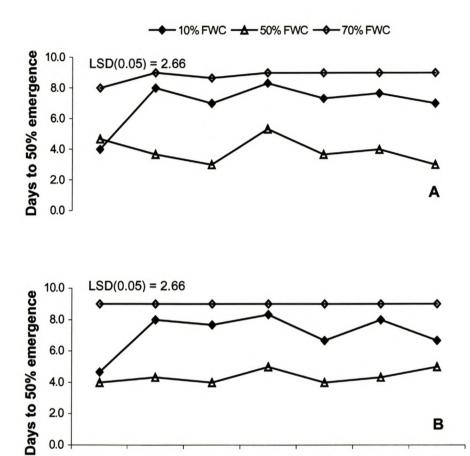
From these results it became clear that in general both chemicals used delayed seed germination when planted in pasteurised soil with sub-optimal (dry) water contents, but not in moist soil. Petch *et al.* (1991) found that emergence was reduced with increasing application rates of fungicide in soil favouring disease development (therefore sub-optimal for seed germination). To avoid this effect and obtain fast germination and establishment it is therefore important to plant shallowly (5 mm) in favourable soil water conditions. These results were similar to those of Day (2000) who found that seedling emergence of sesame was reduced by low soil water content (less than 20%) and by waterlogging.

Table 3 Effect of seed source (S) (SF = freshly treated, SO = treated 15 prior), depth of planting (D), soil water content (M) and seed treatment (T) on time (days) to 50 % of maximum emergence.

S	D	M (%	Seed Treatment (T)						
	(mm)	FWC)	Control	rol Cruiser		SA-combination			
				0.5X	1.0X	2.0X	0.5X	1.0X	2.0X
SF	5	10	4.0	8.0	7.0	8.3	7.6	7.6	7.0
		50	4.6	3.6	3.0	5.3	3.6	4.0	3.0
		70	8.3	10.0	9.3	10.0	10.0	10.0	10.0
		Mean	5.6	7.2	6.4	7.8	7.1	7.2	6.6
	10	10	4.6	8.0	7.6	8.3	6.6	8.0	6.6
		50	4.0	4.3	4.0	5.0	4.0	4.3	5.0
		70	10.0	10.0	10.0	10.0	10.0	10.0	10.0
		Mean	6.2	7.4	7.2	7.7	6.8	7.4	7.2
so	5	10	4.0	6.6	8.0	7.3	6.6	7.3	7.3
		50	4.6	3.6	3.6	4.3	3.0	3.6	4.0
		70	8.3	10.0	10.0	10.0	4.0	9.0	9.3
		Mean	5.6	6.7	7.2	7.2	4.5	6.6	6.8
	10	10	4.6	4.6	7.3	7.0	6.3	7.3	8.0
		50	4.0	3.6	4.3	4.3	3.3	4.0	4.3
		70	10.0	10.0	7.0	10.0	10.0	10.0	10.0
		Mean	6.2	6.1	6.2	7.1	6.5	7.1	7.4
LSD(0	.05):								
	SxI	DχT	2.6						
	SxD	x M x T	3.3						

В

7



4.0

2.0

0.0

1

2

3

Days to 50% emergence of untreated and treated canola seeds in Figure 1 different soil water conditions (FWC = Field water capacity) at a planting depth of (Figure 1A) 5 mm and (Figure 1B) 10 mm.

Seed treatment

5

6

Seed treatments: 1 = Control (untreated seed); 2 = Cruiser X0.5; 3 = Cruiser X1; 4 = Cruiser X2; 5 = SA-combination X0.5; 6 = SA-combination X1; 7 = SA-combination X2.

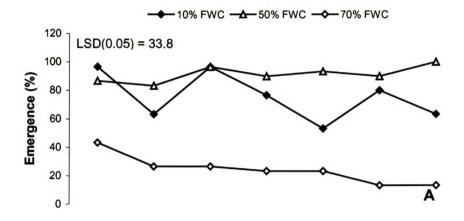
Maximum percentage germination

Maximum percentage germination also showed significant differences due to seed treatment, soil water content and seed source (Table 2). Freshly applied chemical seed treatment was again found to be more toxic (lower maximum percentage germination) compared to the seed treatments 15 months prior to the start of the experiment, especially under extreme soil water (very dry/wet) conditions and in the absence of disease-causing organisms. This is as mentioned somewhat unexpected and needs further investigation. Significant interactions between seed treatment, soil water content and planting depth (Table 2) showed that the phytotoxic effects of the chemical treatments were again affected by soil conditions and planting techniques.

In moist soil (50% FWC) chemicals used again did not have an effect on the maximum germination percentage, irrespective of planting depth (Figure 2A & B) with values of 80–100%. In dry soil (10% FWC) very inconsistent responses were obtained with the different seed treatments when planted at a depth of 5 mm. Some treatments like 0.5X Cruiser as well as 0.5X and 2.0X SA-combination did reduce seedling numbers (% emergence), but no definite trends with regard to either the chemical or application rate used were found. At a planting depth of 10 mm results were more consistent, but did not show any significant differences due to either chemical used or application rate. In wet soil (70% FWC) the maximum emergence percentages were very low compared to that at the other soil water levels. Maximum emergence at 5 mm was only 45% for the untreated

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seeds and 20-40% for the treated seeds. No significant differences due to either chemical or application rate used were found. Maximum emergence at 10 mm was found to be 30% for the untreated seeds with lower values for all treated seeds. Maximum percentage emergence was significantly reduced by double (2.0X) the recommended rate of SA-combination, but no significant differences were found between chemicals used.



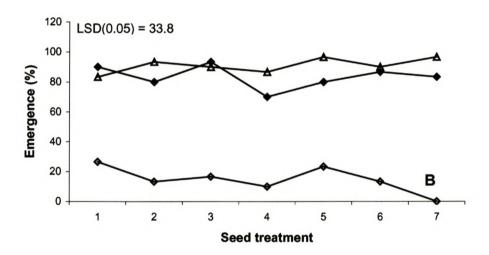


Figure 2 Percentage emergence of untreated and treated canola seeds in different soil water conditions (FWC = Field water capacity), at a planting depth of (A) 5 mm and (B) 10 mm.

Seedling growth

Dry mass per pot is the product of both seedling emergence and seedling vigour.

Dry mass per pot was influenced by soil water content, planting depth and seed treatment, as well as interactions between these factors (Table 2).

In contrast to emergence rate and maximum emergence percentage, 0.5X and 1.0X Cruiser-treatment caused a significant increase in dry mass per pot in moist (50 % FWC) soil at a planting depth of 5 mm (Figure 3A). No differences were found between chemicals and application rates used. In dry (10 % FWC) and wet (70 % FWC) soil, dry mass per pot or chemical-treated seed tended to be lower compared to the untreated seed, but differences were not significant.

At a planting depth of 10 mm no differences due to seed treatment were found in moist (50 % FWC) soil (Figure 3B). In dry soil, seed treatment again tended to reduce dry mass per pot, but was with the exception of the 2.0X Cruiser treatment not significant less than that of the untreated control.

In wet (70 % FWC) soil, dry mass per pot were generally low and did not differ between treatment due to this dominating effect of the high soil water content on dry mass production per pot.

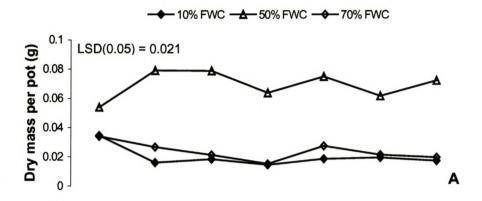
Dry mass per plant, which illustrates seedling vigour, was affected by seed treatment (Table 2), but the response showed significant interactions with planting depth and soil water content. From Figure 4 it is clear that the interaction due to planting depth can be ascribed to the unexpected difference in response found with 0.5X Cruiser treated seed, because similar trends were shown for all

other treatments irrespective of planting depth. These trends showed that in the absence of soil borne pathogens, seedling vigour tended to decrease with an increase in application rate for both chemicals used, but differences were not significant.

If the unexpected low value for 0.5X Cruiser treated seed in the wet (70% FWC) soil was ignored, the results obtained with different soil water contents (Figure 5) confirmed trends found with planting depths, namely a decreasing seedling vigour with increasing application rates for especially SA-combination treated seeds, but re-emphasised the dominant effect of soil water content.

In dry soil (10 % FWC) the seedlings from the untreated seeds produced 0.0042 g dry mass per plant while that of the treated seeds varied between 0.0027 and 0.0037 g (Figure 5), but differences were not significant. Similar to time to 50% germination, maximum emergence and dry mass per pot, treated seeds showed no depression of dry mass production per plant under favourable soil water conditions (50% FWC).

Considering these results it is clear that the effect of seed treatment differs between different conditions (moisture, planting depth and time of application of seed treatment). In the presence of pathogens, Marley & Adeoti (1995) found that plant height and root length of wheat seedlings were significantly greater when a seed treatment was applied, while these were again (for most seed treatments) out yielded in the absence of disease and when no seed treatment was used.



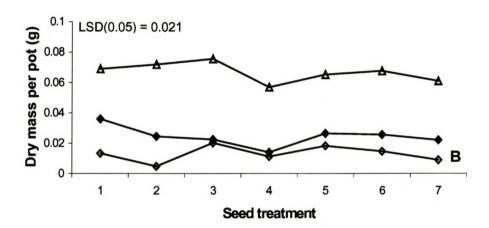


Figure 3 Dry mass per pot of untreated and treated canola seeds in different soil water conditions (FWC = Field water capacity), at a planting depth of (A) 5 mm and (B) 10 mm.

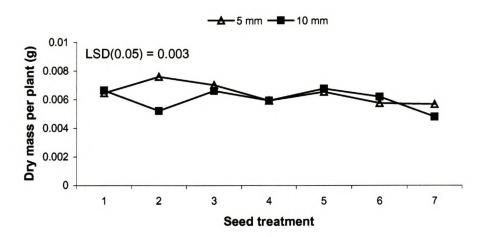


Figure 4 Dry mass per plant of untreated and treated seeds at different planting depths.

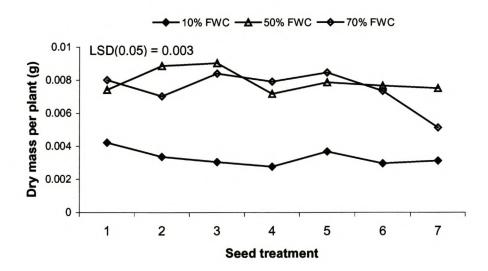


Figure 5 Dry mass per plant of untreated and treated canola seeds at different soil water conditions (FWC = Field water capacity).

Experiment 2

Results of this experiment conducted in soil from different localities (where canola had been produced previously) showed significant interactions between the seed treatments and locality for all parameters tested (Table 4). Because of the large differences and thus dominating effect of the different localities (soil), differences between treatment within a locality (interaction) were not always significant.

Table 4 Significant levels (Pr>F) of main effects.

	% Survival	Plant lengths	Dry mass
Locality (L)	<0.0001	<0.0001	<0.0001
Seed treatment (T)	<0.0001	0.0041	ns
LxT	<0.0018	0.0006	0.0230

Seedling survival and plant growth

Percentage seedling survival (percentage of seed that germinate, emerge and survive until the experiment was terminated 30 days after planting) of control (untreated) seed in soils from Moorreesburg, Leliedam, Koringberg and Hopefield were not different (Table 5). Untreated (control) seeds survived poorly in soils from Elsenburg (16 %) and Napier (53 %) and to a lesser extent in soil from

Tygerhoek (73 %). At these localities the chemically treated seeds showed excellent average survival of 72 %, 90 % and 97 % respectively. Poor survival rates with Cruiser X0.5 (53 %) and Cruiser X1 (63 %) occurred in the soil from Elsenburg indicating that Cruiser was less effective than SA-combination in soil from this locality. This tendency was however not confirmed by the results at other localities. Isolation and identification of pathogens from the soils with poor survival of seedlings showed moderate (Tygerhoek) to high (Elsenburg & Napier) infestations of Rhizoctionia; high infestations of the Pythium F group in the soil from Elsenburg and low infestations of Pythium irregulare in the soil from Tygerhoek (data not shown). Low infestations of Rhizoctonia, Fusarium avenaceum and Pythium irregulare were found in the soils from localities where seedling survival of untreated plots were high. It therefore appears that the seedling survival can be ascribed to the occurrence of pathogens and not differences in soil physical and/or chemical characteristics because no clear correlation could be found between seedling survival and any soil characteristics (Table 1).

The high disease infestations in the soils from Elsenburg and Napier were also reflected in the reduced plant length (seedling vigour) of seedlings from untreated seed. Although on average slightly shorter in the soil from Tygerhoek, length of untreated (control) plants as also found at other lightly infested localities (Moorreesburg, Leliedam, Koringberg & Hopefield) did not differ significantly from that of treated plants, irrespective of chemical or application rate used.

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Significant differences in dry mass produced per pot were found between different localities, with lowest mean values for Elsenburg (0.18 g) and Tygerhoek (0.44 g) and the highest for Koringberg (1.08 g), Leliedam (0.89 g) and Hopefield (0.82 g). Although not significant due to large differences between localities dry mass production of seedlings from untreated seeds at Elsenburg, Tygerhoek and Napier tended to be less than that of chemically treated seed. Differences due to chemicals and application rates used were not significant and therefore needed some further investigation.

Table 5 Percentage survival, plant lengths and dry mass of canola seedlings from untreated and treated seeds in soil from seven localities.

Locality (L)	Treatment (T)	% Survival	Plant length (mm)	Dry mass (g.pot ⁻¹)
Elsenburg	1	16.67	8.67	0.05
	2	53.33	15.00	0.15
	3	63.33	15.00	0.15
	4	90.00	19.33	0.24
	5	73.33	18.00	0.22
	6	70.00	16.67	0.26
	7	83.33	17.67	0.17
	Mean	64.29	15.76	0.18
Tygerhoek	1	73.33	28.00	0.29
	2	93.33	29.67	0.42
	3	100.00	32.00	0.55
	4	93.33	32.67	0.48
	5	100.00	24.67	0.38
	6	96.67	29.33	0.47
	7	100.00	30.33	0.47
	Mean	93.81	29.52	0.44
Napier	1	53.33	37.33	0.33
	2	96.67	47.00	0.71
	3	86.67	42.67	0.59
	4	86.67	54.00	0.66
	5	96.67	40.00	0.68
	6	90.00	40.33	0.58
	7	83.33	36.67	0.49
	Mean	84.76	42.57	0.58

(Table 5 continued)

Locality (L)	Treatment (T)	% Survival	Plant length (mm)	Dry mass (g.pot ⁻¹)
MRB	1	96.67	51.33	0.58
	2	86.67	45.67	0.44
	3	93.33	44.33	0.47
	4	100.00	44.33	0.47
	5	93.33	43.67	0.58
	6	100.00	45.33	0.67
	7	90.00	42.00	0.49
	Mean	94.29	45.24	0.53
Leliedam	1	90.00	48.00	1.00
	2	83.33	46.33	0.75
	3	86.67	55.67	0.87
	4	83.33	51.33	1.01
	5	90.00	44.00	0.87
	6	86.67	43.67	0.83
	7	93.33	41.33	0.91
	Mean	87.62	47.19	0.89
Koringberg	1	96.67	47.33	1.15
	2	93.33	45.67	1.10
	3	86.67	47.67	1.04
	4	90.00	51.00	1.11
	5	93.33	44.67	1.00
	6	73.33	38.00	0.92
	7	80.00	52.67	1.25
	Mean	87.62	46.72	1.08

(Table 5 continued)

Locality (L)	Treatment (T)	% Survival	Plant length (mm)	Dry mass (g.pot ⁻¹)
Hopefield	1	100.00	48.00	0.82
	2	100.00	52.33	0.84
	3	100.00	46.00	0.78
	4	96.67	45.33	0.74
	5	100.00	51.00	0.89
	6	96.67	48.67	0.83
	7	90.00	50.00	0.82
Mean		97.62	48.76	0.82
LSD(0.05):				
Locality (L)		16.6	5.63	0.27
Treatment (T)		16.6	5.63	0.27
LxT		40.6	9.21	0.42

Seed treatments: 1 = Control (untreated seed); 2 = Cruiser X0.5; 3 = Cruiser X1; 4 = Cruiser X2; 5 = SA-combination

X0.5; 6 = SA-combination X1; 7 = SA-combination X2.

Conclusions

The response to seed treatment were affected by soil water content, planting depth and time of application, but from the results it became clear that soil water content had the largest effect.

Experiments conducted in soil from different localities showed significant interactions between the seed treatments and localities indicating that some localities were not infested by the pathogens. For soils where diseases occurred, most of the chemical seed treatments (X0.5, X1, X2) increased the survival percentage, plant length and dry mass per pot. Differences between chemicals and application rates used were, with the exception of percentage survival at

Elsenburg, not significant and no conclusion with regard to the efficiency of different chemicals tested can be made.

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Chapter 4

Effect of chemical seed treatment, seeding rate and row width on the plant populations and yield of canola

Abstract

Field experiments were conducted in 2003 and 2004 at Langgewens and Roodebloem, representing the Swartland and Rûens respectively, in the canola production area of the Western Cape Province to determine the influence of seeding rate, row width and chemical seed treatment on the plant density (plants m⁻²) and yield of canola (cv. Varola 44). Untreated (control) seeds and seeds treated with Cruiser® and a local mixture (SA-combination) were seeded in 17 cm and 34 cm row spacings at seeding rates of 3, 5 and 7 kg ha⁻¹. Results showed that treated seeds produced more plants m⁻² and yielded more than untreated seeds at Roodebloem, while the highest seeding rate produced significantly more plants m⁻² (Roodebloem and Langgewens), but not significantly higher yield than the lowest seeding rate at the same locality. No differences in plant density were found between untreated and treated seeds or due to row width at Langgewens (2004). Row width did not have an effect on plant densities at Roodebloem (2003); yield (Roodebloem 2003) was significant less at wider (34 cm) rows than narrow (17 cm) rows.

Introduction

An increase in seed yield per hectare is of major importance to sustain or improve the production of canola in the Western Cape Province. Poor yields achieved in these areas (average of 0.89 t/ha over the last five seasons (National Department of Agriculture, 2003)) may reduce the area planted or discourage producers from planting canola. One of the main reasons for these poor yields is uneven and patchy canola stands. Uneven emergence of seedlings causes uneven maturity and can be due to many factors such as stands damaged by drought, lodging, insects or disease (Loof, 1972). Injuries inflicted by pests (such as insects and disease pathogens) to rapeseed crops in the seedling or early vegetative stages of development frequently result in loss of plants and thinning of plant populations. This, together with uneven maturity, causes even lower seed yields.

Seedling diseases of canola may cause yield losses of more than 20% (Mikkelson et al., 2003). Pathogens responsible for canola seedling losses includes Fusarium spp. (Calman, Tewari & Mugala, 1986), Pythium spp. (Gugel et al., 1987; Bartlett & Martin, 1995) and Rhizoctonia spp. (Yitbarek et al., 1988; Kataria & Verma, 1992; Khangura, Barbetti & Sweetingham, 1999; Auret, Janse Van Rensburg & Lamprecht, unpublished report to Protein Research Foundation, 2002), while the leaf feeding insects which attack the newly emerged seedlings includes the red legged earth mite (Halotydeus destructor), the blue oat mite (Penthaleus major) (Robinson & Hoffman, 2000) and the flea beetle (Phyllotreta spp.) (Lamb, 1984).

To counter these yield losses by pests several production strategies may be used to obtain a high density and even spaced canola population that is needed for maximum seed yield.

Seed yield of rape is a function of number of pods per unit area, number of seeds per pod and weight per seed (Clarke & Simpson, 1978a), which in turn are affected by production techniques such as seeding rate and row width, while seed treatment may possibly increase the survival of seedlings per unit area.

Reduced plant densities (seeding rates of 1.5 and 3.0 kg ha⁻¹) result in an altered plant form with increased branching (Clarke & Simpson, 1978a), while narrow rows (15 cm) are associated with a more even plant distribution and a lower degree of intra-row competition (Christensen & Drabble, 1984; Morrison, McVetty & Scarth, 1990). McGregor (1987) found that when a canola stand was reduced to as little as 40 plants m⁻² (from 200 plants m⁻²), a seed yield loss of less than 20% occurred. This compensation appeared to be the result of increased and prolonged dry matter accumulation in leaves, stems and pods with a better assimilate availability due to more photosynthetic surface per plant (McGregor, 1987). However, the pods are distributed over a greater depth of the canopy, and light can be rate-limiting for photosynthesis at lower branch positions (Clarke & Simpson, 1978a).

Clarke & Simpson (1978b) also found that the number of seeds per pod and 1000-seed weight are lower on the bottom branches of *B. napus* than on the main raceme. This indicates that adequate seeding rate is still necessary to produce optimal yield and minor losses in plant population can be overcome by

an increase in the production of branch racemes and pods which serves to buffer the effect of a reduced plant density and to maintain yield if there is adequate available soil moisture for yield compensation to occur.

Recommended seeding rates for spring rape cultivars, which are grown in the Western Cape Province are usually high to ensure a large enough plant population at early growth stages to be competitive with weeds. However, the advantage of high interspecies competition may be offset by a higher intraspecies competition, especially at wider rows (23, 31 and 60 cm vs. 15 cm) (Kondra, 1975a), potentially resulting in similar yield losses. The yield component most affected, as seeding rate increases, is the number of pods per plant which decrease as seeding rate increase in a significantly quadratic manner (Clarke & Simpson 1978a; Morrison et al., 1990). In addition, high plant densities (8-12 kg ha⁻¹) produce plants with thinner stems which are less able to support the weight of the pods and seeds, and potentially more susceptible to stem girdling diseases such as Sclerotinia (Sclerotinia sclerotiorum) (Morrison et al., 1990) and lodging (Kondra, 1975a). Progressively less vegetative growth also occurs at increased seeding rates (3-12 kg ha⁻¹) and therefore days to maturity of the first pod significantly decreases (Degenhardt & Kondra, 1981a). According to Christensen & Drabble (1984) competitive mortality became so high that no significant yield differences occurred between seeding rates of 7 and 14 kg ha⁻¹ in experiments in Northwestern Alberta. It is therefore not recommended to plant at a seeding rate of more than 7 kg ha⁻¹. Due to previously mentioned compensation, this seeding

rate can rather be lowered (5 kg ha⁻¹) when canola seeds are treated with a chemical seed treatment to prevent pre- and post emergence seedling losses.

Application of chemical seed treatments can increase the emergence (through protection from disease pathogens) and survival of canola seedlings in the early stages (Chapter 3). Too little of a seed treatment product can result in inadequate protection from disease while too much may impede seed and seedling vigour of emerging seedlings, reduce plant stands and possibly reduce yields or produce yields no higher than those produced by untreated seeds (Ashley *et al.*, 2003).

The objective of this study was to determine whether the response of canola to varying seeding rates and row widths is affected by chemical seed treatments (combinations of fungicides and insecticides) when grown at different localities.

Materials and Methods

Field trials were conducted during 2003 and 2004 on the Agricultural Research (experimental) Farms Roodebloem and Langgewens which respectively represent the Rûens and the Swartland in the canola producing areas of the Western Cape Province of South Africa. Canola (cv Varola 44) was used to compare the plant population (emergence and survival of seedlings) and yield of untreated canola seed to that of seed treated with Cruiser® (3.75 ml 250 g⁻¹ of canola seed) and seed treated with a mixture referred to as SA-combination, which consists of Thiulin® (0.5 g a.i.), Apron® (0.0815 g a.i.), Gaucho® (0.6125 g a.i.) and Rovral® (0.9975 g a.i.), when planted at different seeding rates and row widths. Procedures used for the different seed treatments were described in

Chapter 2. Seeding rates used were 3, 5 and 7 kg ha⁻¹, while 17 and 34 cm row

widths were compared. A factorial design with four replicates was used and both

plant population (plants m⁻²) and grain yield (kg ha⁻¹) were measured.

The experiments were planted with an experimental plot seeder in a weed free

seedbed. Fertiliser was applied according to a soil analysis. Plot sizes of 5 x 3 m

were used to determine yield while emergence was determined by counting the

number of plants in two 50 cm rows per plot, 4 weeks after planting then

calculating the number of plants m⁻² by multiplying it by 5.9 (for 17 cm rows) or

2.9 (for 34 cm rows) respectively.

Statistical analysis

Analysis of variance (ANOVA) was done on the results of the different

treatments. Student's t least significant difference (LSD) values were calculated

at the 5% probability level, using SAS software (SAS Institute Inc., 2000), to

compare treatment means.

Results

Due to very low rainfall, no data was obtained from Langgewens during 2003.

From Table 1 it is clear that plant population (plants m⁻²) was significantly

affected by seeding rate (SR), but not row width (RW) at both localities. Seed

treatment (T) had a significant effect on plant population at Roodebloem during

2004, but not during 2003 or at Langgewens.

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Table 1 Significant levels of main effects namely row width (RW), seeding rate (SR) and seed treatment (T) as well as interactions with regard to the plant population (plants m⁻²) and yield of canola (cv. Varola 44) at two different localities.

	RB 2003	RB 2004	RB 2003	LG 2004
	Plants m ⁻²	Plants m ⁻²	Yield (kg ha ⁻¹)	Plants m ⁻²
RW	ns	ns	0.0006	ns
SR	<.0001	<.0001	ns	<.0001
RW X SR	ns	ns	ns	ns
Т	ns	8000.0	0.0014	ns
RW X T	ns	ns	ns	ns
SRXT	ns	ns	ns	0.0170
RWXSRXT	ns	ns	ns	ns

RB = Roodebloem; LG = Langgewens

An interaction with regard to plant population occurred between seeding rate and seed treatment at Langgewens only. From Table 2 it is clear that this interaction can be ascribed to a significant difference in plants m⁻² for Cruiser-treated seed between seeding rates of 3 and 7 kg ha⁻¹, but not for the control or SA-combination-treated seed. Although Morrison *et al.*, (1990) also found that seed treatments increased competitive mortality (better survival) at higher seeding rates, discussion of the results will focus on main effects because main factor

effects (row width, seeding rate, seed treatment) were much stronger than the interaction (Table 1).

Table 2 Plant populations (plants m⁻²) as affected by seeding rate and chemical seed treatment in 2004 at Langgewens Research Farm.

	Seeding rate (kg ha ⁻¹)			
	3	5	7	
Seed treatment:				
Control	55.6a*	93.5 <i>a</i>	81.4 <i>a</i>	
SA-combination	50.4 <i>a</i>	72.8 <i>a</i>	87.5 <i>a</i>	
Cruiser	48.3 <i>a</i>	73.4a	106.2 <i>b</i>	

^{*}Means followed by the same letter are not significantly different (LSD=0.05).

Yield at Roodebloem (2003) was also significantly affected by seed treatment and row width, but not by seeding rate.

Plant populations (plants m⁻²)

Although plants m⁻² showed a tendency to decrease as row width increased from 17 cm to 34 cm (Table 2) for both years at Roodebloem, differences as found at Langgewens were not significant. This is probably due to the increased interplant competition (more seed planted per row because wide rows meant less rows per unit area).

As expected, due to different amounts of seeds placed, plants m⁻² was reduced as the seeding rate decreased from 7 to 3 kg ha⁻¹ (Table 3) for both years at Roodebloem.

Table 3 Plant populations (plants m⁻²) and yield (kg ha⁻¹) as affected by different row widths, seeding rates and chemical seed treatments during 2003-2004 at Roodebloem and Langgewens Research Farms.

	Roodebloem			Langgewens	
	2003	2004	2003	2004	
	Plants m ⁻²	Plants m ⁻²	Yield (kg ha ⁻¹)	Plants m ⁻²	
Row width (cm):					
17	115.8 <i>a</i>	72.6 a*	1889.5 a	76.8 a	
34	108.1 <i>a</i>	70.6 a	1675.8 <i>b</i>	73.5 a	
Seeding rate (kg.ha ⁻¹):					
3	59.2 c	50.0 c	1748.2 ab	51.4 a	
5	120.9 <i>b</i>	70.6 b	1724.6 b	79.9 b	
7	155.7 a	94.1 <i>a</i>	1875.1 a	91.7 <i>b</i>	
Seed treatment:					
Control	104.3 a	64.3 b	1659.8 <i>b</i>	76.8 a	
SA-combination	112.2 a	76.6 a	1753.7 b	70.2 a	
Cruiser	119.3 a	73.8 a	1934.4 a	78.4 a	

^{*}Means within each year and for row width, seeding rate and seed treatment groups followed by the same letter are not significantly different (LSD=0.05).

The plant population at Roodebloem (2004) was also significantly increased due to the seed treatment (Table 3). No differences were found between the two chemicals used. Similar trends were found in greenhouse studies using soils from different localities in the southern Cape (Chapter 3).

Similar trends in plants m⁻² due to seeding rates were shown at Langgewens (Tables 3), but no clear trend due to seed treatment was found because of the (already explained) interaction between seeding rate and seed treatment. Glasshouse studies with soils from different localities in the Swartland (Chapter 3) also showed little response to seed treatments.

Yield

Where a row width of 17 cm was used at Roodebloem (2003) plants yielded more per hectare than those in 34 cm rows (Table 3). These results are similar to those of Kondra (1975a) and Morrison *et al.* (1990) who found higher yields with row widths of 15 cm compared to 30 cm.

In spite of significant differences in plants m⁻², grain yield at Roodebloem was not affected by seeding rate (Table 3). Similar results were found by Kondra (1975a) and Degenhardt & Kondra (1981b) who used seeding rates of 2, 4 & 8 kg ha⁻¹ and 3, 6 & 12 kg ha⁻¹ respectively. This may be ascribed to the ability of canola to compensate for lower plant populations as found by Clarke & Simpson (1978a) and McGregor (1987).

Although plant populations (plant m⁻²) were not significantly affected by seed treatment at Roodebloem in 2003, Cruiser-treated seed yielded significantly more

than that of both untreated and seed treated with SA-combination. No significant differences were found between untreated and SA-combination treated seed. Yang & Verma (1992) ascribed higher yields from treated seed to higher plant populations, but this study showed that seed treatment might result in higher yields in the absence of significant effects on plants m⁻². Seedling diseases may therefore also reduce yield due to a reduction in seedling and plant vigour.

Conclusions

The effect of seed treatment with regard to plants m⁻² differed between localities and years, probably due to the occurrence of disease-causing organisms in the soil. The effect of chemical seed treatment on plant population was only significant at Roodebloem in 2004. Although seed treatment with both Cruiser and SA-combination resulted in significant more plants m⁻², plant populations of all treatments (including the untreated control) were within the range of 50-80 plants m⁻² set as optimum for the Western Cape Province (Canola Working Group, 2001).

In spite of the absence of significant differences in plants m-2, Cruiser-treated seed produced significantly higher yields compared to the SA-combination and untreated seed at Roodebloem (2003). More field experiments that include more cultivars and localities are however needed to draw any conclusions with regard to the difference in efficiency between chemicals used.

Plant populations at Roodebloem and Langgewens were significantly (predominantly) affected by seeding rate, which caused a progressive increase in plants m⁻² as seeding rate increased.

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Chapter 5

General conclusions

Canola, as a break crop, is a crucial part of crop rotation for economic as well as agronomic benefits in the Western Cape Province. However, to promote the establishment of canola, it must produce consistent yields. Unfortunately, on average, yields obtained are very low (<1.0 ton ha⁻¹) and inconsistent. This may, amongst others, be due to insects and seedling diseases that affect seedling emergence and survival.

As no chemical seed treatments are at present registered in the RSA, the primary objective of this study was to evaluate the efficiency of chemical seed treatments in incubation studies as well as glasshouse and field experiments.

The incubation studies, done in a sterilized (pathogen-free) environment, showed that seed treatment with Cruiser- and SA-combination (Thiulin® (0.5 g a.i.), Apron® (0.0815 g a.i.), Gaucho® (0.6125 g a.i.) and Rovral® (0.9975 g a.i.) at recommended rates (1X = 3.75ml/250g canola seed for Cruiser and 1X = 0.25g; 1g; 3.92ml and 0.875g per 250g canola seed for Apron, Thiulin, Rovral and Gaucho respectively) decreased the rate of germination, but not the percentage germination.

This negative (phytotoxic) effects due to the seed treatment were enlarged during the initial phases of follow-up incubation studies where seeds were preexposed to the Accelerated Ageing test. Results also indicated an increase in phytotoxicity as application rate increased from 0.5X to 1.0X and eventually 2.0X. Because there were, in contrast to SA-combination, no significant differences in germination percentage and plant length after 96 hours of incubation between the untreated (control) seeds and 0.5X and 1.0X Cruiser treatments, it was concluded that seed treatment with SA-combination was more phytotoxic than with Cruiser.

Glasshouse studies confirmed the phytotoxic potential of chemical seed treatments when high application rates were used under unfavourable growth conditions in the absence of pathogens. Most treatments (application rates) of both chemicals used delayed seed germination when planted in dry pasteurized soil, but not in moist (50% field water capacity) or wet soil. Although Cruiser tended to be more phytotoxic to percentage emergence and therefore also dry mass per pot at high (2X) application rates, SA-combination tended to be more harmful to mass per plant at high (2X) application rates.

Surprisingly, freshly treated seed seemed to be more affected by the treatments in comparison to seed treated 15 months prior to the start of the experiment, especially under extreme soil water (very dry/wet) conditions and in the absence of pathogens. This effect of seed source on germination was in contrast to results reported in literature and therefore needs further investigation.

It is however important to note that no phytotoxic effects due to seed treatment were found where the soil was wetted to 50% of field water capacity (moist soil). These results therefore also illustrated the importance of planting in moist soil.

These effects of growth conditions on the efficiency of the chemical seed treatments were confirmed in the second glasshouse experiment where

significant interactions occurred between the seed treatments and soil from different localities. For soils from localities where diseases occurred (especially soils from Elsenburg, Tygerhoek and Napier naturally infested with *Rhizoctonia solani* and *Pythium* spp.), most of the chemical seed treatments (X0.5, X1, X2) increased the survival percentage, plant length and dry mass per pot. In the absence of diseases canola seedlings from treated seeds showed a reduction in survival percentage, plant lengths and dry mass compared to untreated seeds in some soils that can be interpreted as a phytotoxic effect.

The last study was conducted to evaluate the efficiency of these chemical seed treatments under field conditions. From the trials it became evident that the effect of seed treatment differed between the localities (Roodebloem and Langgewens experimental farms), probably due to the occurrence of soil borne pathogens and/or insects. The effect of chemical seed treatment on plants m⁻² was only significant at Roodebloem in 2004, while an interaction occurred between seeding rate and chemical seed treatment at Langgewens. Seedling emergence at Roodebloem and Langgewens was significantly (predominantly) affected by seeding rate, which caused a progressive increase in plants m⁻² as seeding rate increased; however, this did not affect the yield at Roodebloem. No grain yields were obtained at Langgewens during 2003 due to adverse climatic conditions, but higher yields for plants from Cruiser-treated seeds compared to untreated seeds and SA-combination-treated seeds were obtained at Roodebloem. The yield response to seed treatment however was not affected by either seeding rate or row width at this locality.

It can be concluded that applying chemical seed treatments may have phytotoxic effect on plant populations in the absence of soil borne pathogens and especially during conditions of low soil water contents. The advantages of protected seed when diseases do occur is however much greater. Although field studies at more localities should be conducted to undoubtedly prove their efficiency under field conditions, both chemicals used seemed to be effective.

Although significant differences with regard to grain yield in the field experiment were found between the two chemicals used for the purpose of this study, more research is needed to test their efficiency against specific pathogens and with more cultivars and localities (soil and climatic conditions) before the registration of any of these chemicals as a seed treatment can be recommended.