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**Impact of Timing, Rate and Application  
Technology on Biological Control of  
Sclerotinia Stem Rot of Canola caused by  
*Sclerotinia sclerotiorum***

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## **Impact of Timing, Rate and Application Technology on Biological Control of Sclerotinia Stem Rot of Canola caused by *Sclerotinia sclerotiorum***

### **Introduction**

*Sclerotinia sclerotiorum* is one of the most important pathogens affecting canola and other susceptible crops such as dry bean in western Canada. In canola, even a yield loss of 3% would cost producers approximately \$13M yearly. In recent years, disease surveys of commercial bean fields indicate that losses due to white mould cost prairie bean producers approximately \$11M each year. Stem rot of canola and white mould of bean caused by *S. sclerotiorum*, can be managed by fungicide use, but timing of applications is critical. Chemical pesticides will continue to be an important component of the pest management strategy for many crops in future years. However, in recent years, a number of registered fungicides have been removed from the marketplace due to concerns regarding health and international marketing. A high potential exists to reduce fungicide use and to minimize pesticide resistance in canola agroecosystems. The biocontrol agent *C. minitans* (CM) reduces disease and may have long-term benefits for sclerotinia-susceptible rotational crops such as canola, and bean. Timing, rate selection and application strategies for the biocontrol agent CM may affect the development of sclerotinia disease and may have the potential to become important IPM tools for management of sclerotinia stem rot of canola. There is also the potential for disease suppressiveness to develop using *C. minitans*, where the incidence or severity of disease is lower than expected for the prevailing environment, despite the presence of ample inocula of the pathogen. The research outlined below addresses these issues and provides new information regarding the impact of *C. minitans* on canola disease levels, yield, and inoculum potential of *S. sclerotiorum* for subsequent crops and years.

### **Background and Significance of Research**

Sclerotinia disease, caused by *Sclerotinia sclerotiorum*, is a major disease of canola and field beans in western Canada. In canola, with an average yield of 1426 lbs/acre at \$0.15 per lb based on a total acreage of 2.15 million, even a yield loss of 3% would cost producers approximately \$13M yearly. In recent years, disease surveys of commercial bean fields in Manitoba have indicated that white mould caused by *S. sclerotiorum* was responsible for an average loss of 10% in yield each year. With an average yield of 1,500 lbs/acre at \$0.27 per lb based on a total acreage of 270,000 acres, then a 10% yield loss costs prairie bean producers approximately \$11M each year plus the cost of control at \$25/acre. A severe outbreak of white mould in 2004 resulted in greater losses to the producers than in recent years. Timing of fungicide applications is critical for management of this disease. Preliminary results indicate that one well-timed fungicide spray during periods of high risk produced an increase in return of over \$120/acre over the untreated control (McLaren, unpublished). Health and international marketing concerns have resulted in a number of registered fungicides being removed from the market place in recent years. The biocontrol agent *Coniothyrium minitans* reduces disease in bean and may have long-term benefits for sclerotinia-susceptible rotational crops such as canola and peas. Timing, rate selection and application strategies for CM may affect the development of sclerotinia disease and may have the potential to become important IPM tools for the management of sclerotinia stem rot of canola. In future years, the pest management strategy for many crops will continue to involve the use of chemical pesticides. However, in canola and bean agroecosystems, a high potential exists to

minimize pesticide resistance and reduce the use of fungicides. Little information is available in the literature on use of biocontrol agents in cropping systems. Preliminary results indicate that CM reduces disease in canola (McLaren, unpublished). In 2005, application of CM to canola was as effective as a fungicide spray in reducing sclerotinia stem rot. In addition, *C. minitans* has the long-term benefits of reducing the survival of sclerotia, the overwintering bodies of *S. sclerotiorum* in the fields. The judicious use of chemicals, the use of biocontrol agents and research on application rates and technology needs to be further integrated into production systems for reasons of efficiency, environmental preservation and economy. Such research on evolving pest management systems for canola and other sclerotinia-susceptible crops are important in the move towards more environmentally sound and sustainable cropping systems.

Expected short term gains from this research include decreased incidence of sclerotinia in Canadian canola and other sclerotinia-susceptible crops such as bean. Less stem rot, based on the information provided by this study would benefit subsequent canola, bean, soybean and other sclerotinia-susceptible crop production by reducing inoculum build-up in the fields. Lower fungicide inputs would be accepted quickly by many producers, since net returns would be improved by pesticide reduction. Longer term rural and/or community impacts would be felt with the employment of summer students and with the reduction in producer input costs that may be possible with a clear understanding of the impact of CM on sclerotinia disease based on different rates, timing and application technologies in canola and bean crops.

In preliminary studies with canola and the biological control agent *C. minitans*, yield increases due to the application of the biological control agent and fungicide averaged 30%, and 35%, respectively. This translates into significant savings for the producer. The additional benefits with the use of the biocontrol agent include the significant reductions in number of sclerotia that return to the soil as a source of inoculum for subsequent sclerotinia-susceptible crops, and the potential for a “carry-over” effect of *C. minitans*, which would reduce input costs significantly. In addition, the proposed research will help to identify optimal times for spray application and may indicate a larger window of opportunity for control as compared to fungicide application.

#### **Study Objectives:**

1. To determine the impact of the application timing of the biocontrol agent (*C. minitans*) and number of applications on control of sclerotinia disease and on canola crop yield and quality.
2. To determine the impact of rates (CM inoculum concentration) and method of application (soil treatment vs foliar spray) of biocontrol agent on *S. sclerotiorum* and on control of sclerotinia stem rot of canola in order to recommend the most efficient application method and rate of biocontrol for practical use on the Canadian prairies.
3. To determine if disease suppressiveness can be induced using *C. minitans* where the incidence or severity of disease is lower than expected for the prevailing environment, despite the presence of ample inocula of the pathogen.

#### **Experiments:**

1. Experiment 1:
  - a. To determine the impact of application timing (during the flowering period) of the biocontrol agent (*C. minitans*) on sclerotinia stem rot, crop yield and quality of canola.
  - b. To determine the impact of rates (CM inoculum concentration) of biocontrol agent on *S. sclerotiorum* and on control of sclerotinia stem rot of canola in order to determine the most efficient application rate of biocontrol for practical use on the Canadian prairies.

2. Experiment 2:
  - a. To determine the impact of variable rates of soil-applied *C. minitans* on the survival and viability of sclerotia of *S. sclerotiorum*.
  - b. To determine the carry-over effect of previous soil-applications of *C. minitans* on the decomposition and viability of sclerotia of *S. sclerotiorum*.

### **EXPERIMENT 1: Impact of application timing and rate of *C. minitans* on sclerotinia stem rot of canola.**

#### **Research Plan**

1. Research trials for year one were located in Manitoba (Brandon and Morden) on sites that were naturally (Brandon-East) and artificially (Brandon-West, Morden) infested with *S. sclerotiorum*. Soil samples were taken in the spring from all sites to determine the natural population of sclerotia. The number of sclerotia in soil were supplemented at all sites to reach a final concentration of 108 sclerotia m<sup>-1</sup>.
2. During 2006-2007, treatments for canola (cultivar Invigor 2663) included a combination of 3 CM rates and 2 foliar applications times (20-30% bloom and 50-60% bloom) for CM. An untreated control and a fungicide treatment (Ronilan) were also included. The study was established as a randomized complete block design with 8 treatments and four replicates comprising 32 plots at each of the three sites. In 2008, one rate (Rate 2) of CM was selected and treatments included combinations of application type and timing, an untreated control and a fungicide (split application) treatment. Two application times were selected, because the best protection of the crop is achieved when fungicide is applied between the 20-50% bloom stage. At 20-30% bloom, petals have not begun to fall and at 50% bloom, the crop is at its maximum yellow color and is prior to significant petal fall. Details of the 2006 treatments are presented in Tables 1-3, the 2007 treatments in Tables 5-7, and the 2008 treatments in Tables 9-11.
3. Irrigation was not applied for at least 24 hours following treatment applications and after significant rainfall. The goal of the irrigation was to keep the top 2.5 cm of soil moist to promote apothecia development and create canopy conditions conducive to infection by *S. sclerotiorum* and disease development.
4. Data collection included background data (populations of sclerotia in soil), agronomic data (seedling emergence, growth stage data, canopy coverage, grain/seed yield) and disease data (apothecia counts, petal infestation at Brandon sites, disease incidence and severity).
5. To assess petal infestation/colonization by *S. sclerotiorum* and/or *C. minitans*, twelve petals were collected from each of the plots (Brandon-West and Brandon-East sites) of two treatments (untreated check and CM Rate 2) following Time A (20-30% bloom) and Time B (50-60% bloom) applications. An additional twelve petals were collected from flowers (buds) that were not yet open at the application timing for the CM Rate 2 plots at Time #A and #B. These petals were plated onto potato dextrose agar and evaluated for the presence of *S. sclerotiorum* and *C. minitans*.

#### **Difficulties Encountered**

**2006:** The summer of 2006 was very warm and dry. Irrigation was applied frequently to the canola and and bean trials at Brandon to maintain moisture at necessary levels for germination of soil-borne sclerotia and subsequent development of apothecia and ascospores. The canola stand was excellent (Figure 1) but unfortunately, due to high winds during a severe storm in late July, plant lodging occurred. This would enhance moist conditions and possibly aid the development of sclerotinia stem rot. To take into account the impact of lodging on disease development,



measurements of plant height and canopy height were taken in these trials. No lodging occurred at the irrigated site at Morden.

**2007:** The summer was again quite warm and dry. The experiments were irrigated frequently, but few apothecia were observed and disease levels were low. The Brandon-East site required reseeded due to uneven emergence and the late seeding date resulted in a thin stand of canola. The reduced density of the canopy did not produce a favourable microclimate effect with respect to development of sclerotinia stem rot of canola, and as a result, disease levels were quite low.

**2008:** Irrigation application to the canola and bean trials at Brandon was coordinated with rainfall amounts to maintain moisture at necessary levels for germination of soil-borne sclerotia and subsequent development of apothecia and ascospores. The canola stand was excellent at both Brandon sites and only minimal lodging was evident. However, deer damage was a problem and was very evident at the east site, with damage to some outside plots. When the damage was first noticed, electrical ‘shockers’ were placed around each trial, but these did little to deter the deer. This affected the percent flowering of canola in some of the plots, as many of the plant tops were removed. At Brandon, over the 6 day period during canola flowering when the spray applications were applied (both CM and fungicide), the average maximum daily temperatures were 25°C (Time A) and 23°C (Time B). The optimum temperatures for infection by *S. sclerotiorum* are in the range of 20-25°C with growth of mycelia favoured by cooler temperatures of 15-20°C. The low levels of disease in the Brandon trials may be a result of cooler temperatures prevailing during most of the daytime hours, which would impact the growth of mycelia and sclerotial formation. At Morden, disease severity in canola was higher in the check treatments of *S. sclerotiorum* alone than in any previous year of the study. Some of the spray treatments differed at this location compared to the Brandon site, but treatments comparisons were still possible.

## **Results and Discussion**

### **2006 Field Season**

#### ***General information***

At Brandon sites, the daily irrigation period started prior to canopy closure, on June 19, and continued through to late August. The Morden site was irrigated approximately once a week beginning July 6. Apothecia were first observed in the trials on June 29<sup>th</sup> (Brandon-East) and July 4<sup>th</sup> (Brandon-West) (Figure 2). Stem lesions were first observed on July 19<sup>th</sup> for both Brandon sites. The Brandon-East site was rated for stem rot on August 17-18, Brandon-West site on August 23-24 and the Morden site on August 23.

#### ***Disease incidence at the three trial locations***

Levels of sclerotinia stem rot in canola were low in 2006 at the Brandon-West site with 6.8% disease in the untreated control plots (Figure 3, Table 4). The incidence of stem rot was higher at the Brandon-East site where 32.8% stem rot was observed in the untreated control (Figure 4). Levels of sclerotinia stem rot at Morden (Figure 5) were intermediate to those observed at the two Brandon sites with 18.5% disease in the untreated control.

#### ***Impact of C. minitans (CM) on the incidence of sclerotinia stem rot***

Time and rate of application of CM affected sclerotinia stem rot incidence at the Brandon West site. Application of the high rate of CM (Rate 3) at time A resulted in the least amount of disease (Figure 3). At both Brandon sites, application of CM (Time B, Rate 3) appeared to be the least effective biocontrol treatment (Figures 3 and 4). This suggests that the higher rate of CM was only effective when applied at Time A as opposed to Time B. At the Brandon-East site, the two higher rates of biocontrol application at Time A produced lower white mould levels, but the

lowest level numerically was observed with Time B, Rate 2. Clearly, based on the variability of the results, further research in the upcoming years of this study will be helpful in discerning the effects of differences in rates and timing of *C. minitans* application in canola. At the Morden site, no lodging occurred and four biocontrol treatments along with the fungicide treatment produced less disease than the untreated control (Figure 5). The mean disease incidence averaged over rate at the Brandon sites suggests that application timing A produced the lowest levels of sclerotinia stem rot (Figures 6 and 7). However, at the Brandon-East site, this treatment showed a relatively high mean percent lodging (Figure 15) and this would likely contribute to increased disease levels in these plots. At Morden, lowest disease incidences were observed with the biocontrol application at timing B and with the fungicide application (Figure 8).

Three rates of the biocontrol agent were used in this study. Rates 1, 2 and 3 represent low, medium and high rates of CM, respectively. The mean disease incidence averaged over application times at the Brandon sites indicate that numerically, the lowest levels of disease occurred in the plots treated with rates 1 and 2 of *C. minitans* (Figures 9 and 10). However, one plot of the rate 3 treatment at the Brandon-East site had the highest lodging rating, and this likely contributed to the increased disease in this treatment. At Morden, the disease incidence was reduced as the rate of CM increased (Figure 11).

#### ***Petal infestation by S. sclerotiorum***

The mean incidence of petals infested with *S. sclerotiorum* was higher in the untreated control than in the treatments where CM was applied (Figure 12) at both Brandon sites. Less sclerotinia was recovered from petals collected from the Brandon-West site than the Brandon-East site. When the final sclerotinia ratings were conducted, less disease was also observed at the West compared to the East location. The final disease incidence in the untreated controls was 33% and 7% at the Brandon East and West sites, respectively.

Petals were also examined for the presence of *C. minitans*. Few petals were colonized by *C. minitans* in the untreated control plots at both Brandon sites (Figure 13). Of the two remaining treatments assessed, the highest recovery of CM occurred in the *C. minitans*-treated plots (Rate 2) where petals were collected one day after the biocontrol application. The 'CM Rate 2 BUD' treatment refers to petals collected from a flower that was not open at the time of *C. minitans* application. These buds were collected three days following the biocontrol application and the data indicate that *C. minitans* was still viable on the petals, which is a beneficial feature of a biocontrol agent.

#### ***Impact of lodging on the incidence of sclerotinia stem rot***

As previously mentioned, the canola stand was excellent at the Brandon sites, but unfortunately, due to high winds during a severe storm in late July, plant lodging occurred. Overall sclerotinia levels were lower at the West site compared to the East site. Lodging appeared to be fairly consistent across all treatments at the West site (Figure 14). At the East site (Figure 15), more variability in lodging was observed than at the West site and the three treatments with the greatest lodging showed the highest levels of sclerotinia stem rot. One of these treatments was 'Time B-Rate 3'. It is likely that lodging had an impact on the disease incidence at the Brandon-East site.

#### ***Impact of C. minitans on canola seed yield***

All applications of *C. minitans*, excluding the Time A - Rate 2 treatment, tended to increase seed yield over the untreated check at the Morden site (Figure 16). The numerically highest yields were evident in the plots treated with *C. minitans* at Time B and Rate 2. This treatment also had one of the lowest levels of stem rot in the study. Of the Brandon-West site treatments, higher

yields were also observed in the Time B - Rate 2 treatment (Figure 17), and this yield was significantly higher than that observed in the treatment of Time A - Rate 2 (Table 4).

## **2007 Field Season**

### ***General information***

At the Brandon sites (Figure 18), the daily irrigation period started on July 6, prior to flowering and continued through to late August. The Morden site was irrigated approximately once every two weeks beginning July 6. Apothecia were first observed in the Brandon West trial on July 12<sup>th</sup>, but there were no apothecia observed in the Brandon-East trial. Due to the low incidence of stem rot, no stem lesions were observed until the time of rating; Brandon-West on August 23<sup>rd</sup> and Brandon-East on August 30<sup>th</sup>. The Morden site was rated for stem rot on August 10<sup>th</sup>.

### ***Disease incidence at the three trial locations***

Levels of sclerotinia stem rot in canola were low in 2007 at the Brandon-West site with 7.8% disease in the untreated control plots (Figure 19, Table 8). The incidence of stem rot was even lower at the Brandon-East site where 0.6% stem rot was observed in the untreated control (Figure 20). This test was reseeded and the environmental conditions experienced prior to and during flowering were not conducive to stem rot development as indicated by the low disease ratings. Disease levels were so low in the Brandon-East site that it was unlikely that any yield losses would be attributable to sclerotinia stem rot. Levels of sclerotinia stem rot at Morden (Figure 21) were the highest of those observed at the three sites with a 37% disease incidence in the untreated control.

### ***Impact of *C. minitans* (CM) on the incidence of sclerotinia stem rot***

In 2007 at the Brandon-West (Figure 19) and Morden (Figure 21) sites, application of CM (Time A - Rate 3) produced similar or less disease than application of CM at Time B with Rate 3 (Table 8). At the Brandon-West site, there were no differences between disease incidences resulting from application of CM at Time A (Rate 3), Time B (Rate 1) and Time B (Rate 2).

The mean disease incidence averaged over rates at the Brandon-West site indicates that application timing B produced low levels of sclerotinia stem rot that were similar to those observed with timing A (Figure 22). The biocontrol and fungicide treatments resulted in disease levels that were less than that of the untreated control. Of the treatments applied at the Morden site (Figure 23), application of biocontrol at Time B generally produced less disease than at Time A.

At the Morden site, as the rate of CM application at Time B increased, there was a numerical trend towards reduced disease. Of the biocontrol treatments applied at the Morden site, the high rate of application provided the best results (Figure 24). The mean disease incidence averaged over application times at the Brandon-West (Figure 25) site indicates that the lowest levels of disease occurred in the plots treated with rate 3 and rate 1 of *C. minitans*. The similar levels of stem rot observed with all 3 rates of application suggests that lower rates may be as efficacious and less costly than a higher rate of application.

### ***Petal infestation by *S. sclerotiorum****

Petals infested with *S. sclerotiorum* were found in the untreated controls and in the two treatments where CM was applied (Figure 26) at both Brandon sites. Less *S. sclerotiorum* was recovered from petals collected from the Brandon-East site than the Brandon-West site. When the final sclerotinia ratings were conducted, less disease was also observed at the East site compared to the West location. The final disease incidences in the untreated controls were 7.8% and 0.5% at the Brandon West and East sites, respectively.

Petals were also examined for the presence of *C. minitans*. Few petals were colonized by *C. minitans* in the untreated control plots at both Brandon sites (Figure 27). Of the two remaining treatments assessed, the highest recovery of CM occurred in the *C. minitans*-treated plots (Rate 2) where petals were collected late in the same day after the biocontrol application. The 'CM Rate 2 BUD' treatment refers to petals collected from a flower that was not open at the time of *C. minitans* application. These buds were collected three days following the biocontrol application and the data indicate that *C. minitans* was still viable on the petals, which is a beneficial feature of a biocontrol agent.

#### ***Impact of lodging on the incidence of sclerotinia stem rot***

In 2007, lodging was not as serious a problem as in 2006 at all locations and was not expected to have an impact on disease incidence and yield. Low levels of lodging were apparent, but were fairly consistent across all treatments at the Brandon locations.

#### ***Impact of C. minitans on canola seed yield***

Differences in yield between treatments were not significant at the Brandon sites. Numerically the highest yield was evident in the plots treated with *C. minitans* at Time B with Rate 1 at the Brandon-West site (Figure 28, Table 8). This treatment also had one of the lowest levels of stem rot in the study (Figure 19). Of the Morden site treatments, the highest yield value was observed in the Time B - Rate 2 treatment (Figure 29).

### **2008 Field Season**

#### ***General information***

At the Brandon sites (Figure 30), the trials were irrigated prior to flowering, and as required. The Morden site was irrigated approximately once every two weeks beginning prior to flowering. No apothecia were observed at the Brandon East and West sites, but ascospores were present as indicated by petal testing. Disease ratings were conducted on August 27-28, 29 and 10 at the Brandon-West, Brandon-East and Morden sites, respectively. The 2008 treatments at both Brandon sites differed from those of the two previous years. Based on the data collected from 2006-2007, rate 2 of CM application was selected for use in the 2008 experiments. Some of the treatments at the Morden site differed from those at the Brandon sites due to unforeseen circumstances. Petal infestation/colonization by *S. sclerotiorum* and/or *C. minitans*, was assessed by collecting petals from each of the plots of three treatments (untreated check, CM single application at Time A, and CM single application at Time B) at the Brandon sites. As per 2006-07, an additional twelve petals were collected from flowers that were not yet open at the time of CM application.

#### ***Disease incidence at the three trial locations***

Levels of sclerotinia stem rot in canola were low in 2008 at the Brandon location with 3.0% and 11.8% disease in the untreated control plots at the West and East sites, respectively (Figures 31 and 32; Table 12). Disease levels were very low at the Brandon-West site and it is unlikely that any yield losses would be attributable to sclerotinia stem rot. Unfortunately, deer damage occurred at the Brandon sites in 2008. Approximately 35% and 29% of canola flowers were lost at the Brandon-West and Brandon-East sites, respectively, and this may have contributed to the reduced incidence of sclerotinia disease. Levels of sclerotinia stem rot at Morden (Figure 33; Table 13) were the highest of those observed at the three sites with 48% disease in the untreated control.

#### ***Impact of C. minitans (CM) on the incidence of sclerotinia stem rot***

In 2008 at the Brandon-East site, levels of disease in the CM-treated plots ranged from 2.0% to 11.3% (Table 12). The foliar split application of CM produced significantly less disease than the untreated control. The mean disease incidence in the combination treatment of CM soil application followed by a foliar application at Time A was 11.3% and did not differ significantly from 11.8% in the untreated control. The combination treatment of CM applied to the soil + foliar application at time B resulted in 4.3% disease. The disease incidences for the foliar treatments of CM applied at time A+B, and CM applied at time B were similar, and numerically less than the mean disease incidence resulting from the single soil application of CM (Figure 34). Very low levels of disease were observed at the Brandon-West site and there were no significant treatment differences or trends.

At the Morden site, the mean disease incidences of the *C. minitans* treatments as well as the fungicide treatment were significantly lower than the untreated control of 48% (Table 13). The application of CM (1/2 rate at Time B) and the fungicide treatment produced a disease incidence of 20% each. A full application of CM applied at time B resulted in 11.5% disease. When CM was applied to the soil in the spring as well as to the foliage during the field season, the mean disease incidences were 26% and 15.5% in the treatments of soil + foliar (Time A) and soil+foliar (Time A+B), respectively. Although these treatments differences were not significant, the data suggest that the time of CM application is important for management of stem rot of canola – spray application at Time B would provide coverage and protection of a higher percentage of flowers than at Time A.

#### ***Petal infestation by S. sclerotiorum***

As in previous years, petals infested with *S. sclerotiorum* were found in the untreated controls and in the two treatments where CM was applied (Figures 35 and 36) at both Brandon sites. The average infestation rates of petals collected from the Brandon-East and Brandon-West sites were 31% and 39%, respectively.

Petals were also examined for the presence of *C. minitans* (Figures 37 and 38). Generally, few petals were colonized by *C. minitans* in the untreated control plots at both Brandon sites. The highest recovery of CM occurred in the *C. minitans*-treated plots where petals were collected late in the same day after the biocontrol application. The BUD treatment refers to petals collected from flowers that were not open at the time of *C. minitans* application. These buds were collected three days following the biocontrol application and the data indicate that *C. minitans* was still viable on the petals.

#### ***Impact of lodging on the incidence of sclerotinia stem rot***

In 2008, lodging was not as serious a problem as in 2006 at all locations and was not expected to have an impact on disease incidence or yield. Low levels of lodging were apparent, but were fairly consistent across all treatments at the Brandon locations.

#### ***Impact of C. minitans on canola seed yield***

There were no significant treatment differences in yield at the Brandon-West and Brandon-East sites in 2008 (Table 12, Figures 39 and 40). Disease incidences in treatments at the Brandon-West were so low that it would be unlikely that any yield losses could be attributed to sclerotinia stem rot. At the Morden site (Table 13, Figure 41), the treatments of fungicide, CM-FoliarB (1X) and CM-FoliarB (2X) produced yields that were significantly higher than the CM-Soil+FoliarA treatment. Numerically the lowest yields were observed in the untreated control, and in combination treatments where CM was applied to the soil and foliage.

## **EXPERIMENT 2: Carry-over effect of *C. minitans* on sclerotia survival and viability.**

### **Research Plan**

1. This study was established to address a possible carry-over effect of the biocontrol agent *C. minitans* (CM) on survival and viability of sclerotia of *S. sclerotiorum*. The trial was set up as a randomized complete block design with four replicates at two Brandon sites (East and West) and one Morden site. Treatments included 3 CM rates applied to the soil followed by seeding to NW63, a small red bean with a viny growth habit. Details of the treatments are presented in Table 14 (Brandon 2006), Table 15 (Morden 2006), Table 16 (Brandon 2007), Table 17 (Morden 2007), Table 18 (Brandon 2008) and Table 19 (Morden 2008).
2. In 2007, the study was repeated at the three sites, with each treatment applied to the same plots as in 2006. Sclerotia survival and viability were determined by burying mesh bags containing 100 viable sclerotia in each plot. In 2008, no CM was added to the trials. Sclerotia in mesh bags were buried in the plot areas where CM had been applied during 2006-07 to determine the impact of CM on sclerotial viability in year 3 following two previous years of biocontrol application.
3. The mesh bags remained buried at 2 cm for approximately 14 to 15 weeks at all sites (2006-07). In 2008, sclerotia from a different source were used for burial and because they were smaller in size than those used previously, the mesh bags were removed after 8 to 10 weeks of burial. All mesh bags containing sclerotia were carefully washed prior to removing sclerotia for assessment of survival, viability and infection by *C. minitans* under laboratory conditions.
4. To determine the impact of *C. minitans* on apothecia development, 100 viable sclerotia were buried 2 cm deep in a burial area within the treatment area for each plot at the Brandon sites.
5. Measurements included sclerotia survival (intact sclerotia recovered following burial period), viability of sclerotia, recovery of *C. minitans* from buried sclerotia, and apothecia counts.

### **Results and Discussion 2006-2008**

In 2006, the Brandon trials received daily irrigation beginning June 29<sup>th</sup>, prior to canopy closure and continued until the end of August. The Morden site was irrigated periodically starting on July 12 until the end of August. In 2007 and 2008, Brandon trials received weekly irrigation beginning in June, prior to canopy closure and continued until the end of August. The Morden site was not irrigated in 2007 and 2008, but frequent precipitation occurred and therefore the lack of irrigation would not have been expected to be a limiting factor to disease development at this site.

Canopy coverage was assessed throughout each field season. Apothecia counts were taken at both Brandon sites. In all years, the viability of sclerotia was determined prior to burial in the soil. However in 2008, unlike previous years where sclerotia were obtained from sunflower screenings, sclerotia produced in the laboratory were used for burial in mesh bags. This was due to a shortage of sclerotia from natural sources. Mesh bags containing sclerotia were removed from the Brandon and Morden sites and washed prior to removing sclerotia for assessment of viability and infection by *C. minitans* under laboratory conditions. Mesh bags of each treatment from the Brandon site of 2006, 2007 and 2008 are illustrated in Figures 42, 43 and 44, respectively. The small black dots in the compartments of each mesh bag represent sclerotia that were recovered after 15, 14 and 8-10 weeks of burial in 2006, 2007 and 2008, respectively. The reduced time of burial for the 2008 sclerotia was selected because the sclerotia were much smaller in size than those used in the previous two years. In 2006, fewer sclerotia were observed in the mesh bags buried where *C. minitans* was applied than in the untreated control at the Brandon-East

site (Figure 45, Table 20). This was evident at all three locations (Figures 45, 46 and 47) and there appeared to be an effect for different rates of the biocontrol agent at some sites. At the Brandon-West site, the numbers of intact sclerotia recovered from the CM rates 2 and 3 were significantly lower than from the treatment of CM rate 1. The numbers of intact sclerotia recovered from the treatments at the Morden site were higher than at either Brandon site in 2006, but the same trend of fewer intact sclerotia recovered with application (rates 1 and 3) of the biocontrol than in the untreated plots was evident (Table 20). In 2007 at Brandon, fewer sclerotia were observed in the mesh bags buried where *C. minitans* was applied than in the untreated control (Figures 48 and 49) but treatments differences were significant at the Brandon-West site only (Table 21). At both Brandon sites, apothecia production was assessed by treatment. Application of CM significantly reduced apothecia production compared to the untreated control (Figure 50). At Morden, as in 2006, the numbers of intact sclerotia recovered from the treatments (Figure 51) were higher than at either Brandon site. The soil type at Morden differs from that at Brandon and each site would support a different population of soil microflora and fauna which might have influenced the mycoparasitic abilities of *C. minitans*. In addition, the frequency of irrigation and rainfall differed between the Morden and Brandon locations. In 2008, the number of sclerotia recovered from the CM-Rate3 treatment was significantly less than from the treatment of CM-Rate2 at the Brandon-East site (Figure 52, Table 22). However, the number of recovered sclerotia did not differ significantly between the treatments of CM-Rate2 and the untreated control. Although there were no significant treatment differences at the Brandon-West site (Figure 53, Table 22), a numerical trend of fewer intact sclerotia recovered as *C. minitans* rate increased was evident and showed a negative correlation of  $r=-0.62$ . At the Morden site (Figure 54, Table 22), there were more sclerotia recovered from plots treated with CM-Rate2 and CM-Rate 3 than in the untreated control and CM-Rate 1. These results suggest that *C. minitans* may not be as effective at some sites/plots in year 3, after application in years 1 and 2. However, there are indications that the biocontrol agent is able to overwinter and provide control of sclerotia of *S. sclerotiorum*. Therefore, this experiment will continue for the 2009 field season at Morden and Brandon to monitor the ability of *C. minitans* to overwinter and reduce populations of *S. sclerotiorum* in the field.

#### **Summary of all Canola Trials (2006-08)**

In the burial study, the biocontrol agent *C. minitans* reduced the survival of sclerotia of *S. sclerotiorum* under field conditions with fewer sclerotia being recovered in the CM-treated plots compared to the untreated controls at most sites in the 2006-2007 years of application. *Coniothyrium minitans* was applied to the same plots in 2006 and 2007, but was not applied in 2008. In the third year of the study, significant differences between treatments were observed at one site only. However, a numerical trend showing a reduction in the survival of sclerotia as the rate of application of CM increased was observed at the Brandon West site. Because the activity of *C. minitans* is still apparent in some treatments of the 2008 burial trials, the study will continue for 2009 to evaluate the carry-over potential of this biocontrol agent into year 4.

Results of the field trials where *C. minitans* was applied to the aerial parts of canola plants during the flowering stage showed a trend of reduced sclerotinia stem rot of canola in many of the biocontrol treatments compared with untreated controls. In 2006 and 2007, three rates of CM application and two times of application were examined. Significant differences in mean disease incidence between rates and times of application were apparent during three of the six site-years. Similarly, significant differences in seed yield between rates and times of application were observed at four of the six site-years. Numerically, application of CM at Time B, Rate 2 produced higher yields than Time B, Rates 1 and 3 as well as Time A, Rate 2. The single CM treatments selected for year 3 of the study (2008) were applied at Rate 2. Soil applications of CM were included in the 2008 study and the Brandon East disease data suggest that the treatments of CM

application to the soil + foliar application at time A were not as effective as CM application to the soil + foliar application at time B. Moreover, CM applied to the soil and foliage at time A was not as effective in reducing mean disease incidence as CM applied to the foliage at time A and B. One foliar application of CM at the full rate and Time B produced the lowest mean disease incidence at the Morden site. These results suggest that foliar application at Time B appears to be more effective than at Time A and that foliar application may be more effective than soil application in reducing the incidence of sclerotinia stem rot. This study has shown that soil application can reduce the survival and therefore the inoculum potential of *S. sclerotiorum*. However, because the ascospores of this pathogen can be dispersed by wind currents, there is a potential for infection from outside the area of treatment. This study will continue for one additional field season at Morden and Brandon to repeat the treatments applied to the Brandon sites in 2008.

The incidence of sclerotinia stem rot has been positively related to the percentage of flower petals infested with ascospores of *S. sclerotiorum* at the early bloom stage in previous research. In the current study, flower petals were colonized by *S. sclerotiorum*, indicating that inoculum of the pathogen was present. However, the mean disease incidences of stem rot were low at some site-year locations. This may have been related to environmental conditions, such as excessive heat, as well as cultural conditions, such as plant stand and canopy density, that were not always conducive to stem rot development. Previous research has also shown a positive correlation between reduction of lesion development of *S. sclerotiorum* on canola leaves and conidial development of *C. minitans*. In the current study, *C. minitans* was recovered from canola flower petals, and was still viable on the petals after three days. Preventing or reducing colonization of flower petals by *S. sclerotiorum* with the application of *C. minitans* appears to be a valid strategy for management of sclerotinia stem rot, as in the current study, less disease was observed with the application of this biological control agent at some sites in some years.

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## **EXPERIMENT 1: TABLES AND FIGURES 2006**

**Table 1.** Application of *C. minitans* (CM) and Ronilan to canola at the Brandon-East (CE) and Brandon-West (CW) sites in 2006.

<b>Brandon Treatment</b>	<b>Description</b>	<b>Timing</b>	<b>Rate (CM-spores/mL)</b>	<b>Application Date(s)</b>
1	Control	-	-	-
2	Fungicide (Ronilan)	Split: 25-30% and 50% bloom	Rate = 0.2 kg/ac	CE July 6 and July 10; CW July 7 and July 11
3	Foliar CM	A (25-30% bloom)	1 (2.58 x 10 <sup>6</sup> )	CE July 6 and 10*; CW July 7
4	Foliar CM	A	2 (5.15 x 10 <sup>6</sup> )	CE July 6 and 10*; CW July 7
5	Foliar CM	A	3 (7.73 x 10 <sup>6</sup> )	CE July 6 and 10*; CW July 7
6	Foliar CM	B (50-60% bloom)	1 (2.58 x 10 <sup>6</sup> )	CE and CW July 11
7	Foliar CM	B	2 (5.15 x 10 <sup>6</sup> )	CE and CW July 11
8	Foliar CM	B	3 (7.73 x 10 <sup>6</sup> )	CE and CW July 11

\* all applications were to be single applications; however at the Brandon CE site, treatments 3,4 and 5 were inadvertently sprayed with a second application on July 10, 2006. These three treatments were evaluated as split rather than single applications at this site.

**Table 2.** Application of *C. minitans* (CM) and Ronilan to canola at the Morden site in 2006.

<b>Morden Treatment</b>	<b>Description</b>	<b>Timing</b>	<b>Rate (CM-spores/mL)</b>	<b>Application Date(s)</b>
1	Control	-	-	-
2	Fungicide (Ronilan)	Split: 20 and 50% bloom	0.2 kg/ac	July 10 and 17
3	Foliar CM	A (20-30% bloom)	1 (1.63 x 10 <sup>6</sup> )	July 10
4	Foliar CM	A	2 (3.27 x 10 <sup>6</sup> )	July 10
5	Foliar CM	A	3 (6.67 x 10 <sup>6</sup> )	July 10
6	Foliar CM	B (50% bloom)	1 (4.00 x 10 <sup>6</sup> )	July 17

7	Foliar CM	B	2 (8.00 x 10 <sup>6</sup> )	July 17
8	Foliar CM	B	3 (1.17 x 10 <sup>7</sup> )	July 17

**Table 3.** Important dates noted for Experiment 1 (Impact of application timing of *C. minitans* on sclerotinia of canola) at all sites in 2006.

Dates of...	Brandon EAST	Brandon WEST	Morden
Seeding	19-May	19-May	01-Jun
Emergence	27-May	28-May	-
Irrigation Start	19-Jun	19-Jun	-
Canopy Closure	19-Jun	22-Jun	02-Jul
First Apothecia	29-Jun	04-Jul	-
Flowering Start	29-Jun	30-Jun	-
First Petal Drop	04-Jul	05-Jul	-
Treatment Time A	06-Jul	07-Jul	10-Jul
Treatment Time B	11-Jul	11-Jul	17-Jul
Flowering End	25-Jul	25-Jul	-
First Disease Lesion	19-Jul	19-Jul	-
Disease Rating	17&18-Aug	23&24-Aug	23-Aug
Swathing	18-Aug	30-Aug	24-Aug
Combining	07-Sep	11-Sep	05-Sep

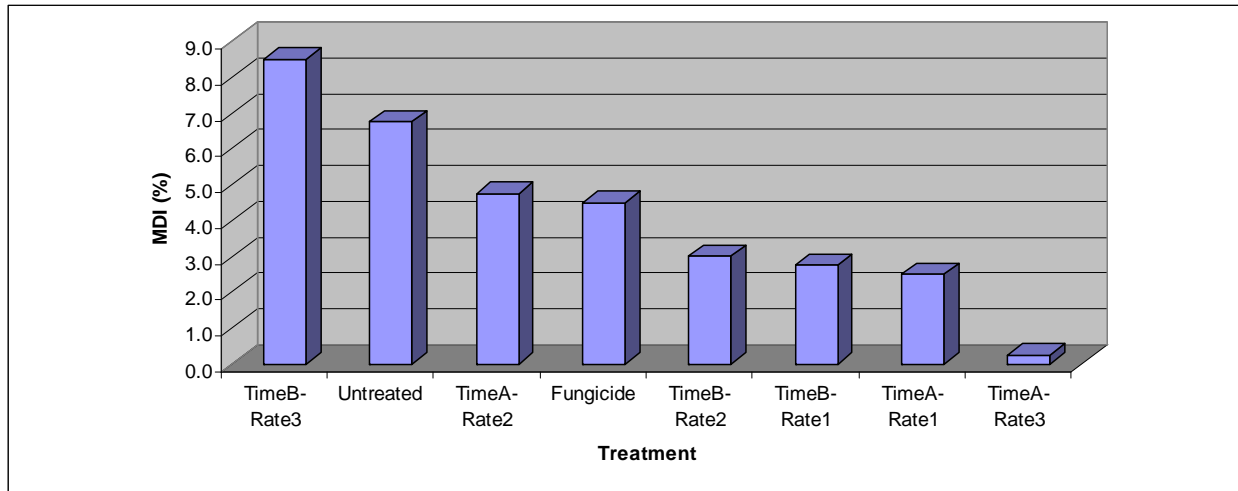
**Figure 1.** Canola trial located at Brandon, July 6, 2006.



**Figure 2.** Apothecia found in the field 2006 (Brandon).



**Figure 3.** Impact of *C. minitans* rate and timing of application, and fungicide on the mean disease incidence (MDI) of sclerotinia stem rot of canola at the Brandon-West site (2006)

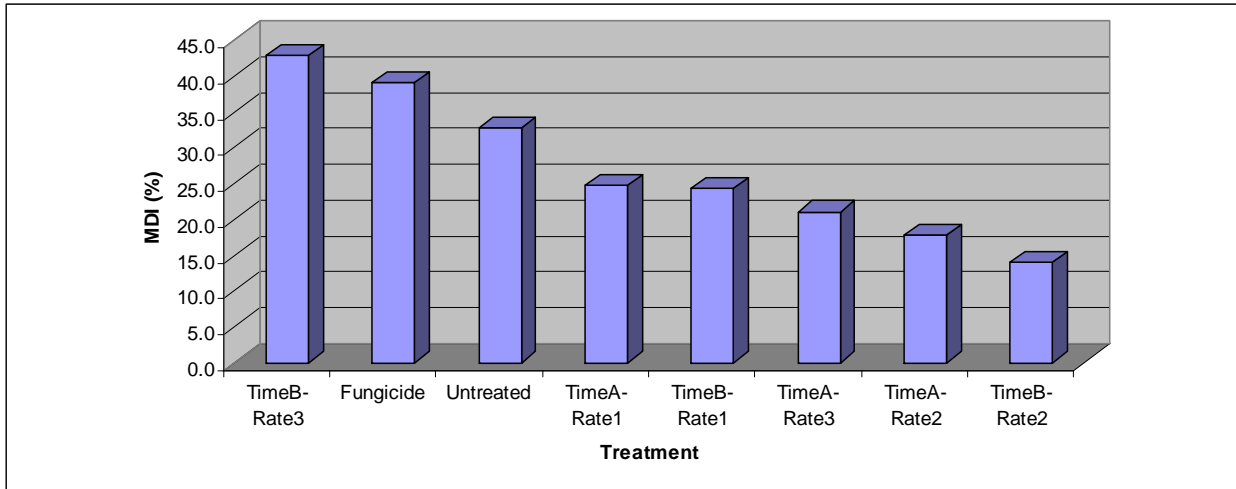


**Table 4.** Impact of *C. minitans* rate and timing of application, and fungicide on the mean disease incidence (MDI) of sclerotinia stem rot and seed yield of canola at three locations in 2006.

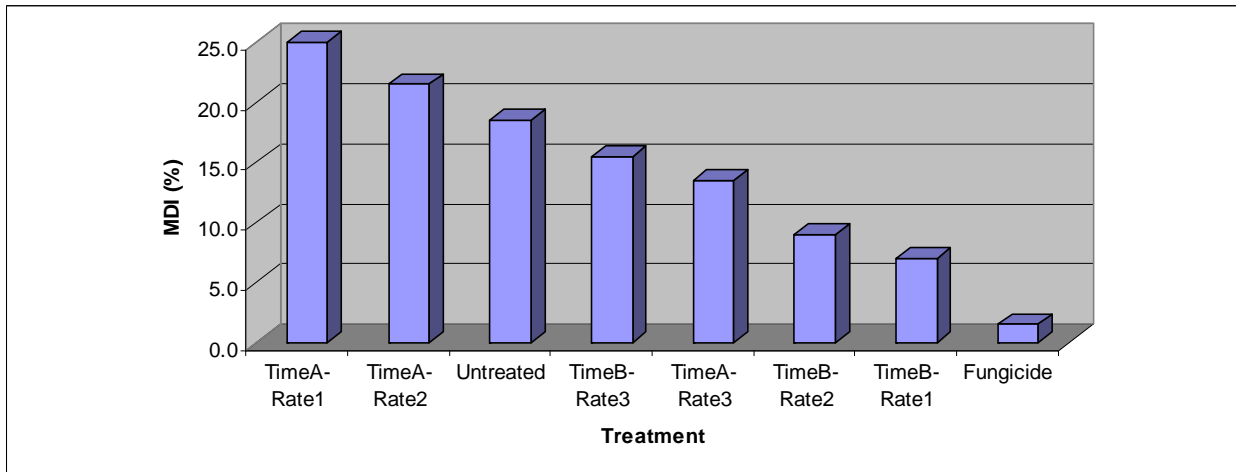
Treatment	Bran-East		Bran-West		Morden	
	MDI	Yield	MDI	Yield	MDI	Yield
Untreated	32.8 <i>Abc</i> *	2001.5 <i>ab</i>	6.8 <i>a</i>	1978.7 <i>b</i>	18.5 <i>ab</i>	3731.0 <i>a</i>
Fungicide	39.0 <i>ab</i>	2011.4 <i>ab</i>	4.5 <i>a</i>	2056.0 <i>b</i>	1.5 <i>b</i>	3894.5 <i>a</i>
Time A, Rate 1	24.8 <i>abc</i>	1937.6 <i>b</i>	2.5 <i>a</i>	2309.1 <i>ab</i>	25.0 <i>a</i>	3917.5 <i>a</i>
Time A, Rate 2	17.8 <i>c</i>	1982.0 <i>ab</i>	4.8 <i>a</i>	2046.1 <i>b</i>	21.5 <i>ab</i>	3574.0 <i>a</i>
Time A, Rate 3	21.0 <i>bc</i>	2184.0 <i>ab</i>	0.3 <i>a</i>	2416.3 <i>ab</i>	13.5 <i>ab</i>	3937.5 <i>a</i>
Time B, Rate 1	24.3 <i>abc</i>	2239.6 <i>ab</i>	2.8 <i>a</i>	2323.0 <i>ab</i>	7.0 <i>ab</i>	3903.0 <i>a</i>
Time B, Rate 2	14.0 <i>c</i>	2299.5 <i>a</i>	3.0 <i>a</i>	2551.1 <i>a</i>	9.0 <i>ab</i>	4016.0 <i>a</i>
Time B, Rate 3	42.8 <i>a</i>	2049.9 <i>ab</i>	8.5 <i>a</i>	2275.8 <i>ab</i>	15.5 <i>ab</i>	3799.0 <i>a</i>

\*means are presented with LSD grouping ( $\alpha=0.05$ ) where treatments with the same letter are not significantly different from each other.

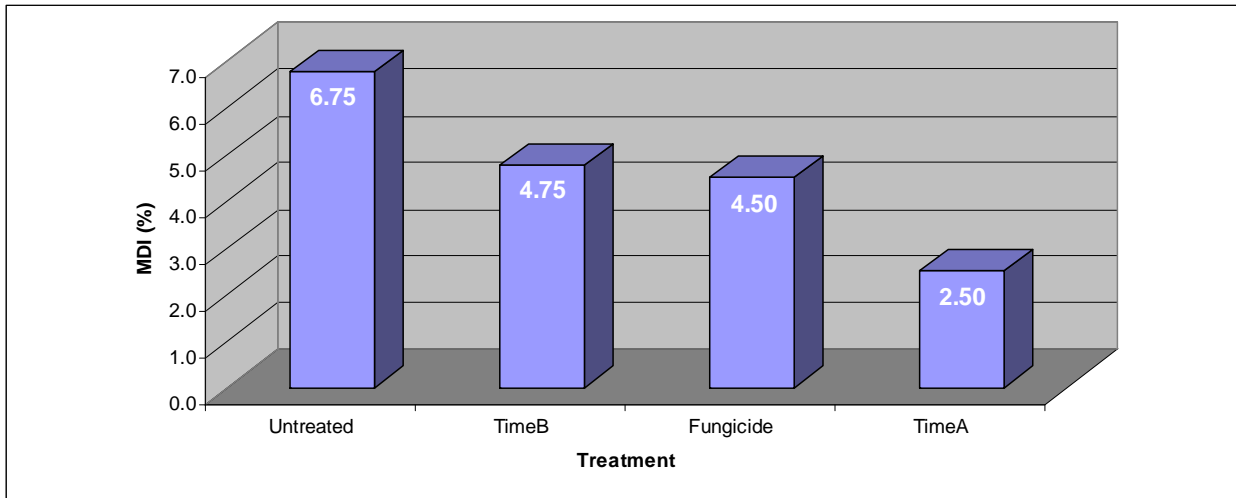
**Figure 4.** Impact of *C. minitans* rate and timing of application, and fungicide on the mean disease incidence (MDI) of sclerotinia stem rot of canola at the Brandon-East site (2006).



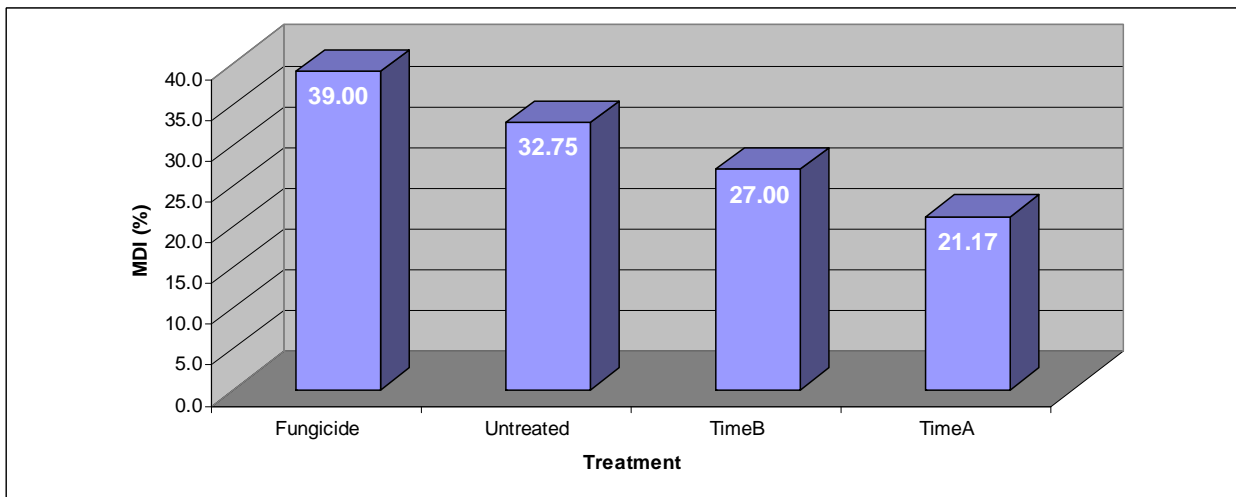
**Figure 5.** Impact of *C. minitans* rate and timing of application, and fungicide on the mean disease incidence (MDI) of sclerotinia stem rot of canola at the Morden site (2006).



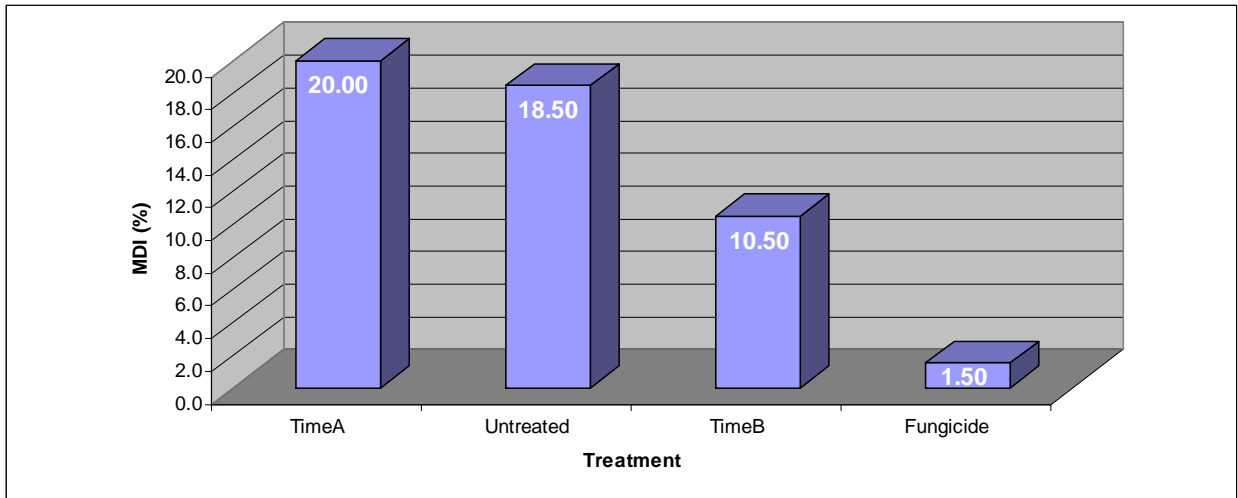
**Figure 6.** Impact of *C. minitans* application timing and fungicide on the mean disease incidence (MDI) of sclerotinia stem rot of canola at the Brandon-West site (2006).



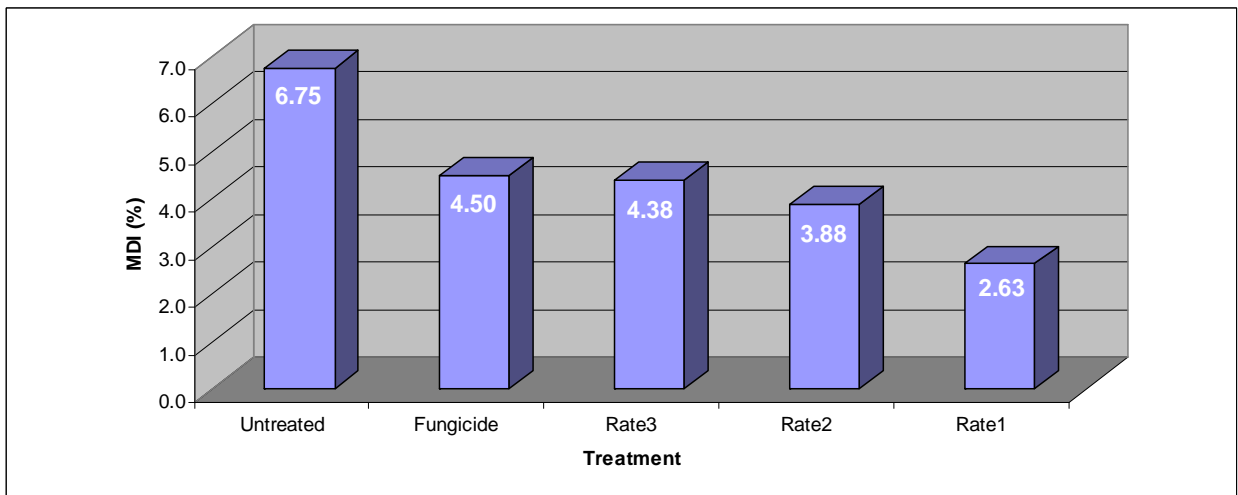
**Figure 7.** Impact of *C. minitans* application timing and fungicide on the mean disease incidence (MDI) of sclerotinia stem rot of canola at the Brandon-East site (2006).



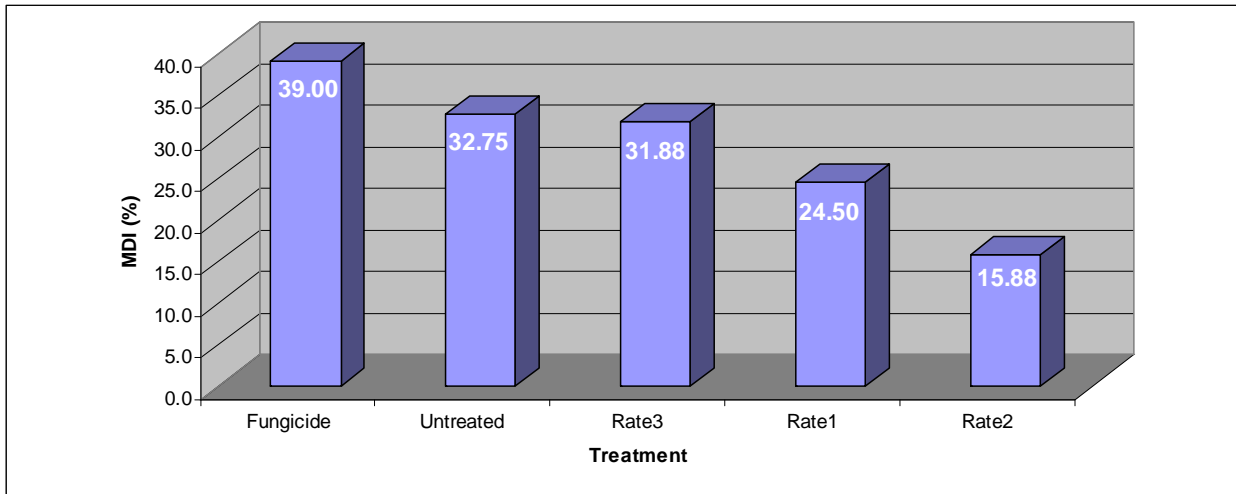
**Figure 8.** Impact of *C. minitans* application timing and fungicide on the mean disease incidence (MDI) of sclerotinia stem rot of canola at the Morden site (2006).



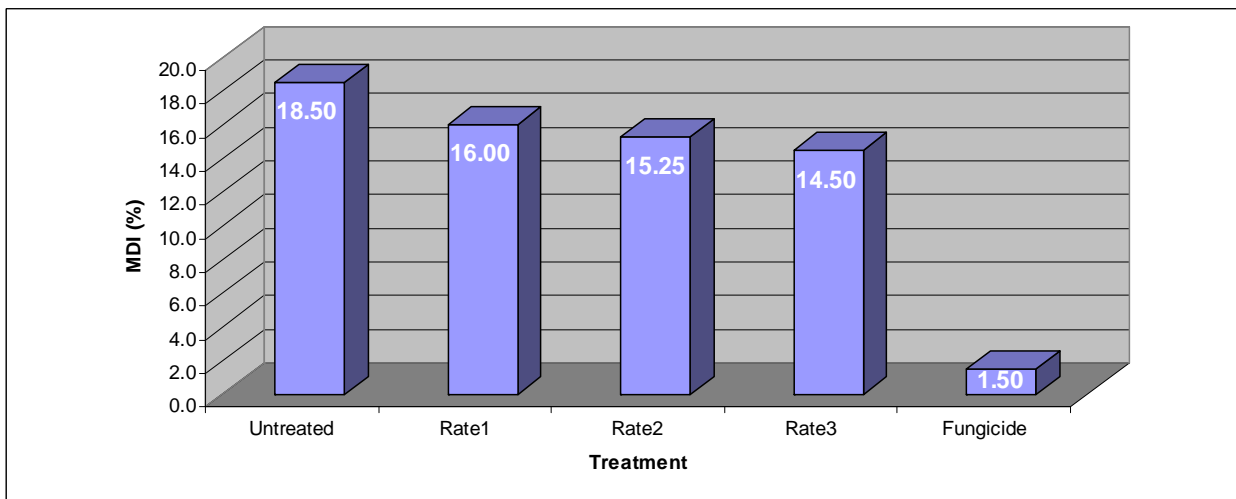
**Figure 9.** Impact of *C. minitans* application rate and fungicide on the mean disease incidence (MDI) of sclerotinia stem rot of canola at the Brandon-West site (2006).



**Figure 10.** Impact of *C. minitans* application rate and fungicide on the mean disease incidence (MDI) of sclerotinia stem rot of canola at the Brandon-East site (2006).

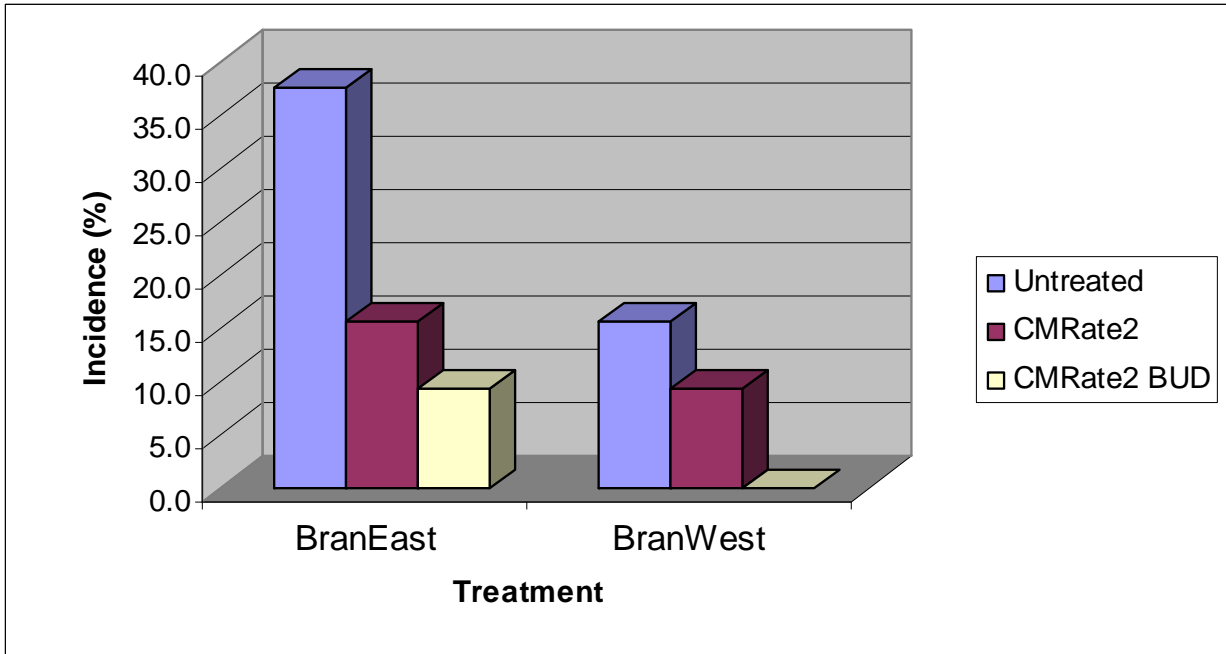


**Figure 11.** Impact of *C. minitans* application rate and fungicide on the mean disease incidence (MDI) of sclerotinia stem rot of canola at the Morden site (2006).

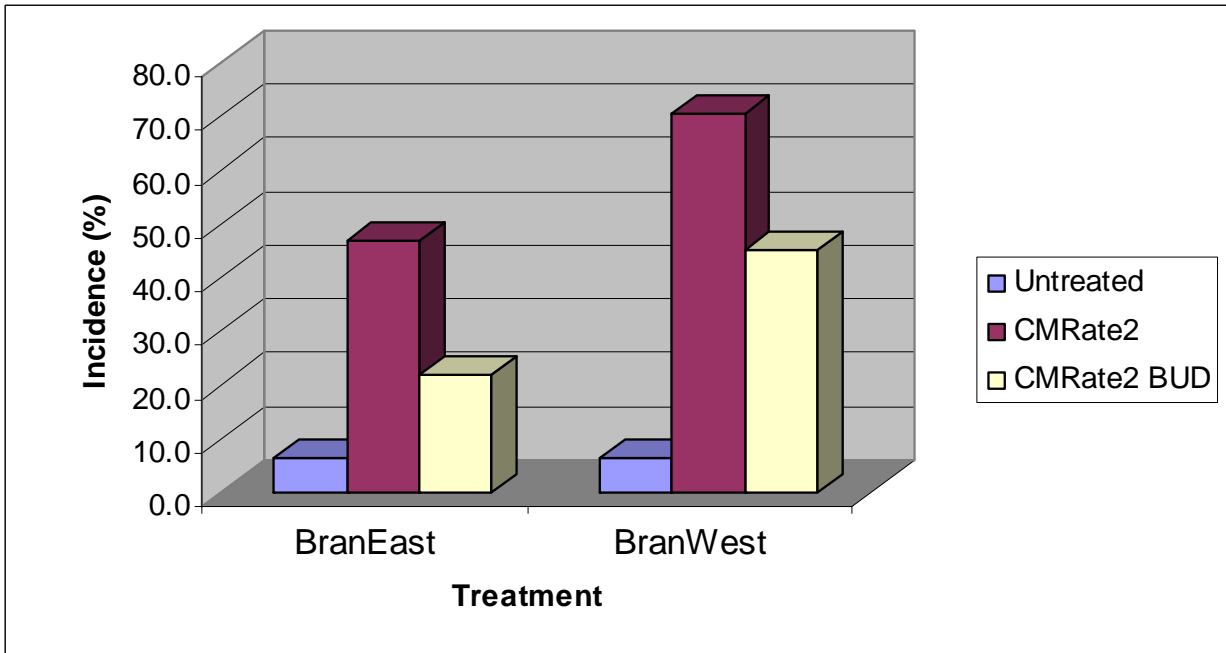


**Figure 12.** Incidence of *Sclerotinia sclerotiorum* cultured from petal samples from select treatments at Brandon (2006).

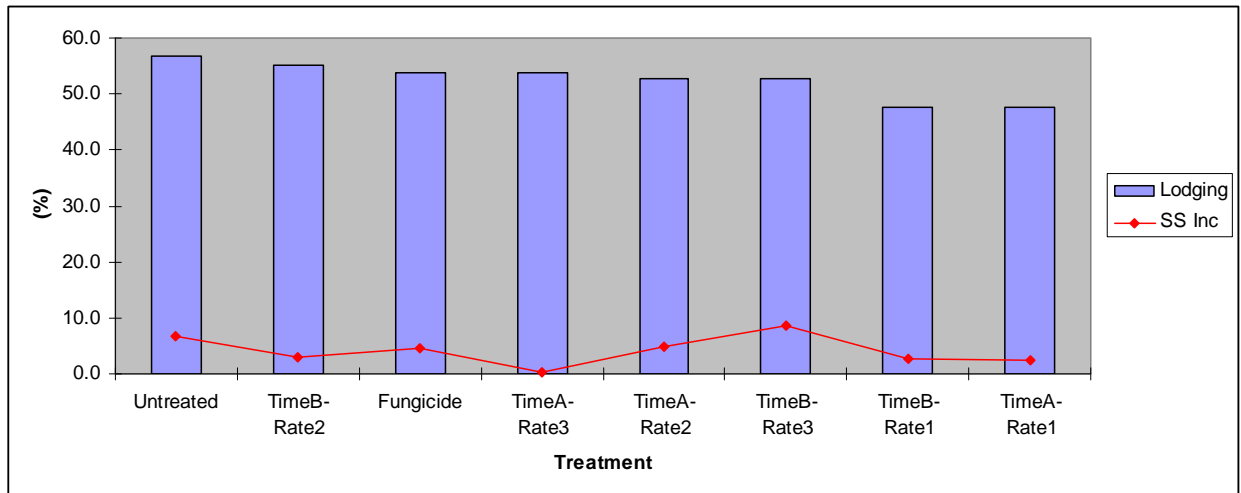




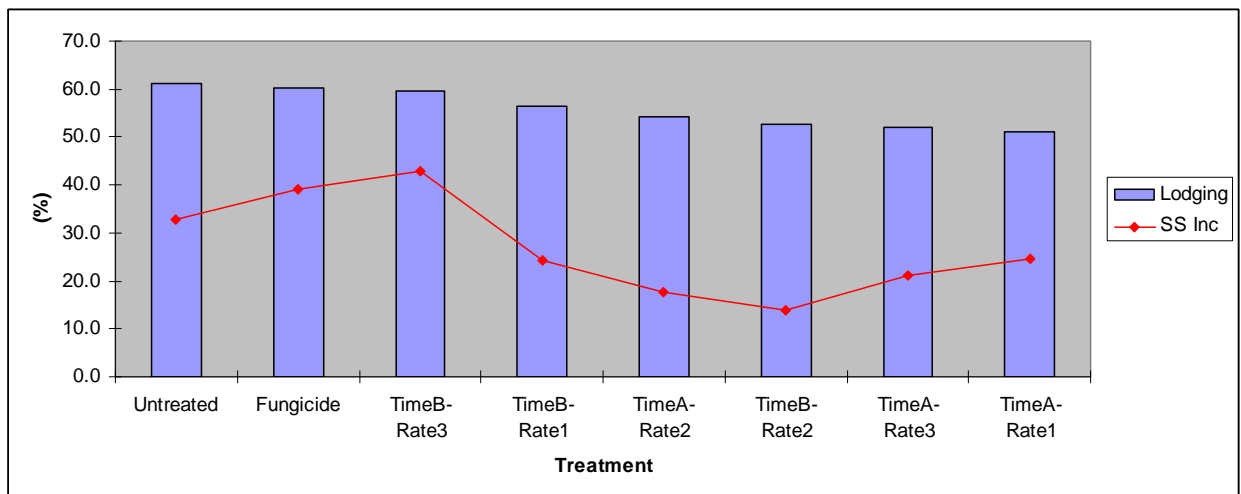
**Figure 13.** Incidence of *Coniothyrium minitans* cultured from petal samples from select treatments at Brandon (2006).



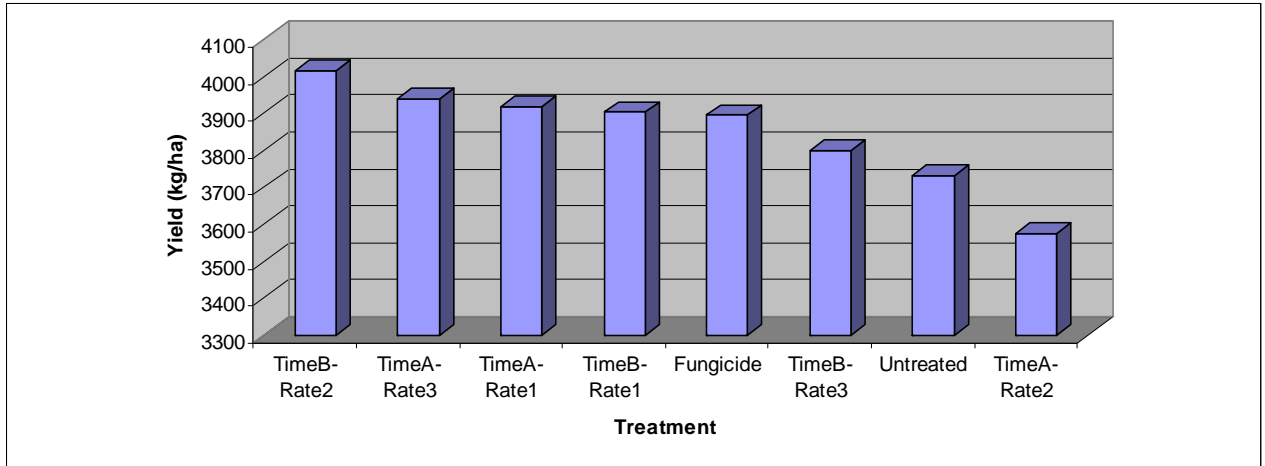
**Figure 14.** Lodging and incidence of sclerotinia stem rot of canola at the Brandon-West site (2006).



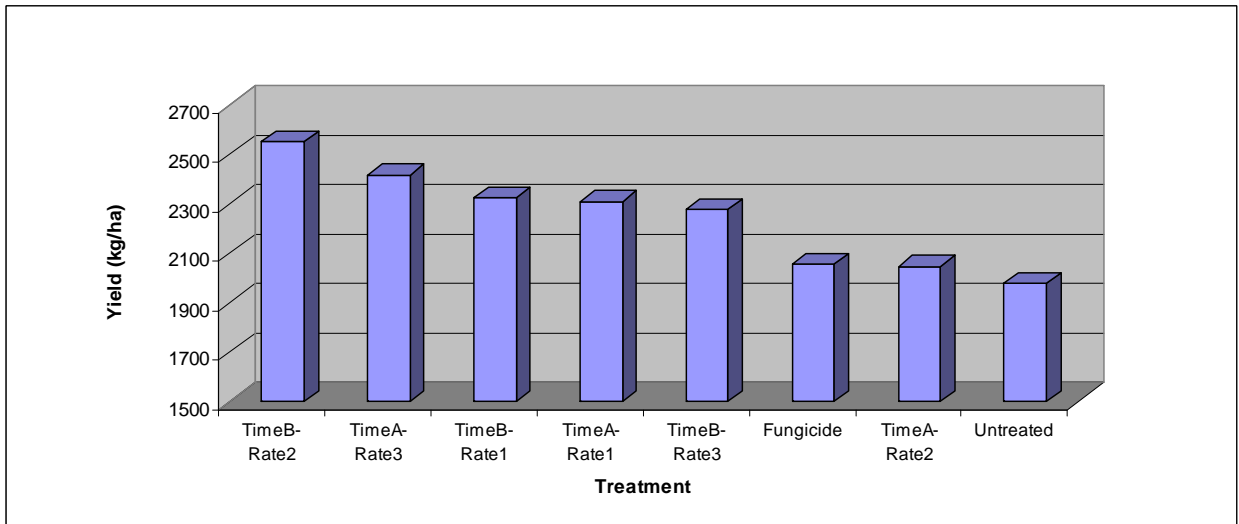
**Figure 15.** Lodging and incidence of sclerotinia stem rot of canola at the Brandon-East site (2006).



**Figure 16.** Impact of *C. minitans* application timing and rate, and fungicide on seed yield of canola at the Morden site (2006).



**Figure 17.** Impact of *C. minitans* application timing and rate, and fungicide on seed yield of canola at the Brandon-West site (2006).



**EXPERIMENT 1: TABLES AND FIGURES 2007**

**Table 5.** Application of *C. minitans* (CM) and Ronilan to canola at the Brandon-East (CE) and Brandon-West (CW) sites in 2007.

Brandon Treatment	Description	Timing	Rate (CM-spores/mL)	Application Date(s)
1	Control	-	-	-
2	Fungicide (Ronilan)	Split: 20-30% and 50-60% bloom	Rate = 0.2 kg/ac	CE July 26 and July 31; CW July 12 and July 16
3	Foliar CM	A (20-30% bloom)	1 ( $2.58 \times 10^6$ )	CE July 26; CW July 12
4	Foliar CM	A	2 ( $5.15 \times 10^6$ )	CE July 26; CW July 12
5	Foliar CM	A	3 ( $7.73 \times 10^6$ )	CE July 26; CW July 12
6	Foliar CM	B (50-60% bloom)	1 ( $2.58 \times 10^6$ )	CE July 31; CW July 16
7	Foliar CM	B	2 ( $5.15 \times 10^6$ )	CE July 31; CW July 16
8	Foliar CM	B	3 ( $7.73 \times 10^6$ )	CE July 31; CW July 16

**Table 6.** Application of *C. minitans* (CM) and Ronilan to canola at the Morden site in 2007

<b>Morden Treatment</b>	<b>Description</b>	<b>Timing</b>	<b>Rate (CM-spores/mL)</b>	<b>Application Date(s)</b>
1	Control	-	-	-
2	Fungicide (Ronilan)	Split: 20 and 50% bloom	0.2 kg/ac	July 4 and 11
3	Foliar CM	A (20-30% bloom)	1 ( $6.5 \times 10^6$ )	July 4
4	Foliar CM	A	2 ( $1.3 \times 10^7$ )	July 4
5	Foliar CM	A	3 ( $2.0 \times 10^7$ )	July 4
6	Foliar CM	B (50% bloom)	1 ( $6.1 \times 10^6$ )	July 11
7	Foliar CM	B	2 ( $1.2 \times 10^7$ )	July 11
8	Foliar CM	B	3 ( $1.8 \times 10^7$ )	July 11

**Table 7.** Important dates noted for Experiment 1 (Impact of application timing of *C. minitans* on sclerotinia of canola) at all sites in 2007.

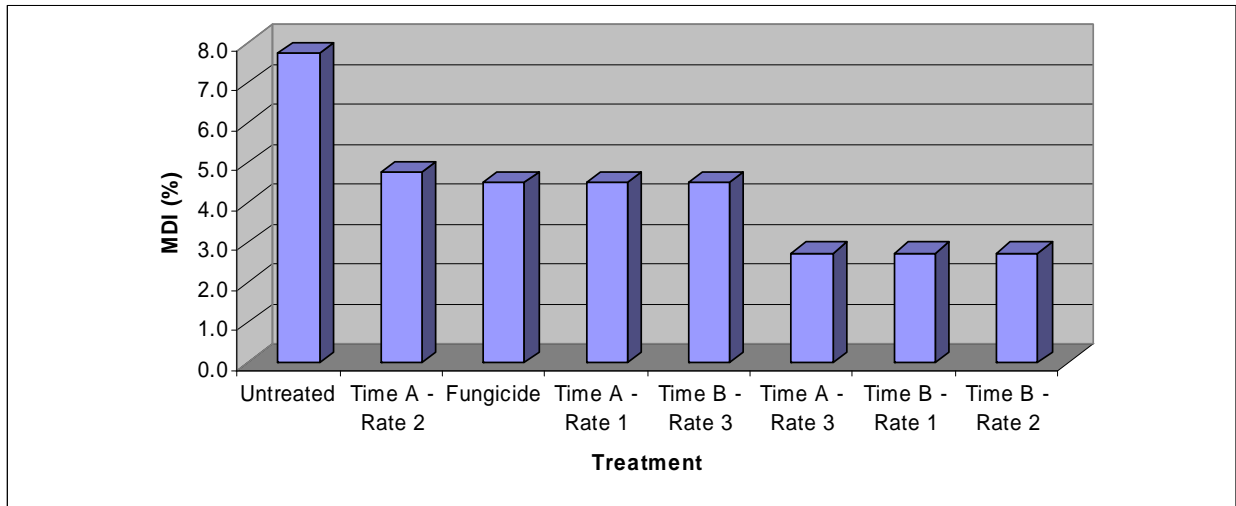
Dates of...	Brandon EAST	Brandon WEST	Morden
Seeding	June 14	May 28	May 10
Emergence	June 21	June 8	May 21
Irrigation Start	July 6	July 6	July 6
Canopy Closure	- *	June 23	-
First Apothecia	-	July 12	-
Flowering Start	July 21	July 8	-
First Petal Drop	July 26	July 13	-
Treatment Time A	July 26	July 12	July 4
Treatment Time B	July 31	July 16	July 11
Flowering End	Aug 17	Aug 3	-
First Disease Lesion	-	-	-
Disease Rating	Aug 30	Aug 22	Aug 10
Swathing	Sept 11	Aug 28	Aug 10
Combining	Oct 3	Sept 11	Aug 22

\* Canopy never fully closed due to thin stand.

**Figure 18.** Canola West (CW) trial located at Brandon, July 20, 2007.



**Figure 19.** Impact of *C. minitans* rate and timing of application, and fungicide on the mean disease incidence (MDI) of sclerotinia stem rot of canola at the Brandon-West site (2007).

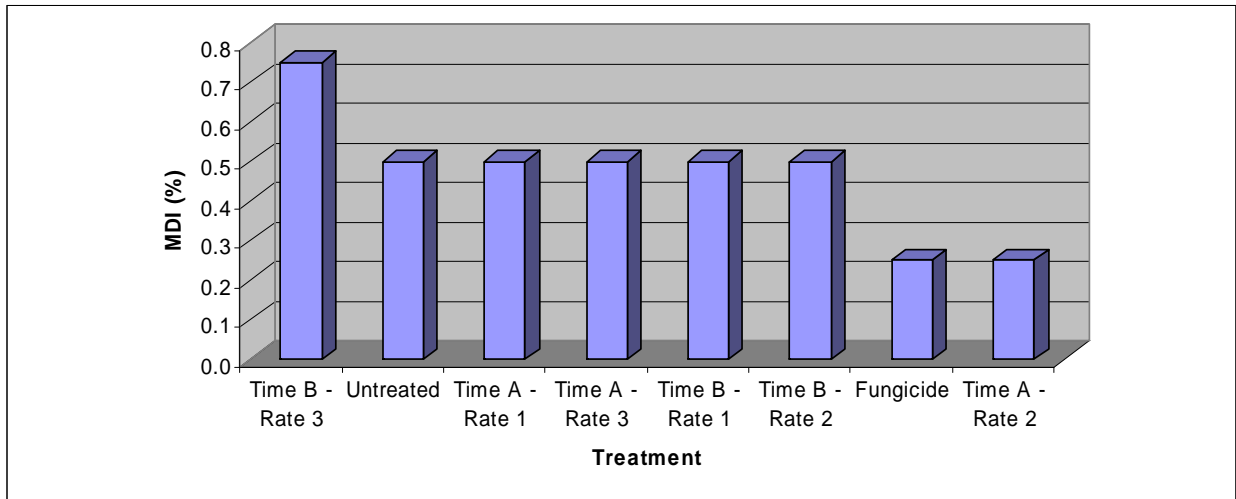


**Table 8.** Impact of *C. minitans* rate and timing of application, and fungicide on the mean disease incidence (MDI) of sclerotinia stem rot and seed yield of canola at three locations in 2007.

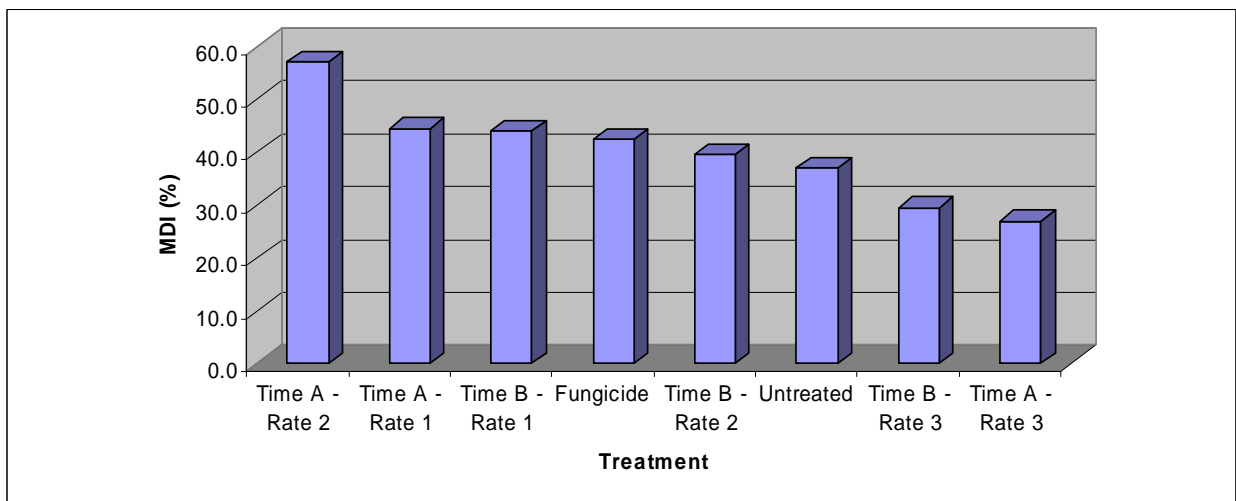
Treatment	Bran-East		Bran-West		Morden	
	MDI	Yield	MDI	Yield	MDI	Yield
Untreated	0.5 <i>a</i>	1423.8 <i>b*</i>	7.8 <i>a</i>	2256.5 <i>a</i>	37.0 <i>ab</i>	2775.0 <i>a</i>
Fungicide	0.3 <i>a</i>	1540.5 <i>ab</i>	4.5 <i>a</i>	2121.7 <i>a</i>	42.5 <i>ab</i>	2458.5 <i>ab</i>
Time A, Rate 1	0.5 <i>a</i>	1665.6 <i>a</i>	4.5 <i>a</i>	2391.5 <i>a</i>	44.5 <i>ab</i>	2620.0 <i>ab</i>
Time A, Rate 2	0.3 <i>a</i>	1508.3 <i>b</i>	4.8 <i>a</i>	2225.2 <i>a</i>	57.0 <i>a</i>	2312.0 <i>b</i>
Time A, Rate 3	0.5 <i>a</i>	1571.4 <i>ab</i>	2.8 <i>a</i>	2245.1 <i>a</i>	27.0 <i>b</i>	2746.5 <i>a</i>
Time B, Rate 1	0.5 <i>a</i>	1463.4 <i>b</i>	2.8 <i>a</i>	2391.9 <i>a</i>	44.0 <i>ab</i>	2499.5 <i>ab</i>
Time B, Rate 2	0.5 <i>a</i>	1575.9 <i>ab</i>	2.8 <i>a</i>	2367.6 <i>a</i>	39.5 <i>ab</i>	2810.0 <i>a</i>
Time B, Rate 3	0.8 <i>a</i>	1532.1 <i>ab</i>	4.5 <i>a</i>	2372.7 <i>a</i>	29.5 <i>b</i>	2642.5 <i>ab</i>

\*means are presented with LSD grouping ( $\alpha=0.05$ ) where treatments with the same letter are not significantly different from each other.

**Figure 20.** Impact of *C. minitans* rate and timing of application, and fungicide on the mean disease incidence (MDI) of sclerotinia stem rot of canola at the Brandon-East site (2007).

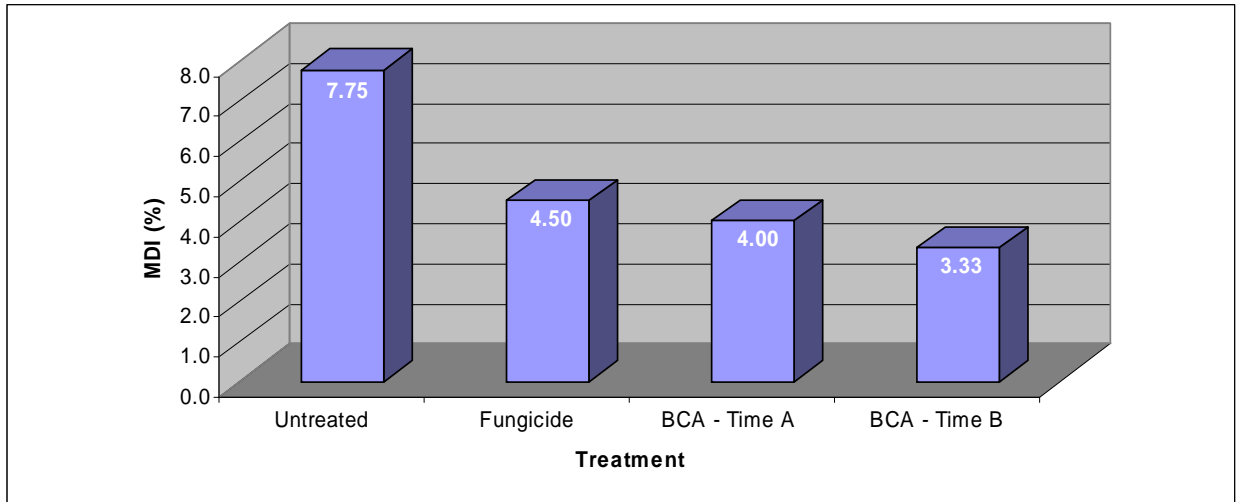


**Figure 21.** Impact of *C. minitans* rate and timing of application, and fungicide on the mean disease incidence (MDI) of sclerotinia stem rot of canola at the Morden site (2007).

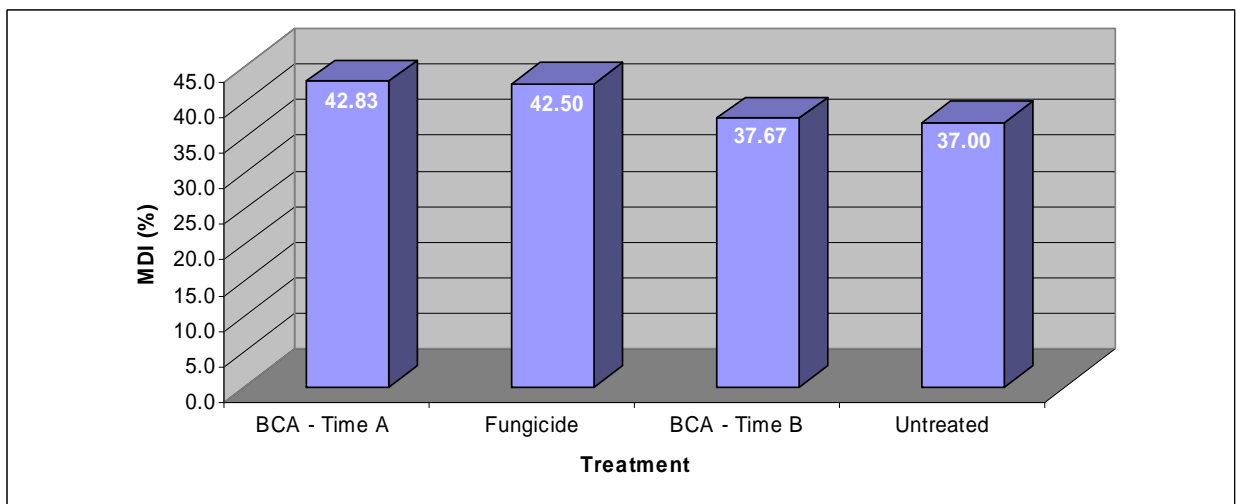




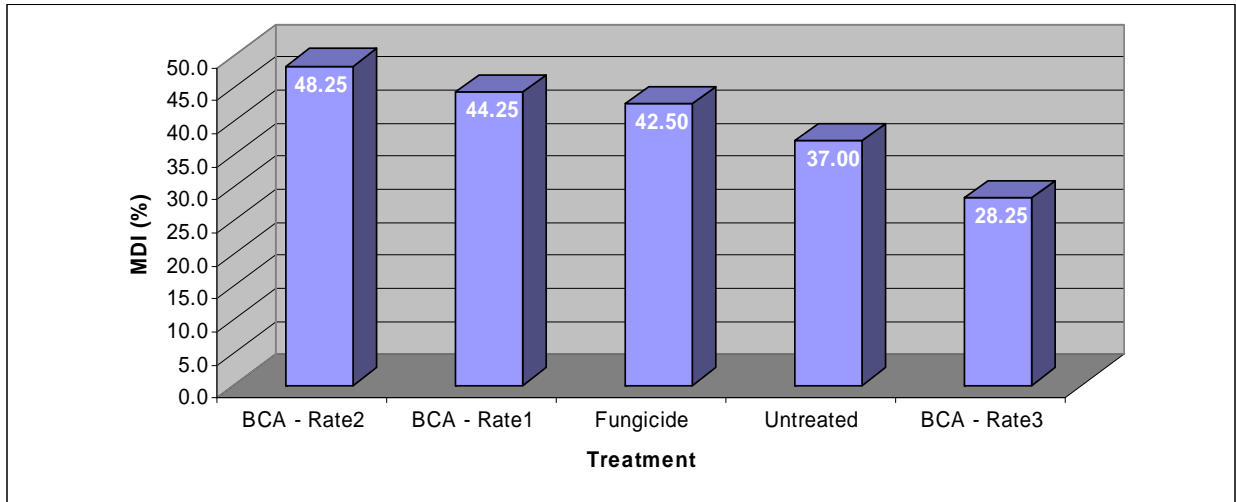
**Figure 22.** Impact of *C. minitans* application timing and fungicide on the mean disease incidence (MDI) of sclerotinia stem rot of canola at the Brandon-West site (2007).



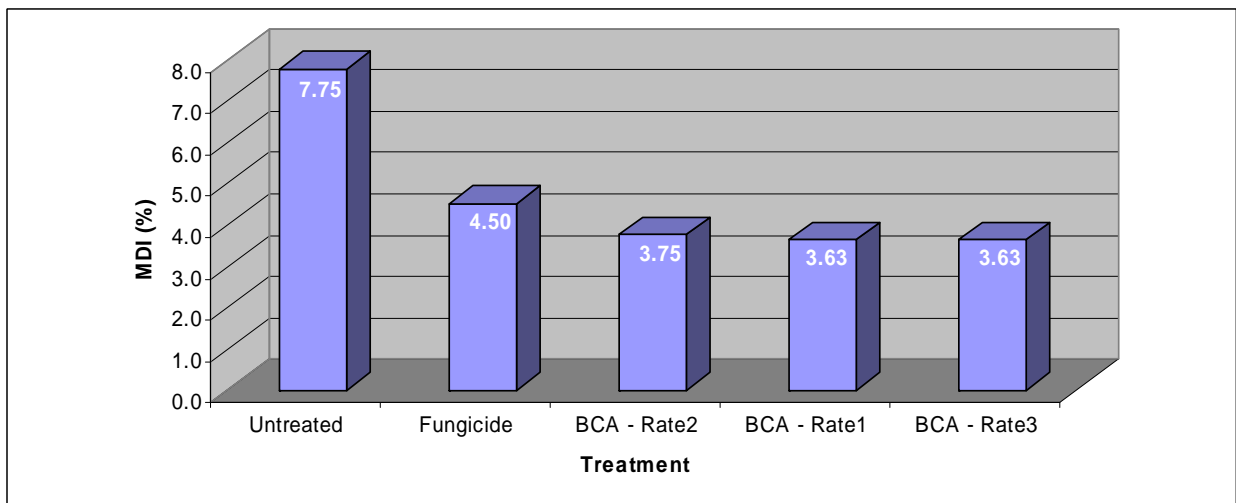
**Figure 23.** Impact of *C. minitans* application timing and fungicide on the mean disease incidence (MDI) of sclerotinia stem rot of canola at the Morden site (2007).



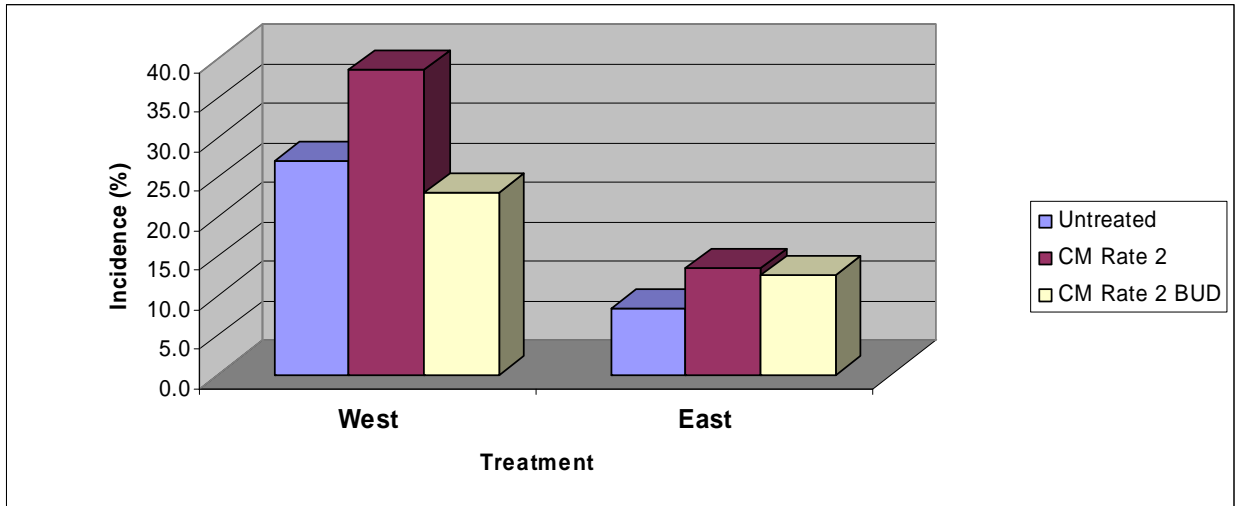
**Figure 24.** Impact of *C. minitans* application rate and fungicide on the mean disease incidence (MDI) of sclerotinia stem rot of canola at the Morden site (2007).



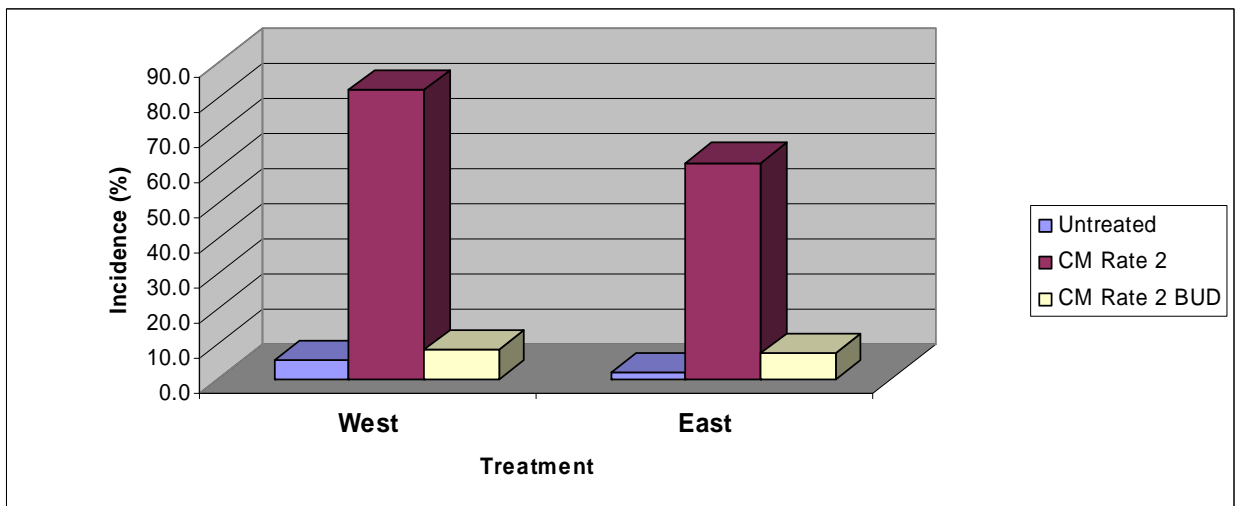
**Figure 25.** Impact of *C. minitans* application rate and fungicide on the mean disease incidence (MDI) of sclerotinia stem rot of canola at the Brandon-West site (2007).



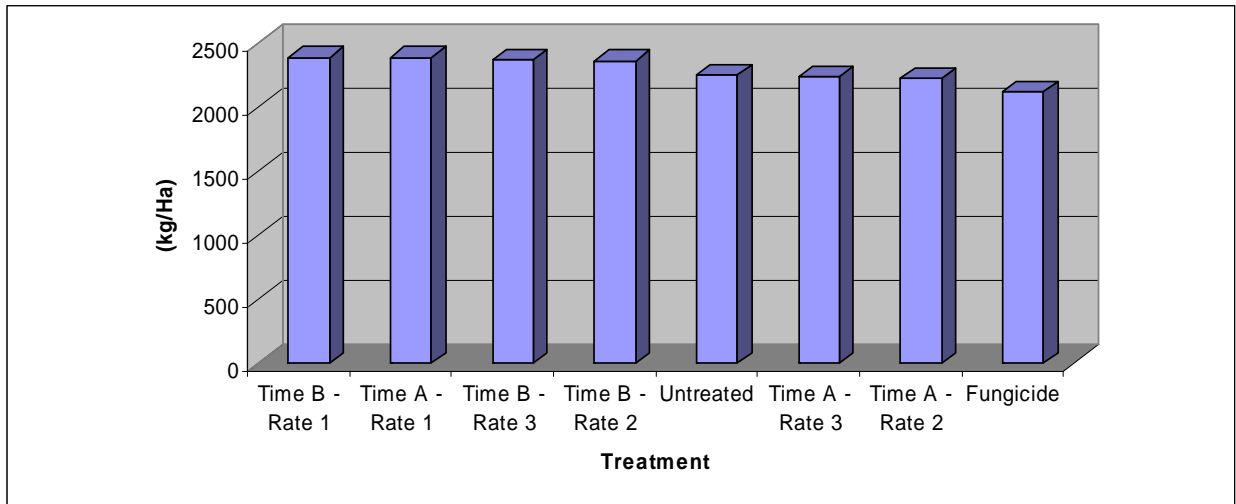
**Figure 26.** Incidence of *Sclerotinia sclerotiorum* cultured from petal samples from select treatments at Brandon (2007).



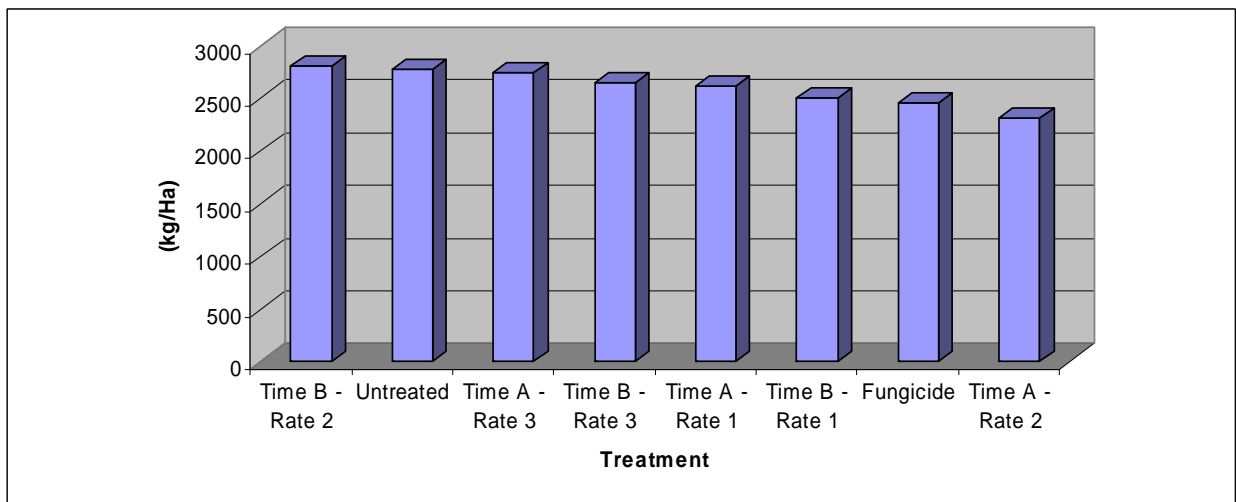
**Figure 27.** Incidence of *Coniothyrium minitans* cultured from petal samples from select treatments at Brandon (2007).



**Figure 28.** Impact of *C. minitans* rate and timing of application, and fungicide on the seed yield of canola at the Brandon-West site (2007).



**Figure 29.** Impact of *C. minitans* rate and timing of application, and fungicide on the seed yield of canola at the Morden site (2007).



**EXPERIMENT 1: TABLES AND FIGURES 2008**

**Table 9.** Application of *C. minitans* (CM) and Ronilan to canola at the Brandon-East (CE) and Brandon-West (CW) sites in 2008.

Brandon Treatment	Description	Timing	Rate (CM-spores/mL)	Application Date(s)
1	Untreated	NA	NA	NA
2	Fungicide, split (Ronilan)	25-30 and 60% bloom	Split Rate = 0.2 kg/ac each	July 10 & July 15
3	Foliar CM single	A (25-30% bloom)	Full Rate Single ( $5.15 \times 10^6$ )	July 10
4	Foliar CM single	B (60% bloom)	Full Rate Single ( $5.15 \times 10^6$ )	July 15
5	Foliar CM split	A & B (25-30 and 60% bloom)	Half Rate Split ( $2.58 \times 10^6$ each)	July 10 & July 15
6	Soil & Foliar CM	Pre-seed & A (25-30% bloom)	Half Rate Split ( $2.58 \times 10^6$ each)	May 23 & July 10
7	Soil & Foliar CM	Pre-seed & B (60% bloom)	Half Rate Split ( $2.58 \times 10^6$ each)	May 23 & July 15
8	Soil CM single	Pre-seed	Full Rate Single ( $5.15 \times 10^6$ )	May 23

**Table 10.** Application of *C. minitans* (CM) and Ronilan to canola at the Morden site in 2008.

Morden Treatment	Description	Timing	Rate (CM-spores/mL)	Application Date(s)
1	Untreated	-	-	-
2	Fungicide, Split (Ronilan)	Split: 20 and 50% bloom	0.2 kg/ac each	July 8 and 15
3	Foliar CM single	B (50% bloom)	0.5X Rate, single ( $3.3 \times 10^6$ )	15-Jul
4	Foliar CM single	B (50% bloom)	1X Rate, single ( $6.6 \times 10^6$ )	15-Jul
5	Foliar CM single	B (50% bloom)	2X Rate, single ( $1.3 \times 10^7$ )	15-Jul
6	Soil and Foliar CM	Pre-seed & A (20-30% bloom)	0.5X Rate, split ( $2.7 \times 10^6$ each)	May 28 and July 8
7	Soil and Foliar CM	Pre-seed, A (20-30%) & B(50%)	0.5X each ( $2.7, 2.7$ and $3.3 \times 10^6$ )	May 28, July 8 & 15
8	Soil and Foliar CM	Pre-seed & A (20-30% bloom)	1X each ( $5.3 \times 10^6$ )	May 28 and July 8

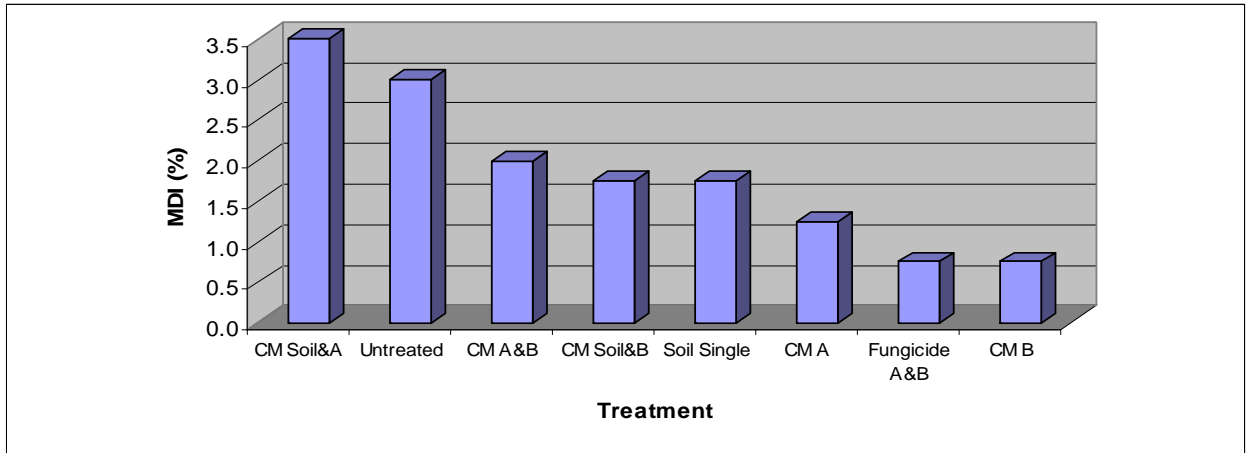
**Table 11.** Important dates noted for Experiment 1 (Impact of application timing of *C. minitans* on sclerotinia of canola) at all sites in 2008.

Dates of...	Brandon EAST	Brandon WEST	Morden
Seeding	May 28	May 28	May 29
Emergence	June 5	June 5	-
Irrigation Start	July 2	-	July 21
Canopy Closure	June 27	June 27	-
First Apothecia	-	July 12	-
Flowering Start	July 6	July 6	-
First Petal Drop	July 10	July 10	-
Soil Treatment	May 23	May 23	May 28
Treatment Time A	July 10	July 110	July 8
Treatment Time B	July 15	July 15	July 15
Flowering End	Aug 5	Aug 5	-
First Disease Lesion	-	-	-
Disease Rating	Aug 28	Aug 27	Aug 10
Swathing	Aug 29	Aug 29	Aug 18
Combining	Sept 25	Sept 25	Sept 9

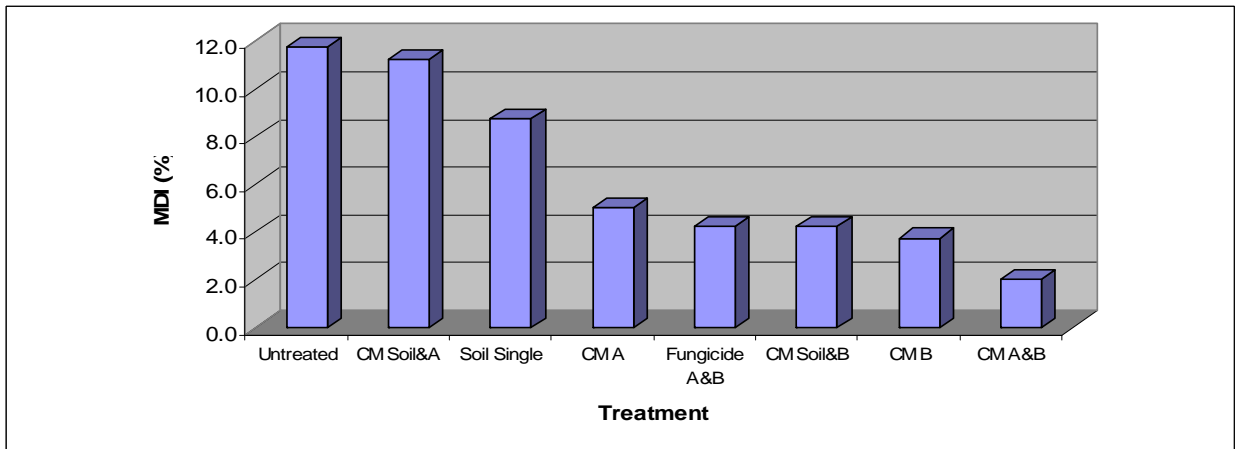
**Figure 30.** Canola trial located at the Brandon-East site, July 2, 2008.



**Figure 31.** Impact of *C. minitans* placement and timing of application, and fungicide on the mean disease incidence (MDI) of sclerotinia stem rot of canola at the Brandon-West site (2008).



**Figure 32.** Impact of *C. minitans* placement and timing of application, and fungicide on the mean disease incidence (MDI) of sclerotinia stem rot of canola at the Brandon-East site (2008).

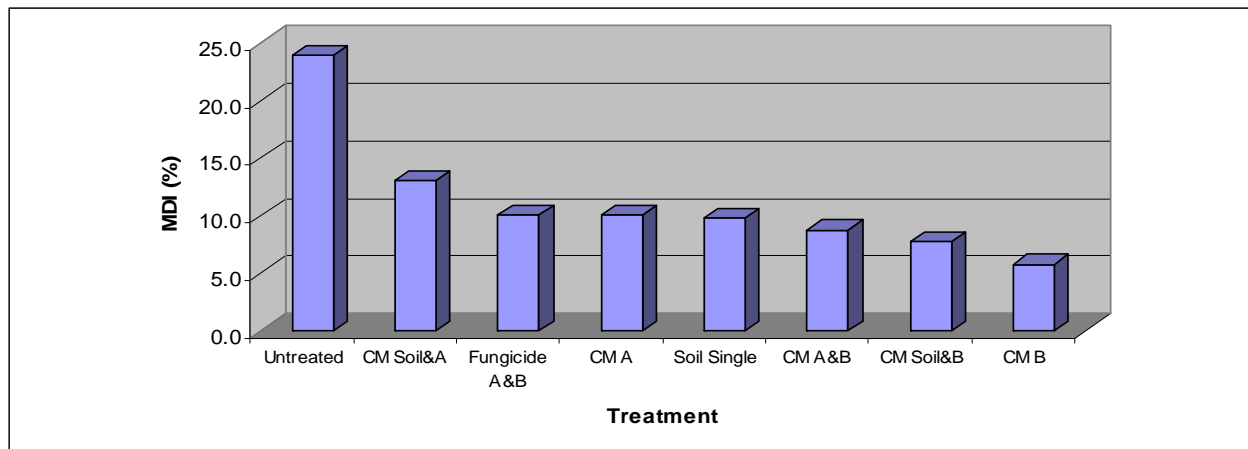


**Table 12.** Impact of *C. minitans* rate and timing of application, and fungicide on the mean disease incidence (MDI) of sclerotinia stem rot and seed yield of canola at the Brandon sites in 2008.

Treatment	Bran-East		Bran-West	
	MDI	Yield	MDI	Yield
Untreated	11.8 a*	2377.9 a	3.0 a	1863.2 a
Fungicide	4.3 ab	2167.8 a	0.8 a	1786.3 a
Time A, Single	5.0 ab	2333.8 a	1.3 a	1768.0 a
Time B, Single	3.8 ab	2068.1 a	0.8 a	1678.2 a
Time A&B, Split	2.0 b	2298.5 a	2.0 a	1742.8 a
Soil & Time A, Split	11.3 a	2432.7 a	3.5 a	1947.4 a
Soil & Time B, Split	4.3 ab	2364.2 a	1.8 a	1913.6 a
Soil, Single	8.8 ab	2330.4 a	1.8 a	1658.1 a

\*means are presented with LSD grouping ( $\alpha=0.05$ ) where treatments with the same letter are not significantly different from each other.

**Figure 33.** Impact of *C. minitans* placement and timing of application, and fungicide on the mean disease incidence (MDI) of sclerotinia stem rot of canola at the Morden site (2008).



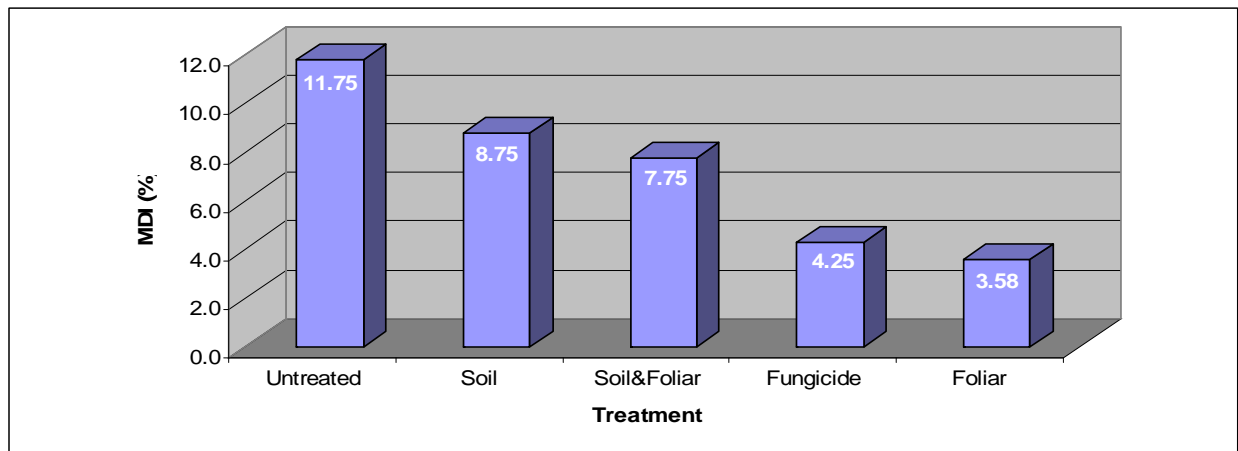


**Table 13.** Impact of *C. minitans* rate and timing of application, and fungicide on the mean disease incidence (MDI) of sclerotinia stem rot and seed yield of canola at the Morden site in 2008.

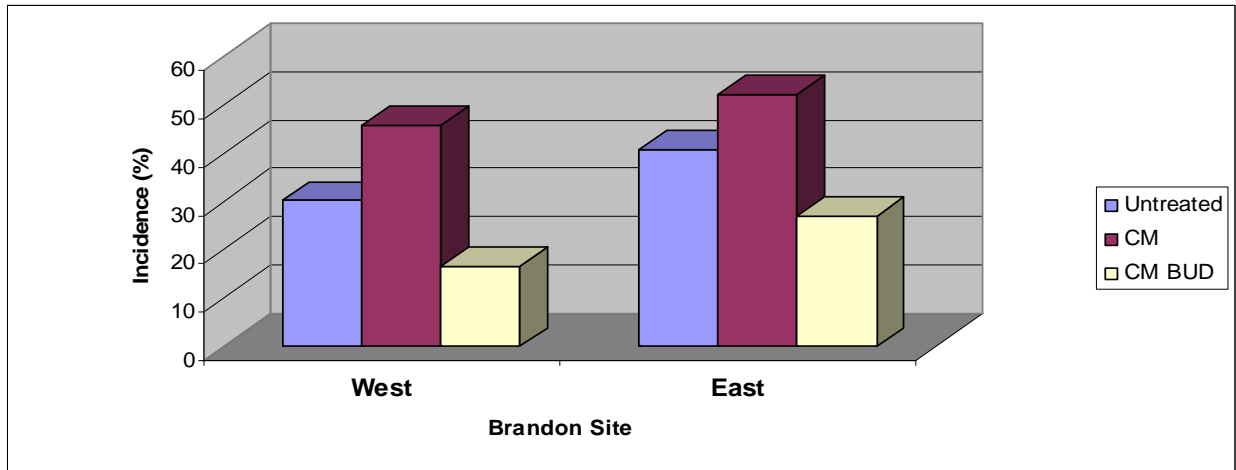
Treatment	MDI	Yield
Untreated	48.0 <i>a</i>	3152.5 <i>ab</i>
Fungicide, Split	20.0 <i>bc</i>	3329.5 <i>a</i>
Time B, 0.5X Rate	20.0 <i>bc</i>	3229.6 <i>ab</i>
Time B, 1X Rate	11.5 <i>c</i>	3256.4 <i>a</i>
Time B, 2X Rate	17.5 <i>bc</i>	3246.7 <i>a</i>
Soil & Time A, 0.5X each	26.0 <i>b</i>	2975.3 <i>b</i>
Soil & Time A&B, 0.5X each	15.5 <i>bc</i>	3146.6 <i>ab</i>
Soil & Time A, 1X each	19.5 <i>bc</i>	3146.0 <i>ab</i>

\*means are presented with LSD grouping ( $\alpha=0.05$ ) where treatments with the same letter are not significantly different from each other.

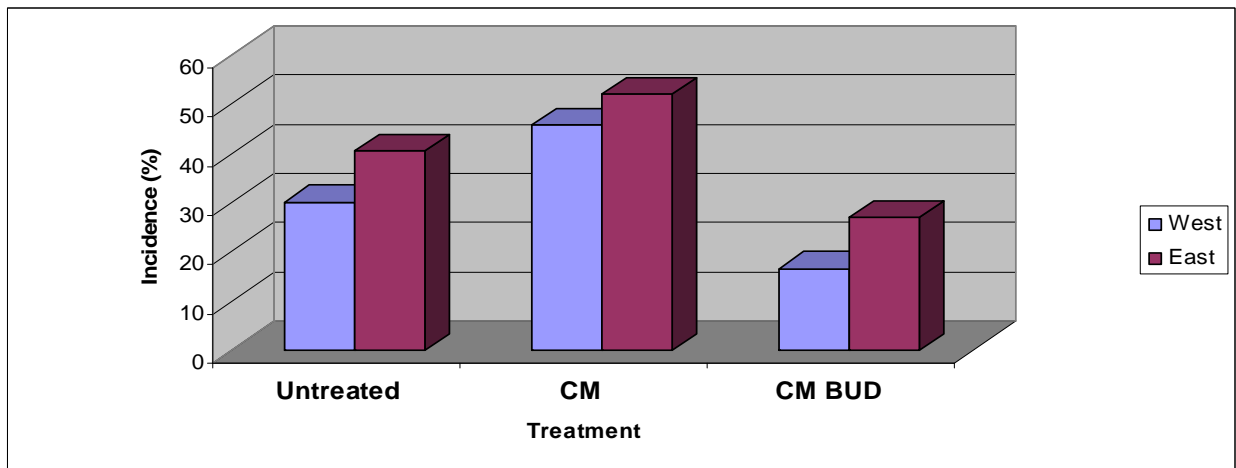
**Figure 34.** Impact of *C. minitans* application type, and fungicide on the mean disease incidence (MDI) of sclerotinia stem rot of canola at the Brandon-East site (2008).



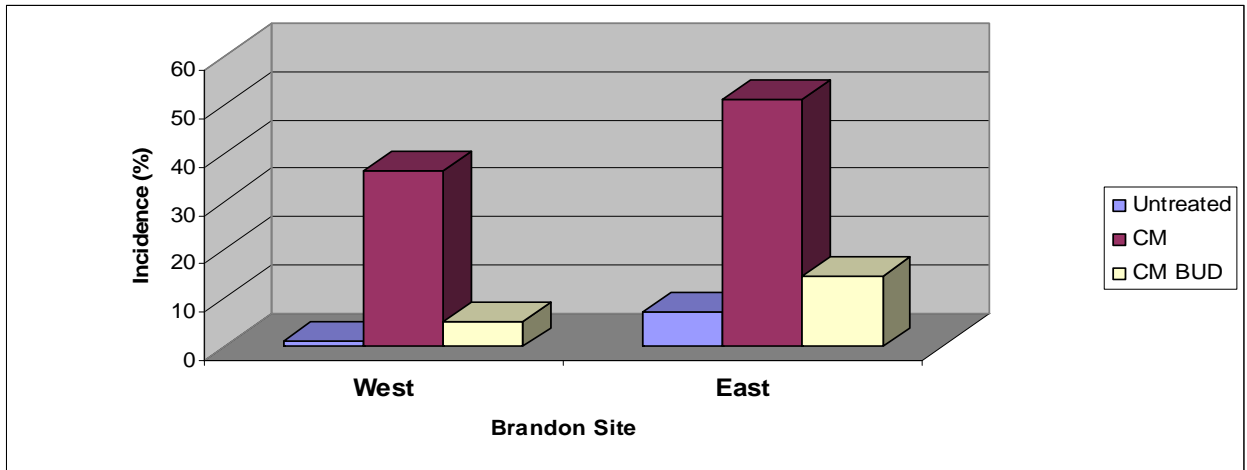
**Figure 35.** Incidence of *Sclerotinia sclerotiorum* cultured from petal samples from select treatments at Brandon (2008).



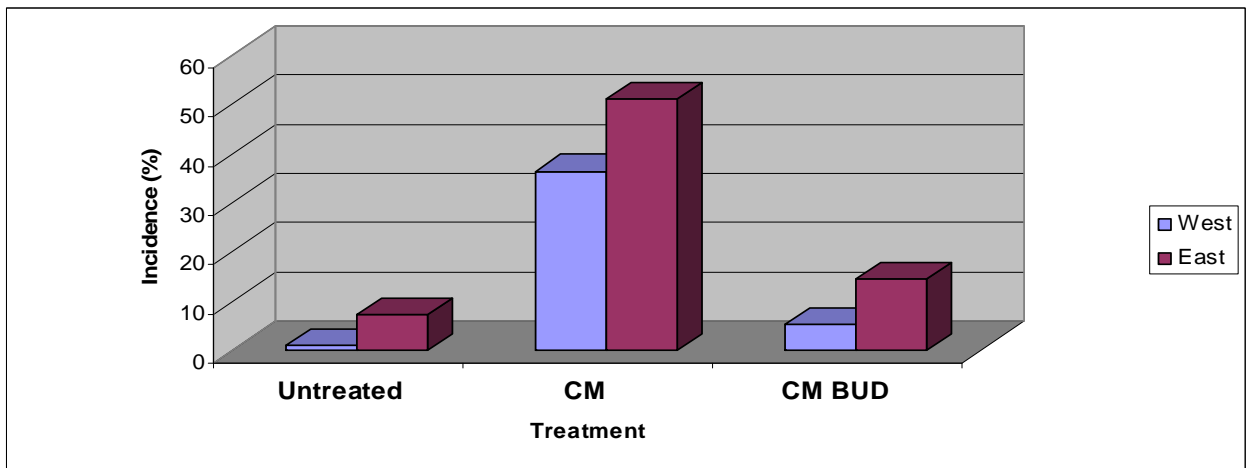
**Figure 36.** Incidence of *Sclerotinia sclerotiorum* cultured from petal samples from select treatments at Brandon (2008).



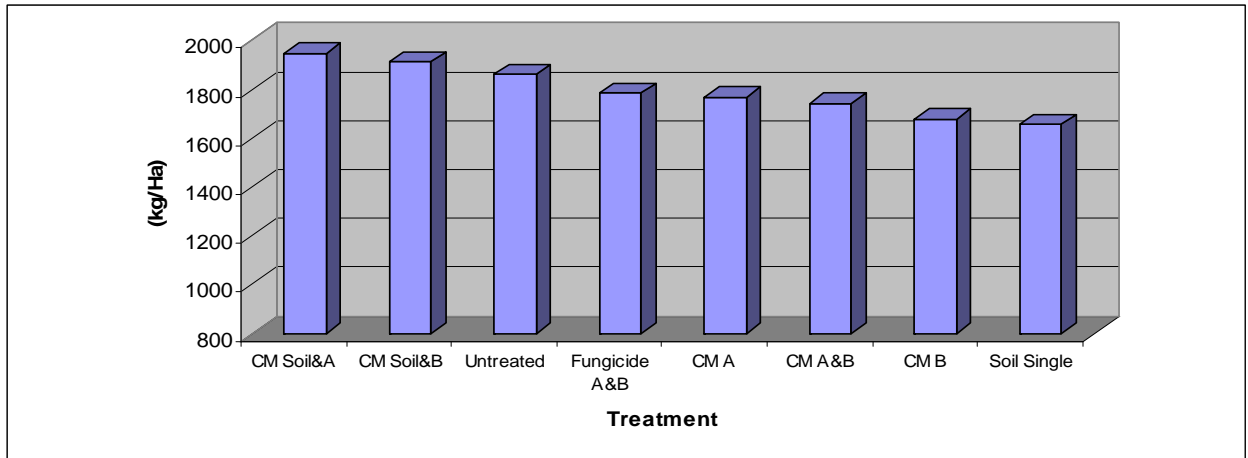
**Figure 37.** Incidence of *Coniothyrium minitans* cultured from petal samples from select treatments at Brandon (2008).



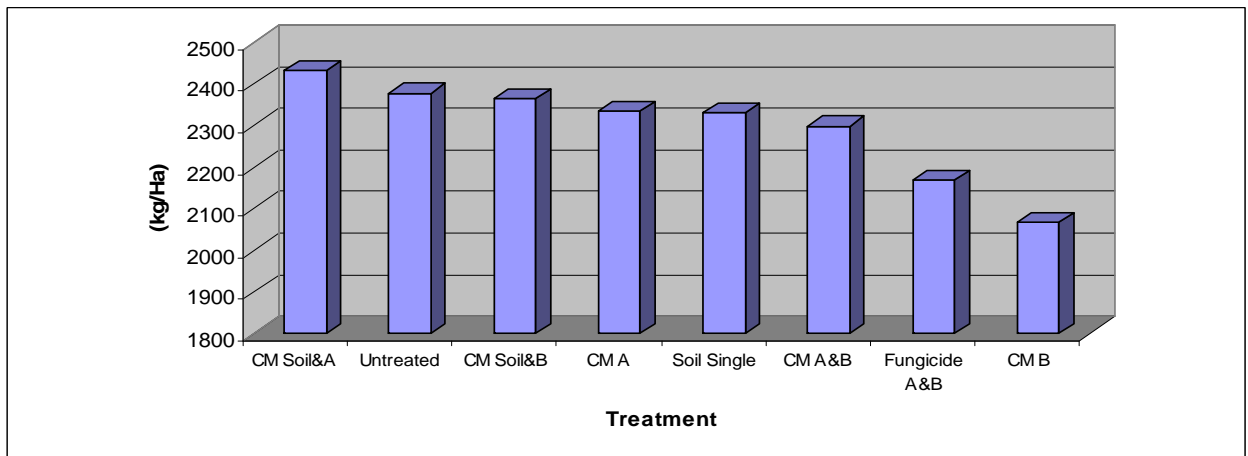
**Figure 38.** Incidence of *Coniothyrium minitans* cultured from petal samples from select treatments at Brandon (2008).



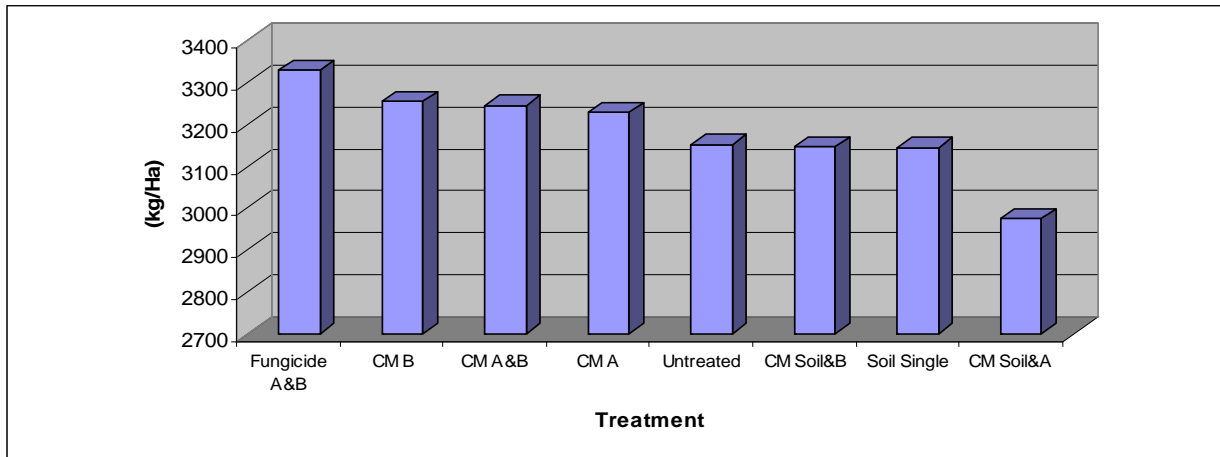
**Figure 39.** Impact of *C. minitans* placement and timing of application, and fungicide on the seed yield of canola at the Brandon-West site (2008).



**Figure 40.** Impact of *C. minitans* rate and timing of application, and fungicide on the seed yield of canola at the Brandon-East site (2008).



**Figure 41.** Impact of *C. minitans* rate and timing of application, and fungicide on the seed yield of canola at the Morden site (2008).



**EXPERIMENT 2: TABLES AND FIGURES 2006-2008**

**Table 14.** Application of *C. minitans* (CM) to soil and burial of sclerotia for the overwintering study at the Brandon-East and Brandon-West sites in 2006.

Brandon Treatment	Description	Rate (spores/mL)	Application Date	Mesh bags burial date	Sclerotia Burial
1	Control	-	-	June 2	June 7
2	Soil CM	1 (1.03 x 10 <sup>6</sup> )	May 31	June 2	June 7
3	Soil CM	2 (5.17 x 10 <sup>6</sup> )	May 31	June 2	June 7
4	Soil CM	3 (2.59 x 10 <sup>7</sup> )	May 31	June 2	June 7

**Table 15.** Application of *C. minitans* (CM) to soil and burial of sclerotia for the overwintering study at the Morden site in 2006.

Morden Treatment	Description	Rate (spores/mL)	Application Date	Mesh bags burial date	Sclerotia Burial*
1	Control	-	-	June 2	N/A
2	Soil CM	1 (1.03 x 10 <sup>6</sup> )	June 1	June 2	N/A
3	Soil CM	2 (5.17 x 10 <sup>6</sup> )	June 1	June 2	N/A
4	Soil CM	3 (2.59 x 10 <sup>7</sup> )	June 1	June 2	N/A

\* sclerotia were buried in mesh bags only at this site.

**Table 16.** Application of *C. minitans* (CM) to soil and burial of sclerotia for the overwintering study at the Brandon-East and Brandon-West sites in 2007.

Brandon Treatment	Description	Rate (spores/mL)	Application Date	Mesh bags burial date	Sclerotia Burial
1	Control	-	-	June 5	June 5
2	Soil CM	1 (1.03 x 10 <sup>6</sup> )	June 5	June 5	June 5
3	Soil CM	2 (5.17 x 10 <sup>6</sup> )	June 5	June 5	June 5
4	Soil CM	3 (2.59 x 10 <sup>7</sup> )	June 5	June 5	June 5

**Table 17.** Application of *C. minitans* (CM) to soil and burial of sclerotia for the overwintering study at the Morden site in 2007.

Morden Treatment	Description	Rate (spores/mL)	Application Date	Mesh bags burial date	Sclerotia Burial*
1	Control	-	-	-	N/A
2	Soil CM	1 (1.03 x 10 <sup>6</sup> )	June 6	CHECK	N/A
3	Soil CM	2 (5.17 x 10 <sup>6</sup> )	June 6	CHECK	N/A
4	Soil CM	3 (2.59 x 10 <sup>7</sup> )	June 6	CHECK	N/A

\* sclerotia were buried in mesh bags only at this site.

**Table 18.** Burial of sclerotia for the *C. minitans* (CM) overwintering study at the Brandon-East and Brandon-West sites in 2008.

Brandon Treatment	Description	2006 & 2007 Rate* (spores/mL)	Mesh bags burial date	Mesh bags removal date	Sclerotia Burial
1	Control	-	June 17	Aug 15	-
2	Soil CM	1 ( $1.03 \times 10^6$ )	June 17	Aug 15	June 17
3	Soil CM	2 ( $5.17 \times 10^6$ )	June 17	Aug 15	June 17
4	Soil CM	3 ( $2.59 \times 10^7$ )	June 17	Aug 15	June 17

\* CM was not applied in 2008 in order to determine the impact of CM application on the year following treatment.

**Table 19.** Burial of sclerotia for the *C. minitans* (CM) overwintering study at the Morden site in 2008.

Morden Treatment	Description	2006 & 2007 Rate <sup>1</sup> (spores/mL)	Mesh bags burial date	Mesh bags removal date	Sclerotia Burial <sup>2</sup>
1	Control	-	June 5	Aug 18	N/A
2	Soil CM	1 ( $1.03 \times 10^6$ )	June 5	Aug 18	N/A
3	Soil CM	2 ( $5.17 \times 10^6$ )	June 5	Aug 18	N/A
4	Soil CM	3 ( $2.59 \times 10^7$ )	June 5	Aug 18	N/A

<sup>1</sup> CM was not applied in 2008 in order to determine the impact of CM application on the year following treatment.

<sup>2</sup> Sclerotia were buried in mesh bags only at this site.

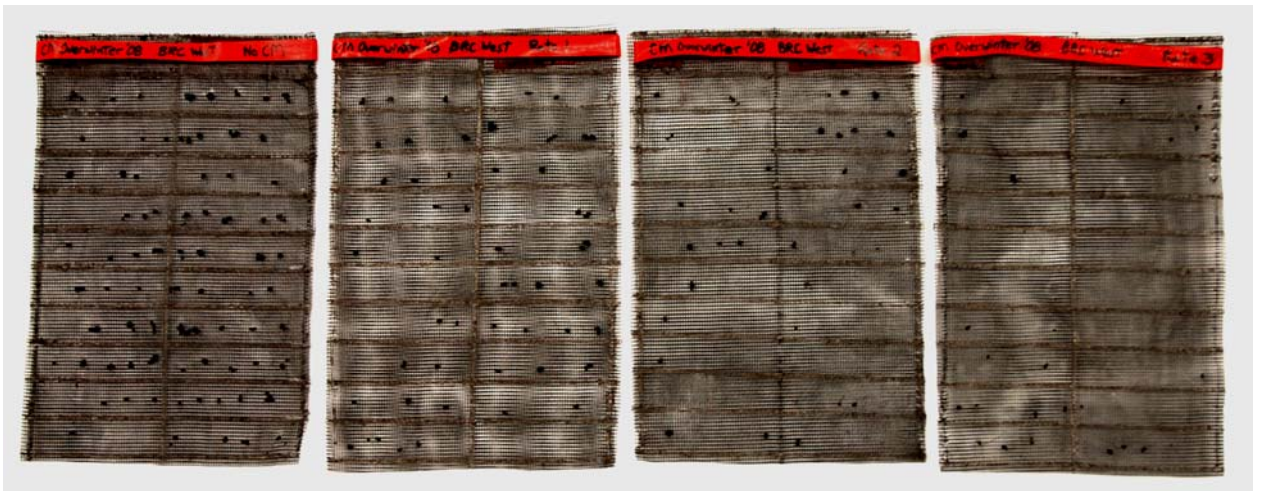
**Figure 42.** Impact of *C. minitans* applied to the soil on recovery of sclerotia of *S. sclerotiorum* at 15 weeks after burial at the Brandon-West site (2006). Treatments from left to right: No biocontrol, *C. minitans* Rate 1, Rate 2 and Rate 3.



**Figure 43.** Impact of *C. minitans* applied to the soil on recovery of sclerotia of *S. sclerotiorum* at 14 weeks after burial at the Brandon-West site (2007). Treatments from left to right: No biocontrol, *C. minitans* Rate 1, Rate2 and Rate 3.

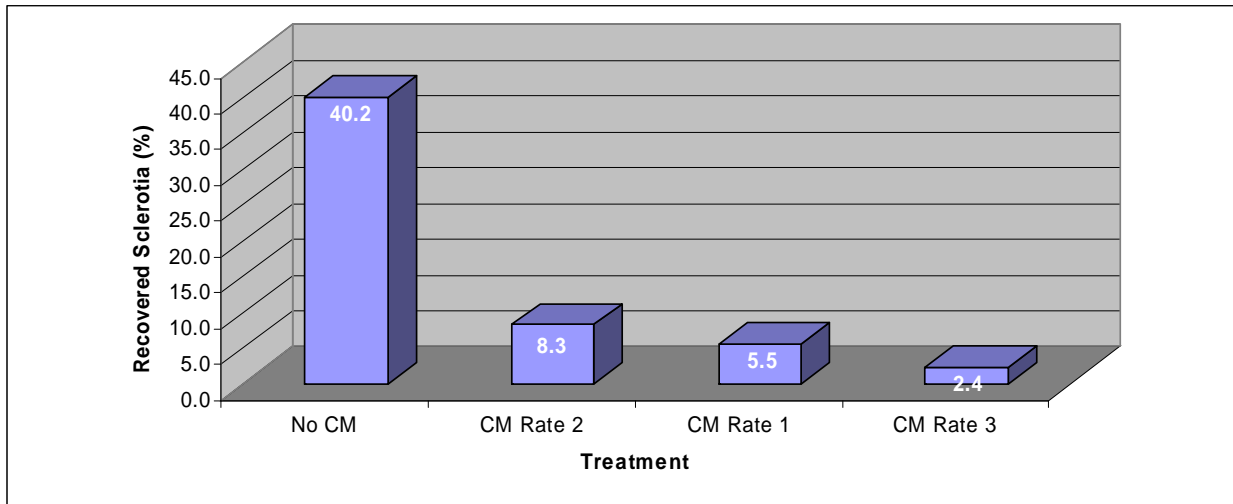


**Figure 44.** Impact of previous year *C. minitans* application to the soil on recovery of sclerotia of *S. sclerotiorum* at 8.3 weeks after burial at the Brandon-West site (2008). Treatments from left to right: No biocontrol, *C. minitans* Rate 1, Rate2 and Rate 3.





**Figure 45.** Mean percent of intact sclerotia recovered from mesh bags in the Brandon-East site in 2006.

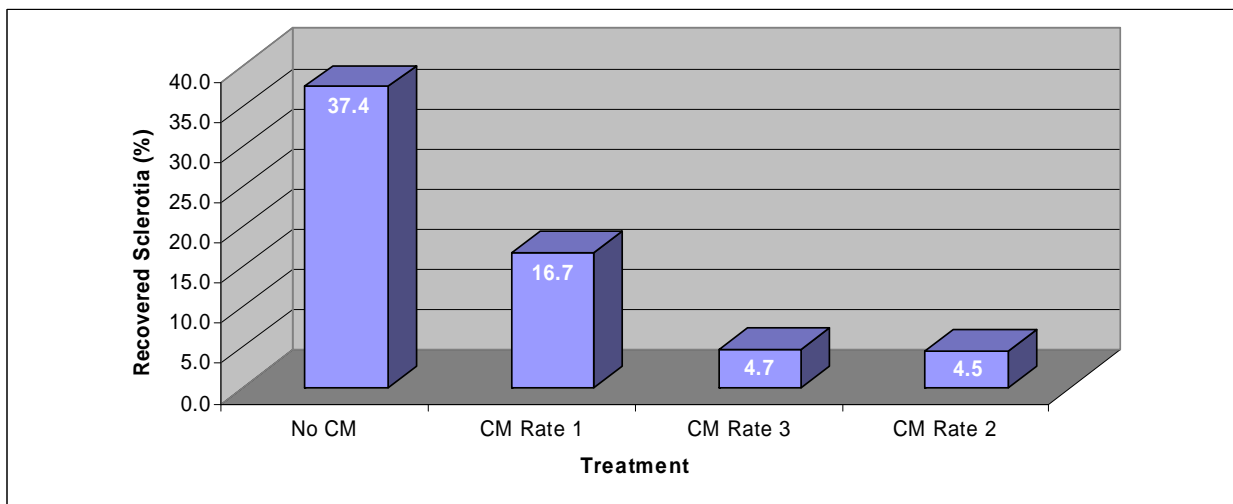


**Table 20.** Effect of *C. minitans* rate of application on percent recovered sclerotia at three locations in 2006.

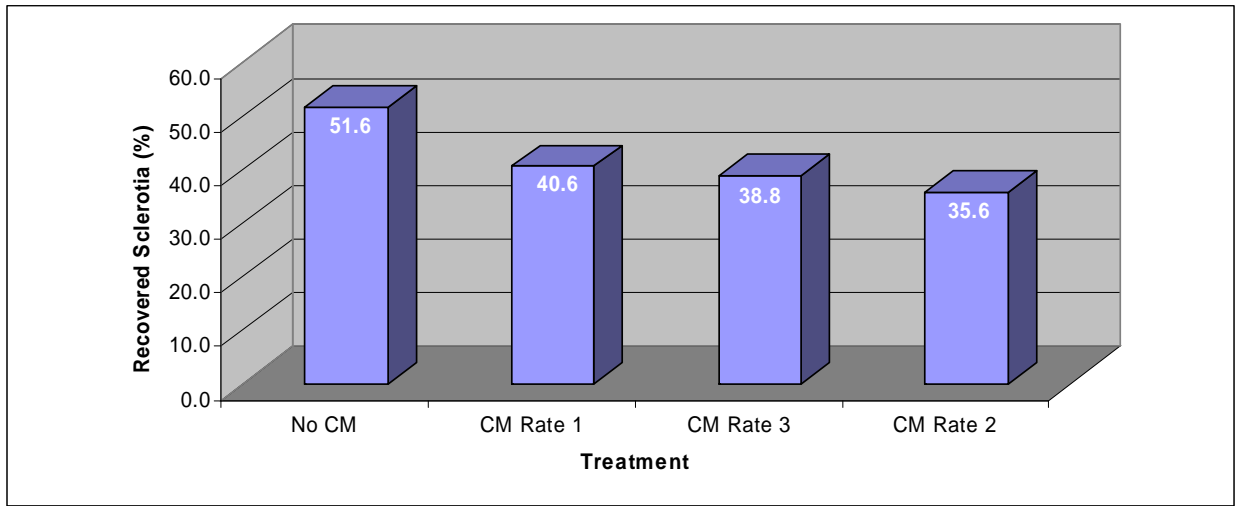
Treatment	Bran-East	Bran-West	Morden
Untreated	40.2 a	37.4 a	51.6 a
Rate 1	5.5 b	16.7 b	40.6 a
Rate 2	8.3 b	4.5 c	35.6 a
Rate 3	2.4 b	4.7 c	38.8 a

\*Means are presented with LSD grouping ( $\alpha=0.05$ ) where treatments with the same letter are not significantly different from each other.

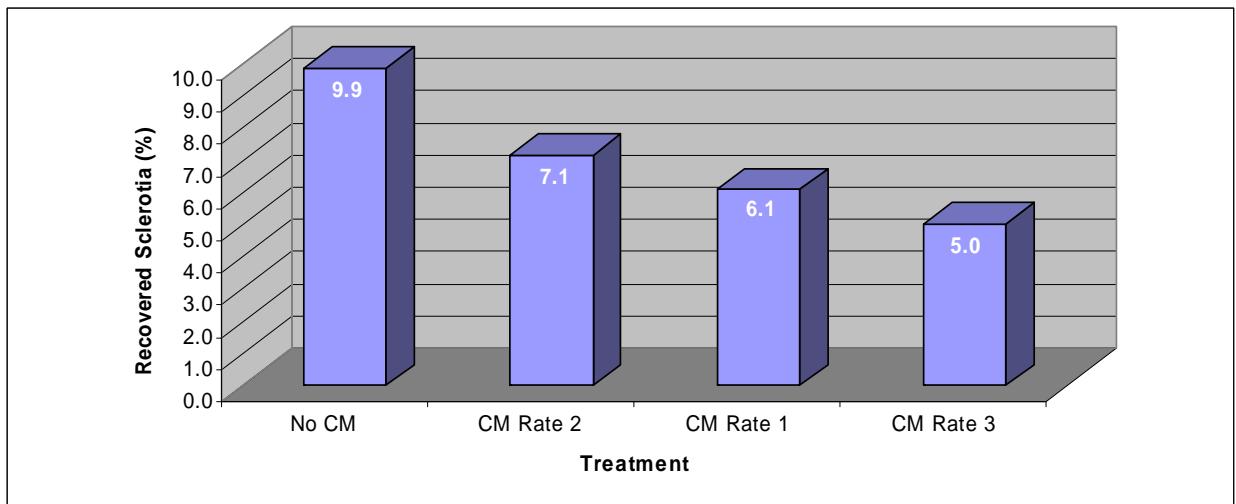
**Figure 46.** Mean percent of intact sclerotia recovered from mesh bags in the Brandon-West site in 2006.



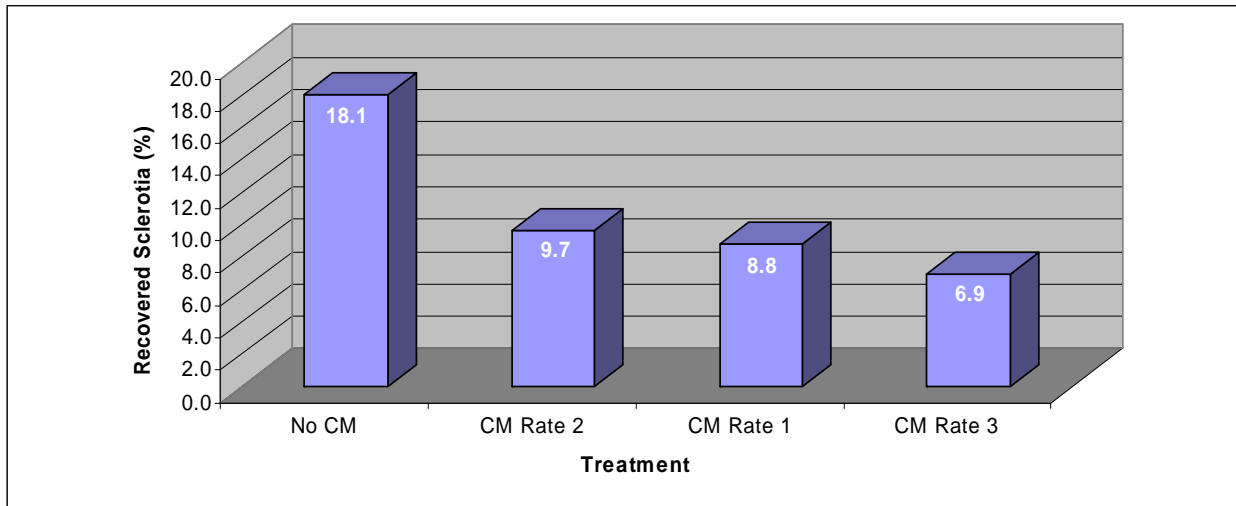
**Figure 47.** Mean percent of intact sclerotia recovered from mesh bags in the Morden site in 2006.



**Figure 48.** Mean percent of intact sclerotia recovered from mesh bags in the Brandon-East site in 2007.



**Figure 49.** Mean percent of intact sclerotia recovered from mesh bags in the Brandon-West site in 2007.

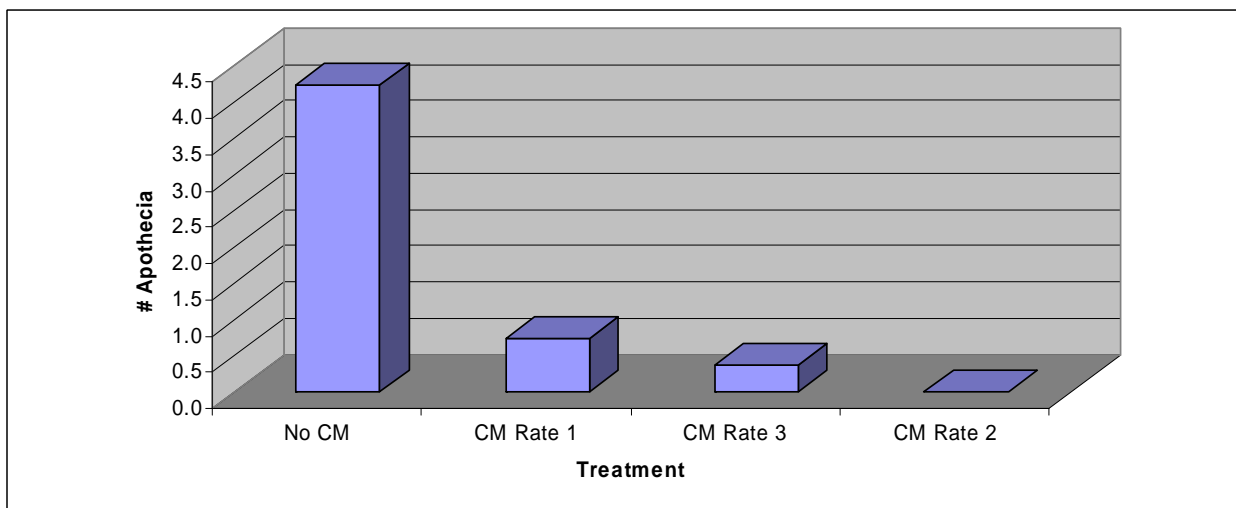


**Table 21.** Effect of *C. minitans* rate of application on percent recovered sclerotia at three locations in 2007.

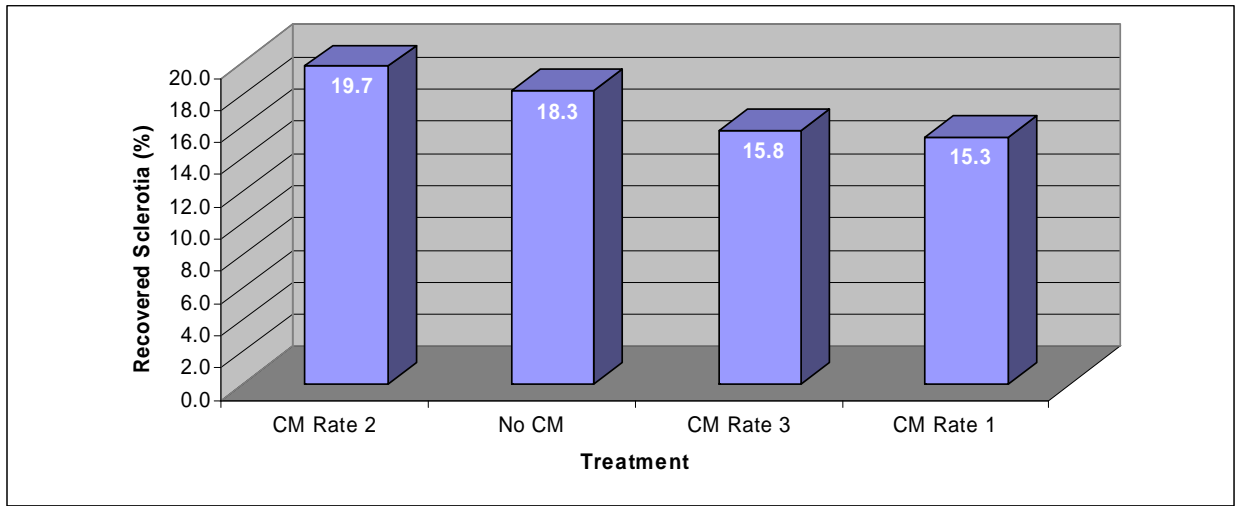
Treatment	Bran-East	Bran-West	Morden
Untreated	9.9 a*	18.1 a	18.3 a
Rate 1	6.1 a	8.8 b	15.3 a
Rate 2	7.1 a	9.7 b	19.7 a
Rate 3	5.0 a	6.9 b	15.8 a

\*Means are presented with LSD grouping ( $\alpha=0.05$ ) where treatments with the same letter are not significantly different from each other.

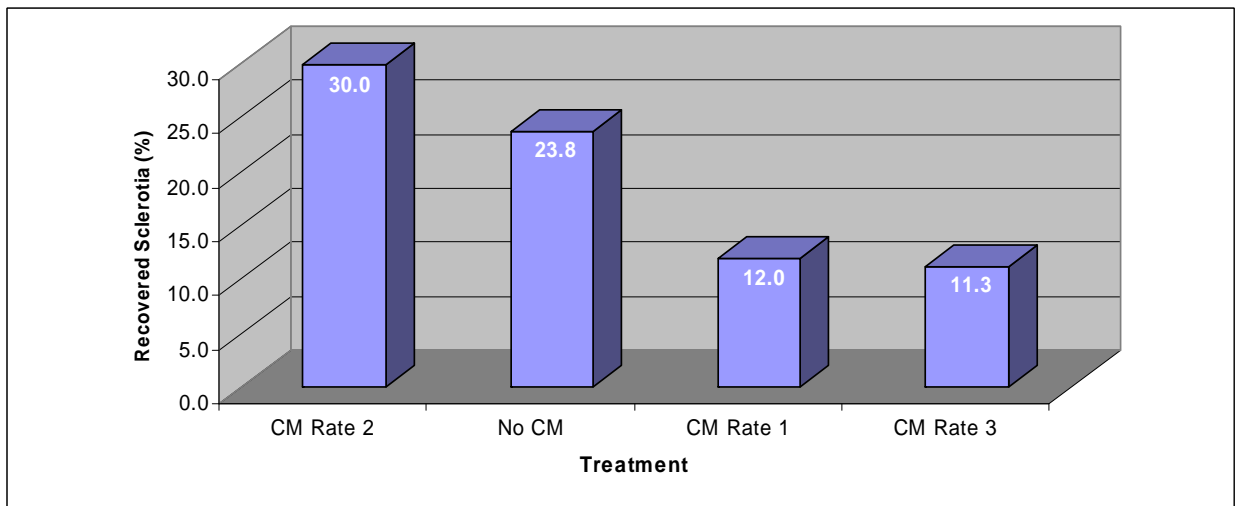
**Figure 50.** Mean number of apothecia in the Brandon sites in 2007.



**Figure 51.** Mean percent of intact sclerotia recovered from mesh bags in the Morden site in 2007.



**Figure 52.** Mean percent of intact sclerotia recovered from mesh bags in the Brandon-East site in 2008.

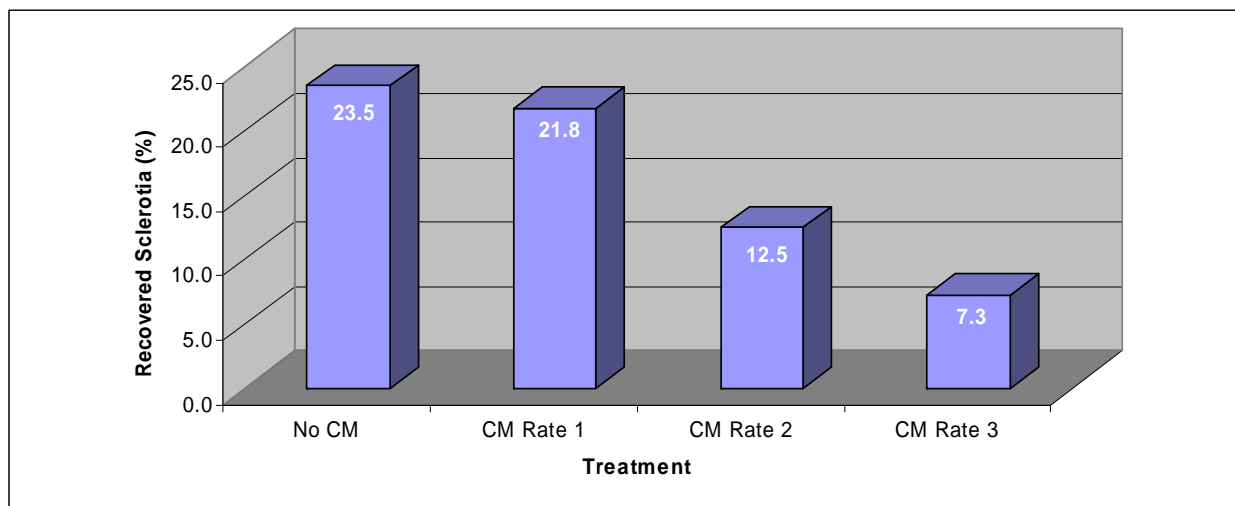


**Table 22.** Effect of *C. minitans* rate of application on percent recovered sclerotia at three locations in 2008.

Treatment	Bran-East	Bran-West	Morden
Untreated	23.8 <i>ab</i>	23.5 <i>a</i>	39.0 <i>a</i>
Rate 1	12.0 <i>b</i>	21.8 <i>a</i>	32.8 <i>a</i>
Rate 2	30.0 <i>a</i>	12.5 <i>a</i>	47.5 <i>a</i>
Rate 3	11.3 <i>b</i>	7.3 <i>a</i>	40.8 <i>a</i>

\*Means are presented with LSD grouping ( $\alpha=0.05$ ) where treatments with the same letter are not significantly different from each other.

**Figure 53.** Mean percent of intact sclerotia recovered from mesh bags in the Brandon-West site in 2008.



**Figure 54.** Mean percent of intact sclerotia recovered from mesh bags in the Morden site in 2008.

