# FINAL REPORT

## CANODEV project
## Alternaria black spot: Studies on control measures and the effect of disease on yield and seed quality in canola

## CSGA project
## Effect of alternaria black spot on seed quality in canola'


**Executive Summary**

In western Canada, alternaria black spot is caused mainly by *Alternaria brassicae* (Berk.) Sacc. and to a lesser extent by *A. raphani* Groves & Skolko. It is most damaging on spring sown Polish canola (*Brassica rapa*).

Results from this study indicate:

1. Alternaria black spot can reduce yields by as much as 40% in Polish canola.
2. The disease also affects seed quality by reducing seed weight and germination, while increasing green seed count.
3. Disease development can be rapid on plants during seed development growth stages.
4. Spraying with a fungicide, such as iprodione, will provide effective control of the disease.
5. Swathing at the optimum time will reduce some losses from black spot compared to swathing at later growth stages.
6. Seed infection by the pathogens occurs mainly in the seed coat, and the fungus dies out over time.
7. Hot water treatment can reduce seed infection while maintaining germination but microwave treatments which reduce seed infection also reduce seed germination
8. The main effect of seed infection by *A. brassicae* is to reduce emergence.

There are several options available to reduce the damage from this disease. Argentine canola (*B. napus*) can be grown where possible, as it usually suffers less damage than that of Polish canola. Iprodione can be applied to control alternaria black spot as it is registered for application at the 30% bloom stage at 500 g a.i./ha. However, the disease can develop rapidly if conditions are favourable and predicting the weather during pod filling so far in advance (at 30% bloom) is not possible. This means growers who consistently have a black spot problem might be advised to spray each year. Timely swathing rather than straight cutting is also recommended when alternaria black spot is a problem because this shortens the time period when damage from the disease can occur.

**Objectives**

The projects involved several studies which were:

1. Assess disease control methods which include the use of fungicides and management practices. The effect of disease on yield was studied using fungicides for control. Control procedures included fungicide rate and timing, and the use of swathing.
2. Determine the effect of alternaria black spot on seed quality. This was a growth room study where inoculation could be controlled to allow confirmation of the damage that the disease appeared to be doing in the field.
3. Determine the effect of seed infection seed vigour. This was a growth room test.
4. Determine the effect of conditions during seed storage on survival of alternaria in seed. Assessments were made to determine where the pathogens were located in seed as well as assessing various methods which may eliminate the fungus while maintaining germination levels. This included microwave and hot water treatments as well as long term storage at different temperatures.
5. Determine disease development of alternaria black spot in the field. This was an additional experiment.

**Details of Experiments**

**1. Assess disease control methods which include the use of fungicides and management practices.**

**1 a. The effect of alternaria black spot on yield and seed quality of *Brassica rapa* in Saskatchewan from 1995 to 1998 using fungicides to control the disease.**

The purpose of this study was to document the effect of alternaria black spot on seed yield and seed quality, and to determine the effect of application timing and the use of lower rates of the fungicide, iprodione (Rovral) on disease development and seed yield.

Materials and Methods

Field trials were conducted in commercial fields of Polish canola from 1995 to 1998. In 1995 the location was Medstead in a field of AC Parkland seeded on May 27. In 1996 the locations were Medstead and Canwood in fields of Tobin, and at Lake Lenore in a field of AC Sunshine seeded at the end of May to early June. In 1997 tests were established at Medstead, Canwood and Kandahar but the dry summer resulted in low disease levels and no effect of treatment on any parameters were observed so results from 1997 are not reported. In 1998 tests were established at Medstead which was hailed out and at Canwood in a field of AC Sunbeam seeded on May 25.

A randomized complete block design with four replicates was established at each location by rotovating a two metre area around each replicate in mid-June. Plots within replicates were five metres long by two metres wide with about one metre of crop on either side to serve as a guard. A one metre area at the centre of each plot was delineated by hoeing out an area 15 cm wide on either side. This created a pathway for spraying and rating to avoid crop damage, and to define the area of the plot to be harvested.

The treatments were a control sprayed with water applied at 30% bloom, Rovral Flo (iprodione

240 or 250 g/L from Rhône-Poulenc) at 250 and 500 g a.i./ha applied at 30% bloom and at 95% petal drop. The water volume was 100 L/ha. All treatments were applied using a hand-held, $CO_2$ pressurized, 4 nozzle boom sprayer at 35 psi fitted with TeeJet 8001VS nozzles. Treatments at 30% bloom were sprayed about mid-July and at 95% petal drop about the end of July.

Naturally occurring inoculum of *Alternaria* spp. was relied upon for infection. Percent disease was visually assessed on the pods of ten main stems collected at random from each plot in mid-late August. Harvesting of standing crop (1 m by 5 m) was done at crop maturity using a Hege combine. Yield was recorded as kilograms per hectare of dry seed.

Sclerotinia stem rot and blackleg basal stem canker incidence were rated on the stubble of 40 randomly collected plants per plot from the water sprayed treatment. Disease levels were low or slight in all years and would have caused very little or no damage.

Seed weight was determined by weighing five lots randomly sampled with a 100 hole seed counter from the harvested plot seed, and this weight was doubled to give a value for thousand seed weight. Percent green seed count was determined by rolling five lots which were randomly sampled with a 100 hole seed counter.

Approximately 15 g of cleaned seed from each plot was surface disinfected for 10 min in 0.6% sodium hypochlorite solution, drained, then placed in an open 9 cm plastic petri dish, and dried with moving air in a laminar flow hood. The incidence of seed contamination by *Alternaria* spp. was determined by placing 300 randomly selected seeds on V-8 agar containing 40 mg/L of rose bengal and 100 mg/L of streptomycin sulphate. The seeds were distributed onto the agar in 9 cm diameter petri dishes using a vacuum plating device that placed 20 seeds in each dish. Dishes were incubated at 18-24°C under cool white fluorescent light for 12 h alternating with 12 h dark for 5 to 7 days. *Alternaria* spp. were identified using colony appearance, and conidial size and shape observed at a magnification of 25-100 X. Seed infestation incidence for each species was calculated as a percentage and the level of *A. brassicae* and *A. raphani* were combined in the analysis. To determine the percent germination of seed, 200 randomly selected disinfected seeds were placed on water agar (1.8%) containing 100 mg/L streptomycin sulphate and 50 mg/L vancomycin hydrochloride with 20 seeds per petri dish. Dishes were incubated at 18-24°C and germination was rated after 5 to 7 days.

Data were analyzed using the general linear model procedure (PROC GLM) and means of treatments were separated using Least Significant Difference and Dunnett's test (SAS Statistics, Version 6.07. SAS Institute, Box 8000, Cary, NC 27511-8000).

Results and Discussion

Fungicide application increased seed yield by 5 to 41% from 1995 to 1998 (Table 1). Relative to the untreated plots foliar application of iprodione decreased disease severity on pods of main stems. In harvested seed, fungicide treatments lowered the infection percentage of *A. brassicae* and *A. raphani* in seed, decreased the percent of green seed, increased seed weight, and increased germination. Differences were not always significant. Based on seed infections, *A. brassicae* was the only pathogen in 1995 and 1996 and accounted for 60% of disease in 1998.

In the combined analysis where all treatments were used (Medstead 1995, Canwood 1998), spraying at low or high rates or at either growth stage resulted in increase in yield, decrease in disease and improved seed quality (Table 1). Not all differences were significantly different. The study showed that alternaria black spot reduces both seed yield and quality including reduced seed weight, reduced

seed germination, and increased green seed count.

Rovral (iprodione) is registered for the control of alternaria black spot at an application of 500 g a.i./ha at the 20-30% bloom stage which corresponds to the registration for the control of sclerotinia stem rot. In the present study, application of iprodione at 250 and 500 g a.i./ha at 20-30% bloom and at 95% petal drop showed a similar response (Table 1). Yields were slightly lower at 20% bloom than at 95% petal drop but this effect was not significant. This data suggests that the lower rate of application of iprodione may be as effect in controlling the disease. This could save growers about $10 per acre for the chemical costs. Control occurs over a wide period and is effective between 30% bloom to 95% petal drop.

If alternaria black spot is present, growers may experience lower yields and could also have seed downgraded because of higher green seed counts. Seed growers suffer additional losses due to reduced seed weight and reduced germination, which may lower the grade as well as contribute to more cleanout.

Fields that had not been seeded to canola for several years previous were chosen for these studies. This should have minimized the disease severity of alternaria black spot for which a four year crop rotation is recommended. Even with this long rotation, alternaria black spot caused considerable damage.

Interim reports have described the use of additional fungicides and have summarized results for 1997, the year with low levels of disease. Appendix 1 is a report summarizing the 1998 test and was submitted for publication in the Pesticide Research Report. The product from Zeneca, ICIA5504, showed activity comparable to that of Rovral (see Appendix 1).

**1 b. Effect of swathing on alternaria black spot in *Brassica rapa*.**

This experiment was written up as a scientific publication and will appear in the Canadian Journal of Plant Science. The abstract is presented below and the full paper is attached as Appendix 2.

From 1990 to 1997 experiments were conducted comparing the effect of swathing versus straight combining on seed infection by *Alternaria* species and on quality of harvested seed. This was done at several locations in Saskatchewan using a number of cultivars of *Brassica rapa* and *B. napus*. The level of *A. brassicae* in harvested seed was significantly higher in straight combined than in swathed treatments. Green seed count and seed weight were not affected by treatment. Seed germination was reduced, but not always significantly, with straight combining compared to swathing. This data supports the recommendation for swathing at the optimal time to reduce alternaria black spot when the potential for disease development is high. Swathing reduces the time for disease development because it reduces the time of ripening compared to straight combining.

**2. Determine the effect of *Alternaria brassicae* inoculation in a growth chamber on seed quality of *Brassica rapa*, cv. R500.**

The following studies were conducted to investigate the effect of *A. brassicae* and *A. raphani* on seed quality following inoculation with the pathogens in a growth chamber. These experiments under controlled conditions would help confirm the effect of disease in the field.

Materials and Methods

Seed of the *B. rapa* cultivar R500 was planted in 30 cm diameter pots in a soil-less mix in a growth chamber with an 18 h day with a temperature of 20°C in the day and 15°C at night. Plants were thinned to one plant per pot and inoculated at 95% petal drop. Half the plants were sprayed to run off with distilled water and the other half with a 3 x $10^4$ spores per mL inoculum of *A. brassicae* or *A. raphani*. Spore suspensions originated from 2-9 week old colonies grown on V-8 juice, rose bengal agar. A plastic tent enclosed the plants for 48 h in the dark with a humidifier positioned inside to maintain leaf wetness. At maximum disease development, main stems of plants were rated as percentage coverage of black lesions. Plants were tagged and at or near maturity they were harvested, dried and threshed individually. Infection rates in seed, seed germination, percent green seed and thousand seed weight were determined following the procedures described in the first study. Two tests were done with *A. brassicae* and one with *A. raphani*.

Data were analyzed using the regression procedure (SAS Statistics, Version 6.07. SAS Institute, Box 8000, Cary, NC 27511-8000). A univariate analysis indicated that no transformation improved the normality of the data so no transformations were done.

Results and Discussion

Increasing black spot disease rating on pods resulted in increasing levels of seed infection with *A. brassicae* or *A. raphani* in seed harvested from those pods (Tables 2, 3). Increasing disease on pods was significantly related to reduction in seed germinability in two of three tests and increasing percentages of green seed in all tests. Infection with *A. brassicae* seemed to result in higher green seed percentages than with *A. raphani*. Disease on pods was related to reduced seed weight in one of three tests. The data support the field results and indicate that alternaria black spot is able to reduce seed quality.

**3. Determine the effect of seed infection with *Alternaria brassicae* on seed vigour in Polish canola.**

The objective of this study was to determine the effect of *A. brassicae* on seed vigour.

Materials and Methods

Seed was obtained from a field of Polish canola, *B. rapa* cv. AC Sunshine, grown at Lake Lenore SK in 1996. Seed was chosen which went through a 4/64 sieve. Since seed infection by *A. brassicae* usually causes grey seed, grey seed was separated from the sieved seed. Seed without grey seed was the clean seed. To assess vigour, seed was planted on soil-less mix and covered with 2 cm of soil-less mix in 50 cm x 26 cm plastic trays as seven rows of 10 seeds each of grey seed alternating with seven rows of clean seed. There were six trays placed in a growth cabinet set at 10°C with a 12 h day/night cycle. Maximum emergence was recorded and this occurred after about 14 days. The test was repeated twice. In each test, seed was also assessed for germination and infection by *A. brassicae* by following the procedure described in the first study.

Results and Discussion

The percentage of seed infection was higher in grey seed while germination and emergence were

lower (Table 4). The main effect of *A. brassicae* is to reduce germination. It is possible that there may be an effect on vigour (emergence at 10°C) but this was not observed in our study where grey seed which had a high level of *A. brassicae* infection was used.

**4. Determine the effect of conditions during seed storage on survival of alternaria in seed.**

**4 a. Infection of parts of canola seed by *Alternaria brassicae.***

The objective of this study was to determine where the fungus is located in seed.

Materials and Methods

Seed samples were taken from *B. rapa* cv. Tobin crops grown at Medstead SK in 1994 and 1996, and at Belbutte in 1996. Seed that passed through a 4.5/64 sieve was used, and grey seed was selected because it has a high level of infection by *A. brassicae*. The seed was surface sterilized for 10 min in 0.6% sodium hypochlorite solution, and then soaked for at least 10 min in sterile distilled water to facilitate dissection. Dissection of seed into five parts was done with a scalpel and tweezers using aseptic techniques in a laminar flow hood with the aid of a binocular microscope at 6-12X. The seed coat was cut in half with the hilum end on one side and the nonhilum end on the other. The embryo was dissected into the hypocotyl, outer cotyledon and inner cotyledon. The parts were placed on V-8 juice agar modified with rose bengal (10 mg/L) and streptomycin sulphate (100 mg/L), then incubated under fluorescent lights (12 h) for one week.

Only seed with one or more of the five parts infected by *A. brassicae* were used in the analysis. There were 60 seeds from the 1994 Medstead crop, 25 from 1996, and 34 from the 1996 Belbutte crop. Actual values summed over samples were assessed using chi-square analysis. A general linear model analysis (SAS Statistics, Version 6.07. SAS Institute, Box 8000, Cary, NC 27511-8000) was done after converting values for each part to a percent of total seed for each sample. Conversion to a percent was done to remove the influence of sample size.

Results and Discussion

There were 19 patterns of infection (Table 5). A chi-square analysis indicated a significant difference in the infection between parts of the seed ($X^2 = 211.9$, df = 4, $P<0.005$) (Table 6). Infection levels were highest in the hilum end of the seed coat followed by the nonhilum end, the outer cotyledon, inner cotyledon and hypocotyl (Table 7). There was no difference between samples which indicates infection in different seed parts was similar between samples.

The data suggests that the fungus first penetrates the seed via the funiculus, grows through the hilum and then generally invades the seed coat. If seed is infected, almost 100% will be infected at the hilum end of the seed coat, 50% at the nonhilum end of the seed coat, and 25-40% of the internal seed parts will be infected (Table 7). Nevertheless, 4% of seed (5 out of 119) was infected only in the embryo and not in the seed coat. The reason for this could be that the fungus was killed in the seed coat during the surface sterilization stage, or that the fungus died out first in the seed coat but not internally in the normal process of the fungus dying out in seed.

**4 b. Survival over time of *Alternaria brassicae* in Polish canola seed stored at various**

**temperatures.**

There is some information indicating that the fungus dies out in seed over time and that this process is related to temperature. The objective of this study was to determine how long *A. brassicae* survives in seed of Polish canola.

Materials and Methods

Seed was obtained from a field of Polish canola, *B. rapa* cv. AC Sunshine grown at Lake Lenore SK in 1996. One hundred gram samples in airtight containers were placed in five incubators which were kept at the following temperatures, -18, 4, 10, 20, 30 °C. Samples of seed were taken at various time periods, 0, 33, 64, 95, 121, 154, 196, 262, 368, and 463 days after the seeds were placed in the incubators on November 25, 1996. At each sample date seed was assessed for germination and seed infection by *A. brassicae* using the procedure outlined in the first study.

Data were analyzed using the general linear model procedure (SAS Statistics, Version 6.07. SAS Institute, Box 8000, Cary, NC 27511-8000). No transformation of data was used since analysis of the distribution (PROC UNIVARIATE) indicated that no transformation improved the normality of the data.

Results and Discussion

Seed germination at the various temperatures was not significantly different (P=0.072). The mean of dates for -18, 4, 10, 20, and 30 °C was 87.4, 87.5, 86.5, 86.8, and 88.9, respectively with an L.S.D. of 1.66 (P=0.05). The plot of the values also demonstrates no change in germination (Figure 1).

The percentage of seed infected with *A. brassicae* was significantly different (P=0.0001) for temperature because of the decline in infection at 30 °C (Figure 2). The mean of dates for -18, 4, 10, 20, and 30 °C was 22.7, 21.7, 21.9, 20.8 and 16.3, respectively with an L.S.D. of 2.61 (P=0.05).

*A. brassicae* infection in seed decreases over time. Seed growers might be able to apply this knowledge if they have seed with low germination which is due to alternaria infection.

**4 c. The effect of hot water treatment on the germination and seed infection by *Alternaria* spp. in Polish canola.**

Materials and Method

A 250 mL beaker containing 200 mL of distilled water was put inside a 400 mL beaker containing 50 mL of water. The water was brought up to the appropriate temperature at a moderate stir speed using a hot/stir plate. The temperature was maintained within 2 °C by adding hot or cold distilled water. Once a stable temperature was attained, 5 g of infected Polish canola seed from Lake Lenore SK (cv. AC Sunshine) in 1996 or Manning AB (cv. Reward) in 1997 was added and treated for the appropriate time. Seed was initially surface disinfested for 10 min with 0.6% sodium hypochlorite and air dried. Ten repeats of each hot water regime was done. The seed was then tested to determine percent germination and percent infection using the procedure described in the first study.

Results and Discussion

Levels of *A. brassicae, A. raphani,* and *A. alternata* were all significantly reduced when subjected to various hot water treatment regimes (Tables 8-10). Infection was nearly eliminated when seed was soaked for 15 to 35 min at 50°C, however, germination was also significantly reduced. The optimum hot water regime for both disease reduction and acceptable germination was 45°C for 15 min. This technology could be used for small seed samples to eliminate seed infection while maintaining seed germination. However, other alternatives such as seed treatment are more practical for commercial production.

**4 d. The effect of microwave treatment on the germination and seed infection by *Alternaria* spp. in Polish canola**

The objective of this study was to determine whether microwave treatment could eliminate seed borne infection of Polish canola by *Alternaria* spp. without affecting seed germinability.

Materials and Methods

Alternaria infected Polish canola seed cv. AC Sunshine collected from Lake Lenore SK in 1996 was put in an open petri dish in a humidity chamber (closed container with water) until the weight of the seed minus the plate increased by 10%. Then the plate and seed were immediately microwaved for 30 sec and within 5 min the seed was disinfected for 10 minutes with 0.6% sodium hypochlorite and air dried. The seed was then tested to determine percent germination and percent infection by the procedure described in the first study. Ten repeats of each treatment were done. Levels of *A. raphani* in this seed sample were very low so only data for *A. brassicae* are presented.

Results and Discussion

Generally the presence of *A. brassicae* in seed decreased when exposed to progressively higher wattage treatments (Table 11). Seed treated for 30 sec at 90 watts showed no significant difference from the control for seed infection or germination. However, seed treated at 277.5 to 925 watts resulted in significantly reduced infection levels and significantly reduced germination levels. Increasing wattage had a detrimental effect on germination levels. It is unlikely that this technology could be used effectively to reduce infection levels while maintaining seed germination.

**5. Determine disease development of alternaria black spot in Polish canola in the field.**

Some reports suggest that disease development is particularly rapid at the time of seed development. This study was done to determine the increase of disease symptoms on pods at the time of seed development during two relatively high disease years, 1996 and 1998.

Materials and Methods

In 1996, the site was at Medstead SK in a field of Tobin, while in 1998 it was at Canwood SK in a field of AC Sunshine. Naturally occurring inoculum was relied upon for infection. In each field, randomly scattered plants were individually tagged. The main stems of these plants were visually assessed for disease symptoms on the pods using the scale of Conn et al. (Can. Plant Dis. Survey 70:119-122, 1990). At each date the growth stage was assessed on plants closely associated with

those being rated. In 1996, rating on 15 plants was done on August 21 (10% seed colour change), August 26 (25%), August 29 (80%), and September 3 (88%); the planting date was May 28. In 1998, rating on 36 plants was done on July 21 (no colour change, green to hard rolled stage), July 23 (1%), July 27 (15%), July 30 (35%), and August 6 (88%); the planting date was May 25.

Results and Discussion

Symptoms of alternaria black spot increased on pods during seed ripening (Figure 3). The increase was fairly linear throughout the time period but perhaps slightly greater after 25% of seeds had turned colour. From the 25% seed colour change stage to 88% seed colour change the disease increased by about 5% for every increase of 20% seed colour change. The data indicate that alternaria black spot increases rapidly during seed ripening in years that favour disease development. Based on assessment of pathogens present in harvested seed, the disease was due to *A. brassicae* in 1996, and 60% of infections were due to *A. brassicae* and 40% due to *A. raphani*; in 1998.

This data has implications for disease prediction relative to using fungicide application for control. Alternaria black spot severity will be difficult to predict especially at early growth stages, 20-30% bloom, when iprodione should be applied. The disease can develop rapidly later if conditions are favourable and longterm weather preditions are not accurate. Because of this, in high value Polish canola crops, in areas where black spot has been a problem, it may be appropriate to apply fungicides every year.

Acknowledgements

The technical support of Sheila Reed and Karyn Sutherland is gratefully acknowledged. The authors also wish to thank R. Prodahl, S. Rude, E. Seidle and J. Vopne for allowing plots to be set up in their fields. Michael Langenberger assessed in the seed dissection study.

S.R. Rimmer, L.J. Duczek and E. Seidle March 12, 1999

**Table 1. The effect of Rovral (iprodione) applied at two rates, 250 and 500 g a.i./ha, and at 20-30% bloom and 95% petal fall on alternaria black spot, grain yield, seed weight, green seed count, seed germination, and in seed infection by *Alternaria brassicae* and *A. raphani* in Saskatchewan, 1995-98.**

| Treatment | Yield | | % | % | Seed Quality | | |
|---|---|---|---|---|---|---|---|
| | kg/ha | % increase | disease on pods | Alternaria in seed | Thousand seed wt. | % green | % germ |
| 1995 at Medstead, SK - iprodione at 250 and 500 g a.i./ha (n=4) | | | | | | | |
| Nontreated | 1579 | - | 7.5 | 20.3 | 2.57 | 12.0 | 90 |
| Rovral 500 - 30% bloom | 2155* | 36% | 4.0 | 15.1 | 2.67 | 10.4 | 91 |
| Rovral 250 - 95% petal | 2140* | 36% | 2.9* | 4.8* | 2.81* | 6.4 | 95* |
| Rovral 500 - 95% petal | 1926 | 22% | 3.3* | 5.8* | 2.74 | 8.5 | 96* |
| L.S.D. (P=0.05) | 330 | | 3.0 | 5.8 | 0.19 | 4.7 | 4 |
| 1996 at Medstead, SK - iprodione at 250 or 500 g a.i./ha (n=4) | | | | | | | |
| Nontreated | 2251 | - | 7.1 | 14.3 | 2.46 | 5.2 | 90 |
| Rovral 250 - 30% bloom | 2426 | 8% | 0.3* | 4.8* | 2.58 | 3.2 | 97* |
| Rovral 500 - 30% bloom | 2357 | 5% | 2.9* | 8.8 | 2.50 | 4.4 | 96 |
| Rovral 250 - 95% petal | 2744* | 22% | 2.4* | 11.8 | 2.59 | 4.3 | 94 |
| Rovral 500 - 95% petal | 2416 | 7% | 3.4* | 8.8 | 2.46 | 4.4 | 93 |
| L.S.D. (P=0.05) | 316 | | 2.7 | 5.3 | 0.15 | 1.8 | 5 |
| 1996 at Canwood, SK - iprodione at 250 or 500 g a.i./ha (n=4) | | | | | | | |
| Nontreated | 1217 | - | 4.0 | 15.5 | 2.00 | 3.7 | 93 |
| Rovral 250 - 95% petal | 1366 | 12% | 1.4* | 7.8 | 2.20* | 1.3* | 96 |
| Rovral 500 - 95% petal | 1372 | 13% | 0.2* | 1.8* | 2.32* | 0.5* | 96 |
| L.S.D. (P=0.05) | 182 | | 1.8 | 7.8 | 0.13 | 2.0 | 5 |
| 1996 at Lake Lenore, SK - iprodione at 250 or 500 g a.i./ha (n=4) | | | | | | | |
| Nontreated | 1126 | - | 21.4 | 21.3 | 2.05 | 9.1 | 88 |
| Rovral 250 - 95% petal | 1357 | 21% | 12.0* | 12.5* | 2.23* | 6.0 | 92 |
| Rovral 500 - 95% petal | 1530* | 36% | 2.8* | 4.8* | 2.48* | 2.2* | 96 |
| L.S.D. (P=0.05) | 333 | | 5.9 | 6.8 | 0.08 | 3.0 | 8 |
| 1998 at Canwood, SK - iprodione at 250 or 500 g a.i./ha (n=4) | | | | | | | |
| Nontreated | 884 | - | 14.5 | 18.1 | 2.35 | 3.3 | 88 |
| Rovral 250 - 30% bloom | 1060 | 20% | 7.0* | 15.1 | 2.43 | 2.6 | 90 |
| Rovral 500 - 30% bloom | 1187* | 34% | 4.9* | 16.1 | 2.58* | 1.6* | 90 |
| Rovral 250 - 95% petal | 1032 | 17% | 6.3* | 13.6 | 2.45 | 1.9* | 93 |
| Rovral 500 - 95% petal | 1243* | 41% | 3.1* | 9.4* | 2.70* | 1.9* | 95 |
| L.S.D. (P=0.05) | 222 | | 4.1 | 5.0 | 0.15 | 0.8 | 5 |
| Combined Analysis of 1995 Medstead and 1998 Canwood (n=8) | | | | | | | |
| Nontreated | 1568 | - | 10.8 | 16.2 | 2.40 | 4.3 | 89 |
| Rovral 250 - 30% | 1743 | 11% | 3.6* | 9.9* | 2.50 | 2.9* | 93* |
| Rovral 500 - 30% | 1772 | 13% | 3.9* | 12.4 | 2.54* | 3.0* | 93* |
| Rovral 250 - 95% | 1888* | 20% | 4.3* | 12.7 | 2.52 | 3.1 | 93* |
| Rovral 500 - 95% | 1830 | 17% | 3.3* | 9.1* | 2.58* | 3.2 | 94* |
| L.S.D. (P=0.05) | 212 | | 3.0 | 3.4 | 0.10 | 0.9 | 3 |

* Indicates a significant difference at P=0.05 between a treatment and the nontreated check using Dunnett's test.

**Table 2. Effect of *Alternaria brassicae* inoculation in a growth chamber on seed quality of *Brassica rapa*, cv. R500.**

**Test 1**

| Disease Rating (% lesions) | % *A. brassicae* | %Germ | Seed Weight (g) | %Green Seed |
|---|---|---|---|---|
| 0 | 0.0 | 100 | 4.8 | 0.0 |
| 1 | 0.0 | 100 | 5.1 | 0.6 |
| 2 | 1.7 | 99 | 5.2 | 2.0 |
| 3 | 0.7 | 100 | 5.8 | 0.2 |
| 4-5 | 4.3 | 100 | 5.2 | 0.6 |
| 6-7 | 15.3 | 99 | no seed | no seed |
| 8-10 | 20.7 | 87 | 5.0 | 12.4 |
| 11-19 | 24.0 | 95 | 5.2 | 12.2 |
| 20-29 | 38.3 | 86 | 4.3 | 20.8 |
| 30-39 | 37.0 | 90 | 4.5 | 75.5 |
| 40-49 | 54.0 | 65 | no seed | no seed |
| 50-59 | 57.3 | 47 | no seed | no seed |
| Regression analysis between Disease Rating and other variables: | | | | |
| $R^2$ | 0.927 | 0.893 | 0.327 | 0.682 |
| Prob>F | 0.0001 | 0.0001 | 0.063 | 0.004 |

**Test 2**

| Disease Rating (% lesions) | % *A. brassicae* | %Germ | Seed Weight (g) | %Green Seed |
|---|---|---|---|---|
| 0 | 0.0 | 99 | 5.6 | 0.0 |
| 1-9 | 2.3 | 100 | 5.3 | 1.8 |
| 10-19 | 9.3 | 97 | 5.7 | 7.2 |
| 20-29 | 34.3 | 95 | 5.3 | 8.6 |
| 30-39 | 43.5 | 83 | 4.7 | 24.4 |
| 40-49 | 35.0 | 94 | 5.1 | 18.2 |
| 50-59 | 32.0 | 91 | 5.2 | 16.9 |
| Regression analysis between Disease Rating and other variables: | | | | |
| $R^2$ | 0.619 | 0.329 | 0.221 | 0.656 |
| Prob>F | 0.022 | 0.104 | 0.161 | 0.017 |

**Table 3. Effect of *A. raphani* inoculation in a growth chamber on seed quality of *Brassica rapa*, cv. R500.**

| Disease Rating (% lesions) | % *A. raphani* | %Germ | Seed Weight (g) | %Green Seed |
|---|---|---|---|---|
| 0 | 0.0 | 100 | 6.3 | 0.0 |
| 1-9 | 24.7 | 97 | 6.4 | 0.1 |
| 10-19 | 50.7 | 96 | 5.9 | 2.0 |
| 20-29 | 49.0 | 98 | 5.4 | 1.4 |
| 30-39 | 79.4 | 97 | 5.3 | 2.8 |
| 40-49 | 76.0 | 92 | 5.0 | 3.5 |
| 50-59 | 81.0 | 94 | 4.5 | 4.3 |
| Regression analysis between Disease Rating and other variables | | | | |
| $R^2$ | 0.774 | 0.496 | 0.969 | 0.894 |
| Prob>F | 0.006 | 0.047 | 0.0001 | 0.0008 |

**Table 4. Effect of *Alternaria brassicae* (Ab) infection on Polish canola seed germination and emergence at 10°C.**

| Assessment | Seed | Test 1 | Test 2 | Test 3 | Mean | S.E. |
|---|---|---|---|---|---|---|
| % Infection with Ab | Clean | 1.2% | 1.3% | 1.0% | 1.2% | 0.09 |
| | Gray | 70.7% | 65.0% | 71.8% | 69.2% | 2.11 |
| Seed germination | Clean | 99.7% | 99.0% | 100.0% | 99.6% | 0.30 |
| | Gray | 20.7% | 23.0% | 15.5% | 19.7% | 2.22 |
| Emergence at 10°C | Clean | 97.6% | 97.3% | 98.6% | 97.8% | 0.39 |
| | Gray | 6.0% | 4.6% | 8.1% | 6.2% | 1.02 |

**Table 5. The presence of *Alternaria brassicae* in various parts of infected seed of Tobin canola in seed samples from Medstead in 1994 (M94), in 1996 (M96), and from Belbutte in 1996 (B96).**

| Patterns of infestation | Seed Coat | | Embryo | | | Number of seeds | | |
|---|---|---|---|---|---|---|---|---|
| | Hilum end | Nonhilum end | Hypocotyl | Outer cotyledon | Inner cotyledon | M94 | M96 | B96 |
| 1 | x | | | | | 15 | 8 | 13 |
| 2 | x | x | | | | 25 | 2 | 1 |
| 3 | x | x | | x | | 4 | 2 | 5 |
| 4 | x | x | | x | x | 0 | 2 | 1 |
| 5 | x | x | x | x | x | 7 | 4 | 2 |
| 6 | x | x | x | x | | 1 | 1 | 1 |
| 7 | x | x | | | x | 0 | 0 | 1 |
| 8 | x | | x | x | x | 1 | 0 | 1 |
| 9 | x | | | x | x | 1 | 0 | 2 |
| 10 | x | | x | | x | 0 | 0 | 1 |
| 11 | x | x | x | | | 3 | 0 | 1 |
| 12 | x | | x | | | 0 | 2 | 1 |
| 13 | x | | x | x | | 1 | 0 | 1 |
| 14 | x | | | | x | 0 | 0 | 1 |
| 15 | x | | | x | | 0 | 0 | 2 |
| 16 | | x | | | x | 1 | 0 | 0 |
| 17 | | | | x | | 0 | 1 | 0 |
| 18 | | | x | x | x | 1 | 2 | 0 |
| 19 | | | x | | | 0 | 1 | 0 |
| Total | 113 | 64 32 | 43 | 28 | 60 | 25 | 34 | |

**Table 6. Chi-square analysis of *Alternaria brassicae* in various parts of infected Tobin canola in seed samples from Medstead in 1994 and 1996, and Belbutte in 1996.**

| Seed Part | Observed | Expected | Deviation | Dev. $^2$ | Dev. $^2$ / Expected |
|---|---|---|---|---|---|
| Seed coat | | | | | |
| Hilum | 113 | 119 | 6 | 36 | 0.3 |
| Nonhilum | 64 | 119 | 55 | 3025 | 25.4 |
| Embryo | | | | | |
| Hypocotyl | 29 | 119 | 90 | 8100 | 68.1 |
| Outer cot. | 43 | 119 | 76 | 5776 | 48.5 |
| Inner cot. | 28 | 119 | 91 | 8281 | 69.6 |
| Total | 119 | | | | 211.9* |

* $x^2 = 211.9$, df = 4, $P < 0.005$

**Table 7. Levels of *Alternaria brassicae* in various parts of infected Tobin canola in samples from Medstead in 1994 and 1996, and Belbutte in 1996.**

| **Seed Part** | **% Infection** |
|---|---|
| Seed coat | |
| Hilum | 93.6 a |
| Nonhilum | 49.2 b |
| Embryo | |
| Hypocotyl | 24.9 c |
| Outer cot. | 39.6 bc |
| Inner cot. | 25.6 c |
| L.S.D. ($P = 0.05$) | 22.0 |

| GLM | F Value | Pr > F |
|---|---|---|
| Part | 17.46 | 0.0005 |
| Seed Sample | 0.02 | 0.9840 |

[a-c] Values with different letters indicate significant differences (P=0.05) with L.S.D.

**Table 8. The effect of various hot water regimes on infection by *Alternaria* spp. and germination of *B. rapa* cv. AC Sunshine collected from Lake Lenore in 1996.**

| Treatment | | | | | |
|---|---|---|---|---|---|
| Time(min.) | Temp.(°C) | *A. brassicae* (%) | *A. raphani* (%) | *A. alternata* (%) | Germination(%) |
| 0 | n/w | 15.2a** | 0.2bc** | 0.9a** | 90.1ab** |
| 35 | 23 | 14.8a | 0.8a | 0.9a | 92.9a |
| 15 | 40 | 5.0b | 0.2bc | 0.7a | 93.3a |
| 20 | 40 | 4.3b | 0.3b | 1.0a | 92.8a |
| 15 | 45 | 0.6c | 0.3b | 0.2b | 91.0ab |
| 15 | 50 | 0.1d | 0.0c | 0.1b | 86.6b |
| 25 | 50 | 0.0d | 0.0c | 0.0b | 72.2c |
| 35 | 50 | 0.0d | 0.0c | 0.0b | 71.5c |

*Values in the same column which are not followed by the same letter are significantly different at $P$=0.05 according to Duncan's Multiple Range Test.
**Variables were transformed using a square root transformation.

**Table 9. The effect of various hot water treatments on seed infection by *Alternaria* spp. and germination using *B. rapa* cv. Reward seed collected from Manning AB in 1997.**

| Treatment | | | | | |
|---|---|---|---|---|---|
| Time(min.) | Temp.(°C) | *A. brassicae* (%) | *A. raphani* (%) | *A. alternata* (%) | Germination(%) |
| 0 | n/w | 8.5a** | 9.6b** | 4.4a** | 82.4b |
| 35 | 23 | 7.4b | 22.3a | 4.0a | 85.8a |
| 15 | 40 | 1.3c | 7.4bc | 2.6b | 87.2a |
| 20 | 40 | 0.2d | 4.1cd | 0.4d | 85.9a |
| 15 | 45 | 0.1d | 1.9de | 1.2c | 87.9a |
| 15 | 50 | 0.0d | 0.2e | 0.3d | 79.9b |

*Values in the same column which are not followed by the same letter are significantly different at $P$=0.05 according to Duncan's Multiple Range Test.
**Variables were transformed using a square root transformation.

**Table 10. The effect of various hot water treatments on seed infection and germination on a combination of the two seed lots.**

| Treatment | | | | | |
|---|---|---|---|---|---|
| Time(min) | Temp.(°C) | *A.brassicae* (%) | *A. raphani* (%) | *A. alternata* (%) | Germination(%) |
| 0 | n/w | 11.9a** | 4.9b** | 2.7a** | 86.3ab |
| 35 | 23 | 10.7a | 12.7a | 2.6a | 88.9a |
| 15 | 40 | 3.2b | 3.8b | 1.7a | 90.3a |
| 20 | 40 | 2.5b | 2.0bc | 0.7b | 89.7a |
| 15 | 45 | 0.4c | 1.1bc | 0.7b | 89.5a |
| 15 | 50 | 0.1c | 0.1c | 0.2bc | 83.2b |
| 25 | 50 | 0.0c | 0.0c | 0.0c | 72.2c |
| 35 | 50 | 0.0c | 0.0c | 0.0c | 71.5c |

*Values in the same column which are not followed by the same letter are significantly different at $P$=0.05 according to Duncan's Multiple Range Test.
**Variables were transformed using a square root transformation.

**Table 11. The effect of microwave treatment on seed infection by *A. brassicae* and germination of *B. rapa* cv. AC Sunshine collected from Lake Lenore in 1996.**

| Wattage(w) | *A. brassicae* (%) | Germination(%) |
|---|---|---|
| 0 | 18.3a* | 89.7a* |
| 90 | 19.0a | 88.6a |
| 277.5 | 10.2b | 66.9b |
| 508.8 | 6.8c | 47.2c |
| 647.5 | 5.1d | 48.8c |
| 925 | 2.6e | 27.4d |

* Letters in the same columns distinguish significant differences at a level of $p$=0.05 using Duncan's multiple range test. Results are based on square root transformation in the case of *A. brassicae* levels and log transformations in the case of germination.

Appendix 1.

**PMR REPORT NO.** **SECTION L: DISEASES OF CEREALS, FORAGES AND OILSEEDS**
**STUDY DATA BASE NUMBER:** 375-1411-8719

**CROP:** *Brassica rapa*, cultivar AC Sunbeam
**PEST:** Alternaria black spot, *Alternaria* spp.

**NAME AND AGENCY:**
REED S L, RIMMER S R, and SEIDLE E
Agriculture and Agri-Food Canada, Research Centre, 107 Science Place, Saskatoon, Saskatchewan S7N 0X2
**Tel:** (306)956-7200 **Fax:** (306)956-7247 **Email:** rimmerr@em.agr.ca
Seidle Seed Farm, Box 146, Medstead, Saskatchewan S0M 1W0

**TITLE: EFFECT OF FUNGICIDE APPLICATION ON ALTERNARIA BLACK SPOT IN AC SUNBEAM CANOLA, 1998.**

**MATERIALS:** ROVRAL FLO (iprodione 250g/L), ICIA5504 (azoxystrobin 800g/kg).

**METHODS:** A randomized complete block test with four replicates was established in a commercially grown field of AC Sunbeam canola, at Canwood, Saskatchewan in 1998. The crop was seeded on May 25. The test plots were established on June 11, by rotovating a two metre area around each replicate. Plots were five metres long by two metres wide, with a one and one half metre guard area on either side of each plot. As the site was air seeded, a one metre area at the centre of each plot was delineated by hoeing out an area 15 cm wide on either side. This created a pathway for spraying to avoid crop damage and to define the area of the plot to be harvested. Naturally occurring inoculum of *Alternaria* spp. was relied upon for infection. All treatments were sprayed using a hand-held, $CO_2$ pressurized, four nozzle boom sprayer at 35 psi fitted with TeeJet 8001VS nozzles. The water volume was 100L/ha for all treatments. ROVRAL FLO (250 gai/ha, 500gai /ha), ICIA5504 (125 gai/ha, 250gai/ha) were sprayed on June 30 when the plants were at full bloom. All other treatments including a water control were sprayed on July 10 at 95% petal drop. Ten main stems from each plot were visually assessed for disease on pods on July 30, when the seed was at 20-50% colour change stage. Plots were harvested (7 rows x 5 m long) on August 17 and yield was recorded as kilograms per hectare of dry grain. Subsamples were taken from each plot and the seed was surface disinfested for 10 min with 0.6% sodium hypochlorite and then air dried. This seed was then used to determine percent germination and percent infection by the three *Alternaria* spp. - *A. brassicae*, *A. raphani*, and *A. alternata.* To determine percent germination 200 seeds/plot were vacuum plated (20 seeds/plate) onto 1.8% water agar amended with 100mg/L streptomycin and 50mg/L vancomycin. These plates were incubated at 20 C for 3-5 days, at which time germinated seed was counted and calculated as a percentage of the total seeds plated. Three hundred seeds/plot were plated (20 seeds/plate) on V-8 juice agar amended with 40mg/L rose bengal and 100mg/L streptomycin. After seven days under fluorescent lights (12 hour day/night cycle) at 18-24 C, the plates were examined for presence of the two *Alternaria* spp. The species were differentiated by examining colony morphology, and by determining spore shape and size under a compound microscope. Results were reported as the percentage of total seed infested. Percent green seed was determined by crushing 500 seeds/plot and counting the number of green seeds. Thousand kernel weights were determined by weighing 500 seeds and multiplying by two. Data was analysed using an analysis of variance procedure.

**RESULTS**: See Tables 1 and 2 below. All treatments significantly decreased disease levels (Table 1). Spraying ROVRAL FLO at full rate and ICIA5504 at both full and half rates at 95% petal drop resulted in the greatest disease control. These treatments also resulted in the greatest increase in yield compared to the check. All treatments resulted in a significant increase in yield except ROVRAL FLO 250gai sprayed at either growth stage. Thousand kernel weight was greater in all treatments compared to the check. Levels of all species of *Alternaria* and percent green seed were decreased with all treatments but not always significantly. Treatment did not significantly affect germination of the seed.

**CONCLUSIONS:** All fungicide treatments applied decreased the incidence of black spot in canola. Yield increased, but not always significantly with all fungicide treatments. The greatest improvement in yield was 42% with ICIA5504 sprayed at full bloom. All fungicide treatments generally improved seed quality by decreasing percent green seed and seed infestation with *A. brassicae*, and increasing seed weight. Only ROVRAL FLO (500gai at 95 % petal drop) decreased the seed infection with *A. raphani*. None of the fungicide treatments decreased the incidence of *A. alternata*. Both ICIA5504 and ROVRAL controlled disease on the pods and decreased seed infestation by *A. brassicae* and *A. raphani* when applied at full rates at 95% petal drop.

**Table 1.**The effect of foliar applied fungicides on mean percent disease of alternaria black spot on main stem pods and yield of AC Sunshine canola.

| Product | Rate (/ha) | Growth Stage* | Alt. Blk. Spot (% disease)** | Yield (kg/ha)** | 1000 KWT (g)** |
|---|---|---|---|---|---|
| Control | ----- | 2 | 14.4a*** | 1280b*** | 2.4d*** |
| ROVRAL FLO | 500gai | 1 | 4.9bcd | 1720a | 2.6bc |
| ROVRAL FLO | 500gai | 2 | 3.1cde | 1800a | 2.7ab |
| ROVRAL FLO | 250gai | 1 | 7.0b | 1540ab | 2.4cd |
| ROVRAL FLO | 250gai | 2 | 6.3bc | 1500ab | 2.5cd |
| ICIA5504 | 250gai | 1 | 4.0bcde | 1820a | 2.6bc |
| ICIA5504 | 250gai | 2 | 1.2e | 1740a | 2.6bc |
| ICIA5504 | 125gai | 2 | 1.9de | 1750a | 2.8a |

* 1=full bloom; 2=95% petal drop.

**Based on the mean of four replicates.

***Values in the same column which are not followed by the same letter are significantly different at P=0.05 according to T tests.

**Table 2.** The effect of foliar applied fungicides on thousand kernel weight, *A. brassicae* infection , *A. raphani* infection, *A. alternata* infection and percent green seed of AC Sunbeam canola.

| Product | Rate (/ha) | Growth* Stage | *A. brassicae* (% infection)** | *A. raphani* (% infection)** | *A. alternata* (% infection)** | Green Seed(%)** |
|---|---|---|---|---|---|---|
| Control | ----- | 2 | 10.8a*** | 7.4ab*** | 2.7c*** | 3.3a*** |
| ROVRAL FLO | 500gai | 1 | 9.0abc | 7.1a | 4.5b | 1.6d |
| ROVRAL FLO | 500gai | 2 | 5.5d | 3.9c | 3.9bc | 1.9bcd |
| ROVRAL FLO | 250gai | 1 | 9.1abc | 6.0abc | 3.5bc | 2.6ab |
| ROVRAL FLO | 250gai | 2 | 6.6dc | 6.4abc | 4.2bc | 1.9bcd |
| ICIA5504 | 250gai | 1 | 10.0ab | 5.8abc | 3.9bc | 1.7cd |
| ICIA5504 | 250gai | 2 | 6.6cd | 8.4a | 6.8a | 1.2d |
| ICIA5504 | 125gai | 2 | 7.5bcd | 7.9a | 5.0b | 1.5d |

* 1=full bloom; 2=95% petal drop.

** Based on the mean of four replicates.

*** Values in the same column which are not followed by the same letter are significantly different at P=0.05 according to T tests.