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Table of Contents

List of Tables

Chapter I

Chapter II

List of Figures

Chapter I

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GENERAL INTRODUCTION

Clubroot (*Plasmodiophora brassicae* Woronin) is a serious threat to the canola (*Brassica napus* L., *B. rapa* L.) industry in western Canada (Tewari et al. 2005; Strelkov et al. 2006). Clubroot of canola was first identified in Alberta canola fields in 2003 (Tewari et al. 2005) and has since spread to more than 400 fields in the province (Cao et al. 2009).

Several clubroot management strategies are used in high value horticultural crops, including drench application of fungicide (Shimotori et al., 1996; Donald et al., 2001) and soil amendment with lime (Mukarami et al., 2002a) to increase the soil pH which may not be practical in in canola production systems. Reduction in clubroot severity is also possible with the application of fungicides (Mitani et al. 2003) and soil amendments (Hwang et al. 2008). Therefore, a range of alternative management strategies are being assessed for their usefulness in canola production, including timing of seeding (Gossen et al. 2009), biological control (Peng et al. 2009), and use of bait crops (Kroll et al. 1984; Ikegami 1985; Mukarami et al., 2001).

As with most diseases, clubroot development is regulated by the dynamic interaction of the host, the pathogen, and the environment (Browning et al. 1977; Crute 1986; Williamson 1987; Wallenhammar 1996). To better understand this interaction in canola, the effect of seedling age and inoculum density on clubroot severity, plant height, and seed yield were investigated. While the influence of seedling age and inoculum density have been examined in Brassica vegetable crops (Horiuchi and Hori 1980; Timila et al. 2008), there is little or no data available regarding their impact on the growth and yield of canola and other oilseed crops. Such information is required to design effective management practices for clubroot of canola in western Canada.

Besides host plants, non-host plants are known to enhance resting spore germination of *P. brassicae* (Friberg, 2006). Some studies indicated root hair infection of some plants, e.g. *Holcus lanatus*, *Lolium perenne* and *Tropaeolum majus* (Webb, 1949; Macfarlane, 1952), and cortical infection and the subsequent development of resting spores in non-crucifers e,g. *T. majus* and *Beta vulgaris* (Ludwig-Müller et al., 1999). Moreover, laboratory experiment revealed root exudates of *L. perenne*, *Allium porrum*, *Secale cereale* and *Trifolium pretense* has been shown to induce resting spore germination (Friberg, 2006). In absence of host plant germinated spores are considered to survive only for short periods of time (Suzuki et al., 1992; Takahashi, 1994b). The possibility of inducing resting spore germination in absence of host plants is suggested to be effective to control clubroot (Friberg, 2006). Based on this principle crop that are sensitive to clubroot are allowed to grow as bait crop to stimulate resting spore germination, and then ploughed down before the pathogen completes it life cycle thus reducing the resting spore population in that field. We often recommend cultural practices like baiting crop and crop sequences have effect on the clubroot of crucifers. However, empirical data in this aspect is conflicting and inadequate (Friberg, 2006).

OBJECTIVES

The overall objective of this project was to understanding the relationship of inoculum density on clubroot severity and seed yield, and development of alternative strategy for the sustainable management of clubroot.

The specific objectives were to:

1) establish the effect and relationships of inoculum density and seedling age of clubroot severity and plant growth parameter and yield,

2) to determine the effect of baiting, and sequencing host and non-host crops on resting spore density and clubroot of canola,

3) to evaluate the effect of seeding dates on clubroot and seed yield of canola at field conditions.

EXECUTIVE SUMMARY

Under this project we studied the effect of inoculum density and seedling age on clubroot severity and seed yield; effect of cultural practices such as baiting crop, crop rotation and seeding date on clubroot resting spore and clubroot severity; and the effect of inoculum density on root hair colonization. Studies revealed that increasing inoculum density increased root hair infection, clubroot severity and decreased plant height seed yield. Younger seedlings are more prone to develop higher clubroot severity with higher yield loss which indicates that seed treatment fungicides with a long residual period (4 weeks or more) may be useful for the management of clubroot. Baiting crops were found effective to reduce clubroot severity under greenhouse conditions only. The rotation of canola-fallow-canola was consistently reduced clubroot severity.

CHAPTER I

Seedling Age and Inoculum Density Affect Clubroot Severity and Seed Yield in Canola

Abstract

Clubroot, caused by *Plasmodiophora brassicae*, is a serious threat to canola (*Brassica napus, B. rapa*) production in western Canada because of its long-lived resting spores, high spore production potential, and negative impact on seed yield when inoculum pressure is high. The effect of inoculum density was studied by diluting heavily infested field soil with pathogen-free soil-less potting mix at seven increments, ranging from completely pathogen-free to 100% infested soil, and also by incorporating resting spores into the soil-less mix at concentrations of 1×10^5 to 1×10^8 spores cm^{-3}, along with a non-inoculated control. Seed of the susceptible canola cultivar '34 SS 65' was planted in soil of each treatment, grown to maturity, and rated for plant height, seed yield, and clubroot severity (0–3 scale) at harvest. Clubroot severity increased and plant height and seed yield decreased with increasing inoculum density. To assess the effect of seedling age on reaction to clubroot, resting spores of *P. brassicae* were inoculated onto roots of 1-, 2-, 3- and 4-week-old seedlings of '34 SS 65'. In addition, seed (i.e., 0-week-old seedlings) was sown into infested soil. Inoculation of young seedlings resulted in higher clubroot severity, shorter plants and lower yield than inoculation of older seedlings. These results indicate that seed treatment fungicides with a long residual period (4 weeks or more) may be useful for the management of clubroot.

Introduction

Clubroot (*Plasmodiophora brassicae* Woronin) is a serious threat to the canola (*Brassica napus* L., *B. rapa* L.) industry in western Canada (Tewari et al. 2005; Strelkov et al. 2006) because of its long-lived resting spores, ability to rapidly build up high spore populations in the soil, and impact on canola yield where spore populations are high. Clubroot of canola was first identified in Alberta canola fields in 2003 (Tewari et al. 2005) and has since spread to more than 400 fields in the province (Cao et al. 2009). Acidic soils and high soil moisture are conducive to clubroot development (Karling 1968), but clubroot has also been reported in fields where soils are neutral to basic (Strelkov et al. 2007).

Clubroot is characterized by the development of galls on infected roots. Large galls disrupt water and nutrient uptake and transport in affected plants (Dixon 2006), which causes wilting, stunting, and premature ripening that result in yield and quality losses (Wallenhammar et al. 1999; Strelkov et al. 2006). Each large gall contains millions of resting spores that can survive in the soil for many years. Repeated cultivation of susceptible hosts favours the rapid build-up of spores in the soil. As a result, the rotation breaks of four or more years between susceptible crops that are required to reduce losses to acceptable levels are far longer than producers on the Canadian prairies deem acceptable (Strelkov et al. 2006). Even more importantly, this strategy might not be effective because clubroot development on *Brassica* spp. that grow as weeds or volunteers may be sufficient to maintain spore populations at high levels.

A canola cultivar that is resistant to clubroot is now commercially available in Canada (Anonymous 2009), and several others are in development. However, the number

of genes for resistance that are available to breeders at the present time is limited, and substantial genetic and pathotype variation is present in the *P. brassicae* populations of western Canada (Strelkov et al. 2006; Xue et al. 2008; Cao et al. 2009). Production of a cultivar with single-gene resistance against a genetically diverse pathogen on a large acreage imposes a strong selection pressure for pathogen genotypes that are able to overcome the resistance. Breakdowns in resistance to clubroot have occurred in winter canola and other crops (Kuginuki et al. 1999), so cultivar resistance should be only one component for the management of clubroot in canola on the Canadian prairies.

Several clubroot management strategies are used in high value horticultural crops, including drench application of fungicide (Shimotori et al., 1996; Donald et al., 2001) and soil amendment with lime (Mukarami et al., 2002a) to increase the soil pH. However, these management options are not practical or cost effective for use in field crops. For example, several tons of lime per hectare would be required to increase soil pH to 7.2 or higher, where clubroot severity is reduced (Campbell et al. 1985; Myers and Campbell 1985; Webster and Dixon 1991). These large tonnages are not only too expensive to be cost-effective for a field crop, but are also physically impractical to source, apply and incorporate over hundreds of fields each year. Reduction in clubroot severity is also possible with the application of fungicides (Mitani et al. 2003) and soil amendments (Hwang et al. 2008), but the rates used for horticultural crops are not cost-effective for canola. As a result, a range of alternative management strategies are being assessed for their usefulness in canola production, including timing of seeding (Gossen et al. 2009), biological control (Peng et al. 2009), and use of bait crops (Kroll et al. 1984; Ikegami 1985; Mukarami et al., 2001).

As with most diseases, clubroot development is regulated by the dynamic interaction of the host, the pathogen, and the environment (Browning et al. 1977; Crute 1986; Williamson 1987; Wallenhammar 1996). To better understand this interaction in canola, the effect of seedling age and inoculum density on clubroot severity, plant height, and seed yield were investigated. While the influence of seedling age and inoculum density have been examined in Brassica vegetable crops (Horiuchi and Hori 1980; Timila et al. 2008), there is little or no data available regarding their impact on the growth and yield of canola and other oilseed crops. Such information is required to design effective management practices for clubroot of canola in western Canada.

Methods

Seedling age, inoculum density and clubroot

The trials were conducted under greenhouse conditions with a 16-h photoperiod (20°C day/16°C night) at the Crop Diversification Centre North (CDCN), Edmonton, Alberta. Canola cv. '34 SS 65' was seeded every week for 4 weeks into 450-mL Jiffy® cups (2 seeds/cup) filled with soil-less potting mixture (Sunshine Mix 4; pH 6.5; SUN GRO® Horticulture Canada Ltd., Seba Beach, AB) to generate young plants of different ages. The plants were thinned to one plant per cup after seedling establishment. In the fifth week, clubroot-infested soil (pH 6.73) collected from a commercial field near Leduc, AB, was mixed with sterilized soil to produce the inoculum density treatments, based on 1:0, 1:1, 1:2, and 0:1 ratios (v:v) of infested to sterile soil. The soil mixtures were distributed into 26-cm-diam. pots and seedlings that were 1, 2, 3 and 4 weeks of age were transplanted into the pots (1 seedling/pot). Prior to transplanting, as much as possible of the soilless-mix associated with the plant roots was gently removed. Ungerminated seeds,

representing a fifth seeding date, were planted directly into the pots at a rate of one seed per pot. The pots were maintained in a tray filled with water adjusted to pH 6.5 at the bottom for the first 2 weeks after planting. They were then transferred to a greenhouse bench, where they were watered (pH 6.5) daily from above to maintain a high level of soil moisture. Seven replicate pots for each seedling age were placed on the greenhouse bench in a split-plot design, where inoculum density was the main plot treatment and the sub-plots were seedling age. Trays of the different inoculum concentrations were separated by at least 30 cm to avoid cross-contamination during watering. The plants were fertilized once per week until flowering with a 0.1% solution of 20:20:20 (N: P: K). Plant height was assessed after flowering, while clubroot severity was assessed at maturity on a 0-3 scale, where: 0 = no galling, 1 = a few small galls, 2 = moderate galling and 3 = severe galling (Kuginuki et al. 1999). Seed yield was also recorded at this time. The experiment was conducted twice.

Inoculum density (infested soil), clubroot and seed yield

In a second trial, soil from a field with high populations of resting spores was diluted with a soil-less mix as previously described to produce 0, 2, 4, 9, 17, 25, 33, and 50% of the resting spores present in the original infested soil. The soil mixtures were placed into ten pots (13-cm-diam.) per treatment, and five seeds of canola cv. '34 SS 65' were sown per pot. The pots were placed on trays with water on a greenhouse bench as described above, and arranged in a randomized complete block design with ten pots per treatment. Half of the replications were uprooted 6 weeks after sowing, and individual plants from each pot were scored for clubroot severity (0-3 scale) and plant height. The remaining

replications were grown to maturity, and seed yield was recorded. The disease severity data was used to calculate an index of disease (ID) using the formula of Horiuchi and Hori (1980) as modified by Strelkov et al. (2006):

$$\text{ID (\%)} = \frac{\Sigma\,(n \times 0 + n \times 1 + n \times 2 + n \times 3)}{N \times 3} \times 100 \qquad \text{(Eq.1)}$$

Where: n is the number of plants in each class, N is the total number of plants, and 0, 1, 2 and 3 are the symptom severity classes. The experiment was conducted twice.

Inoculum density (artificially inoculated), clubroot and seed yield

To relate the results from the soil dilution experiments to specific spore densities, a trial was conducted using inoculated soil-less potting mixture. The soil-less mix was inoculated with a resting spore suspension prepared from air-dried canola galls as described by Strelkov et al. (2006), and adjusted to a concentration of 1×10^9 spores cm^{-3}. Aliquots of the spore suspension were added to the potting mix to produce final resting spore densities of 0, 1×10^5, 1×10^6, 1×10^7, and 1×10^8 spores cm^{-3}. The study was conducted in a greenhouse, as described previously, and the treatments were arranged in a randomized complete block design with seven replications. Each treatment consisted of seven pots (replicates) of inoculated soil-less mix with five seeds of '34 SS 65' per pot. After 6 weeks, four pots of each treatment were assessed for clubroot severity (0-3 scale, converted to ID% as in Experiment 2) and plant height and the remaining pots were maintained until maturity to evaluate seed yield. The experiment was conducted twice.

Statistical analysis

Exploratory analyses were performed to determine the normality of each data set, and disease severity and plant height data were square root transformed to minimize heterogeneity of variance (Gomez and Gomez, 1984; McArdle and Anderson, 2004). The runs of each experiment were pooled after an initial analysis revealed no effect of run and run × treatment interaction. The pooled data were subjected to analysis of variance using the General Linear Model and mean separation was performed based on Fishers' protected least significant difference test using SAS software (SAS version 9.1.3). Differences were considered significant at $P \leq 0.05$ unless otherwise specified. Regression analysis was used to quantify the relationships among inoculum concentration, clubroot severity, plant height and seed yield (Exps.2 & 3).

Results

Seedling age, inoculum density and clubroot

In the study where sterilized soil was used to dilute infested field soil, both the main effects and interaction of seedling age × inoculum density had an impact on clubroot severity, plant height and seed yield. However, most of the variance was associated with the main effects; inoculum density accounted for 92%, 46%, and 65% of the variance in clubroot severity, plant height, and yield, respectively. Seedling age accounted for 7%, 44%, and 28% of variance in clubroot severity, plant height, and yield, respectively. In contrast, seedling age × inoculum density accounted for only 1%, 10%, and 7% of the variance in clubroot severity, plant height, and yield, respectively.

Clubroot severity decreased ($P \leq 0.01$) as seedling age (at first exposure to the pathogen) increased (Fig. 1A). Symptoms were most severe in plants grown from seed planted directly into infested soil and in plants transplanted to infested soil as 1-week-old seedlings. Symptoms were intermediate in plants transplanted as 2-week-old seedlings and lowest in those transplanted as 3- and 4-week-old seedlings. Similarly, seed yield increased ($P \leq 0.01$) as seedling age at first exposure to the pathogen increased (Fig. 1B).

Plant height was low for plants grown from seed sown directly into infested soil, higher in plants transplanted as 1-week-old seedlings, and intermediate for 2- to 4-week-old seedlings.

As expected, canola plants grown in sterilized soil did not develop clubroot galls, grew taller, and produced more seed than those that were exposed to the pathogen (Fig. 1D-F). Inoculum density across the range of dilutions assessed (infested: sterilized soil at 1:0 to 1:2) had little or no impact on clubroot symptom severity or plant height (Fig. 1D, F), but seed yield declined as inoculum density increased ($P \leq 0.01$, Fig. 1E). Plants seeded in infested soil undiluted with sterilized soil ripened prematurely and had lower yields than plants seeded in infested soil diluted with sterilized soil, irrespective of plant age.

Inoculum density (infested soil), clubroot and seed yield

In the second trial, the impact of inoculum density on clubroot severity, plant height, and seed yield was assessed using naturally infested soil diluted with soil-less mix as in the previous experiment. Plant height at 50% inoculum density (1 part infested soil: 1 part soil-less mix) was reduced by 56% relative to plant height at 2% inoculum density (1 part

infested soil: 49 parts soil-less mix). Regression analysis indicated a strong linear and negative relationship between inoculum density and plant height (y= 0.5888x + 58.356; R^2= 0.90) (Fig. 2A; Table 1). The maximum possible clubroot severity (ID of 100%) was obtained at 50% inoculum density; severity decreased with declining inoculum density (y= 34.067Ln(x) – 29.936; R^2= 0.95) and no clubroot symptoms were observed at 2% inoculum density (Fig. 2B; Table 1). Seed yield declined dramatically with increasing inoculum density from 2 to 10%, and dropped to zero at 50% density (y= -0.6866 Ln(x) + 2.584; R^2= 0.89) (Fig. 2C; Table 1). Plant height was 53% lower at an ID of 100% relative to an ID of 0%. Regression analysis revealed that plant height decreased with increasing clubroot severity, and the equation that best explained the model was y= -0.005x^2 + 0.2408x + 55.98; R^2= 0.82 (Fig. 3A). Likewise, seed yield decreased with increasing clubroot severity and reached zero at an ID of 100% (y= 0.0001x2- 0.0331x + 2.0361; R^2= 0.92; Fig. 3B).

Inoculum density (artificially inoculated), clubroot and seed yield

In the study where known concentrations of resting spores were added to soil-less mix, plant height was not substantially reduced at 1×10^5 spores cm^{-3}, but declined with further increases in spore density (Fig. 2D; Table 2). For example, there was a 47% reduction in height relative to the non-inoculated control at 1×10^8 resting spores cm^{-3}. Clubroot severity increased with increasing inoculum density (y= 0.3792 Ln(x) + 0.044; R^2= 0.99), while plant height (y= 0.6629- Ln(x^2) + 1.5038 Ln(x) + 64.028; R^2=98) and seed yield (P > 0.05; y=0.0381 Ln(x^2) – 0.6952 Ln(x) + 3.1436; R^2= 0.99) decreased with

increasing inoculum density (Fig. 2D-F; Table 2), although seed yield was not significantly different among the inoculated treatments ($P \leq 0.05$). A similar trend of relationships was observed between ID (%) and plant height ($y= -0.0047x^2 + 0.1692x + 63.971$; $R^2= 0.96$) and between ID and seed yield ($y= 0.21022x^2 – 1.6752x + 3.1435$; $R^2= 0.99$) (Fig. 3 C&D), as in the previous trial.

Discussion

Clubroot severity generally declined and seed yield consistently increased as seedling age at first exposure to *P. brassicae* increased across all of the assessments. A similar pattern has also been reported for Chinese cabbage (*B. rapa* L. subsp. *Chinensis* (Rupr.) var. *utilis* Tsen and Lee) and turnip (*B. rapa* L. var. rapa), where young seedlings were more susceptible to clubroot than older plants (Horiuchi and Hori 1980). When the initial infection is delayed, *P. brassicae* has a shorter period between infection and plant maturity to affect host development. This reduced period of exposure to the pathogen may be the principal factor responsible for reduced symptom expression. It is also possible, however, that the susceptibility of canola roots to infection by *P. brassicae* declines with increasing age, perhaps due to the thickening of cell walls (Mellano et al. 1970) and formation of other barriers that limit pathogen ingress. Direct histological examination of the infection process in canola is required to clarify this issue, but that research is beyond the scope of the current study.

The relationship between plant height and seedling age at the time of first exposure to the pathogen was complex. For example, the plants were shortest when the seeds were sown directly into clubroot-infested soil, and tallest when 1-week-old seedlings were

transplanted in to the soil; height was intermediate when 2- to 4-week-old seedlings were transplanted. This pattern may have resulted from an interaction between *P. brassicae* infection and transplant shock. Young plants, although more susceptible to clubroot, recover quickly when transplanted. Older seedlings, while more resistant to infection (Horiuchi and Hori, 1980), take longer to recover after transplanting and may have reached a physiological age at which reproductive growth is initiated rather than vegetative growth (Schrader, 2008); thus, they were shorter than those that were moved as young seedlings.

In the initial trial, seed yield declined as inoculum density increased, but plant height and final clubroot severity were not significantly affected, probably because the clubroot-infested soil used in the study had enough resting spores to produce clubroot galls even after the dilutions. The probability of multiple infections on each individual plant increased with increasing inoculum density, and more infections likely result in earlier and more rapid development of galls. The stage of plant maturity achieved before large galls develop is expected to have an impact on plant growth and reproduction, because large galls disrupt water and nutrient flow.

Early development of large root galls should have an impact on vegetative growth parameters as well as yield, since water and nutrient uptake would be impaired throughout most of the lifecycle of the host plant. In contrast, if large galls develop only later in the growing season, then vegetative growth may not be as severely affected, since root function will have been fairly normal during the earlier stages of the plant lifecycle. By the time the host plant begins reproductive growth, however, the impact of the enlarging galls will likely be greater, and hence could still negatively influence yield.

The extent of this impact on yield would be related to the specific timing of gall development. If large galls develop after most seedset has taken place, for instance, then the impact on yield may be minimal. Therefore, treatments could have similar clubroot severity ratings, based on root symptoms, but differ in yield and above-ground vegetative growth parameters such as height.

The models that best fit the data differed slightly between the trials (Fig. 2A-F), but the general effects of inoculum density on plant height, clubroot severity and seed yield were similar across all three trials. The current study revealed that clubroot severity on canola increases with increasing density of *P. brassicae* inoculum between 1×10^5 and 1×10^8 resting spores cm^{-3} and decreases with increasing age of the seedlings at the time of first exposure to the pathogen. This supports previous reports that clubroot disease severity on cruciferous vegetables is influenced by resting spore density, as well as soil type, soil pH, and plant species or cultivar (Murakami et al. 2001; 2002a; 2002b; 2003). The current results also indicate that the disease response curves vary with the type of inoculum used.

The observation that seed yield increased with seedling age indicates that treatments that delay infection for several weeks after seeding are likely to reduce clubroot severity and have a positive impact on seed yield in susceptible canola genotypes grown in infested soil. A treatment that only delays infection by one or two weeks will have a much smaller impact on yield. These results indicate that seed treatment fungicides that have a residual period of four weeks or more have the greatest potential for the management of clubroot. Moreover, early seeding was found to be effective in reducing

clubroot severity in canola and Brassica vegetables (Gossen et al., 2009). The adjustment of seeding date therefore represents another option for controlling clubroot of canola.

The use of resting spores from clubroot galls as inoculum was a superior alternative to infested soil for studies of clubroot of canola, because the concentration of resting spores in the growing medium could be determined precisely, including the lowest inoculum density needed to induce clubroot development. Infested soil from a field represents a bulk sample taken from several locations in that field. A second sample from the same field will likely also have a different spore concentration, making comparisons challenging. Also, it is very difficult to obtain accurate estimates of the concentration of resting spores in infested soil. The small size and lack of ornamentation of these spores makes them difficult to extract from soil and identify with a high degree of certainty. Quantitative PCR techniques for measuring spore concentrations in soil are under development (S.E. Strelkov, unpublished data), but they are not yet available. Nonetheless, in the inoculum density trial with infested soil, clubroot severity at a 1:5 dilution was roughly equivalent to that obtained with a concentration of 1×10^5 spores cm^{-3} of soil mix.

We conclude that accurate assessments of inoculum density, either directly via techniques such as qPCR or indirectly via dose response curves, are essential for the development of an integrated management strategy for clubroot (Murakami et al., 2002a). Further research on the inoculum dose response of canola will therefore be important in developing an effective clubroot management plan for western Canada.

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Table. 1. Effect of inoculum density of *Plasmodiophora brassicae* (based on dilution of infested soil with soil-less mix) on clubroot severity (index of disease), plant height and seed yield of canola in a greenhouse study.

Inoculum density[a]	Index of Disease [b] (%)	Plant height[b] (cm)	Seed yield g/plant[b]
0:1	0.0 f	53.4 ab	2.14 a
1:50	0.0 f	57.2 a	2.36 a
1:25	31.2 e	56.2 ab	1.70 b
1:10	52.1 d	48.2 bc	0.53 c
1:5	74.1 c	49.3 abc	0.49 c
1:3	81.2 bc	47.9 bc	0.40 cd
1:2	85.9 b	42.9 c	0.44 c
1:1	100.0 a	24.9 d	0.00 d

[a] Ratio of clubroot-infested field soil to commercial Sunshine Mix L4 soil-less mix (pH 6.5).

[b] Data are the mean of four replications; means with in a column followed by the same letters do not differ according to Fisher's protected least significant different test at $P \leq 0.05$.

Table. 2. Effect of the density of *Plasmodiophora brassicae* resting spores in the growth medium on clubroot severity (index of disease), plant height and seed yield of canola under greenhouse conditions.

Spore conc. (cm^{-3})	Index of Disease[a] (%)	Plant height[a] (cm)	Seed yield[a] (g/plant)
0 (control)	0.0 e	63.9 a	0.65 a
1×10^5	42.9 d	56.8 b	0.13 b
1×10^6	84.7 c	47.3 c	0.07 b
1×10^7	97.6 b	41.4 cd	0.02 b
1×10^8	100.0 a	34.5 e	0.01 b

[a] Data are the means of six replications; means with in a column followed by the same letter do not differ according to Fisher's least significant different test test at $P \leq 0.05$.

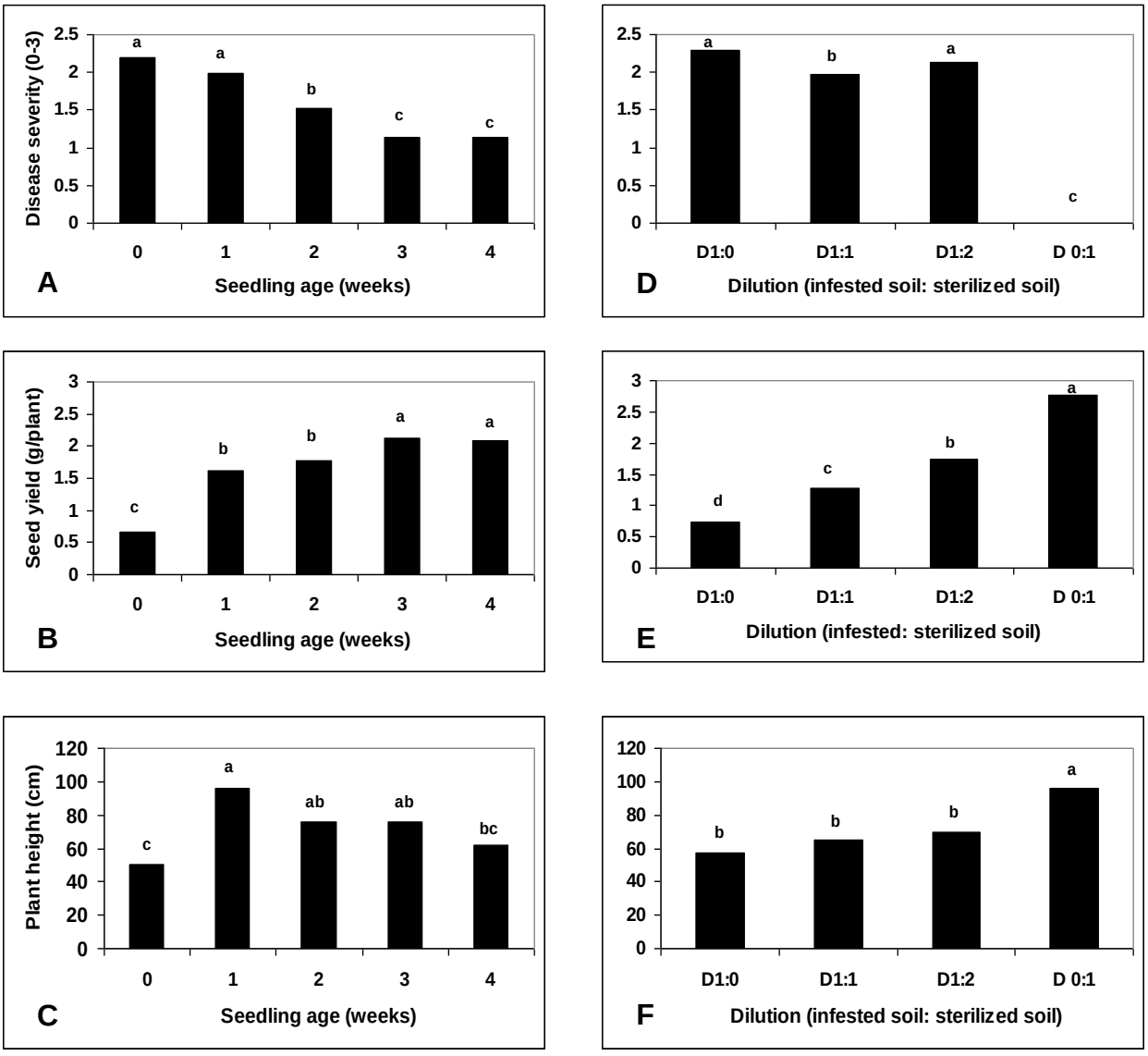

Fig. 1. Effect of seedling age (in weeks) at first exposure to infection and pathogen inoculum density (infested soil: sterilized soil (v/v)) on clubroot severity, plant height and yield of canola under greenhouse conditions. No clubroot developed in the sterilized soil control, so these data were dropped from the analysis. Bars with the same letter within a graph do not differ according to Fisher's protected least significant difference (LSD) test at $P \leq 0.05$.

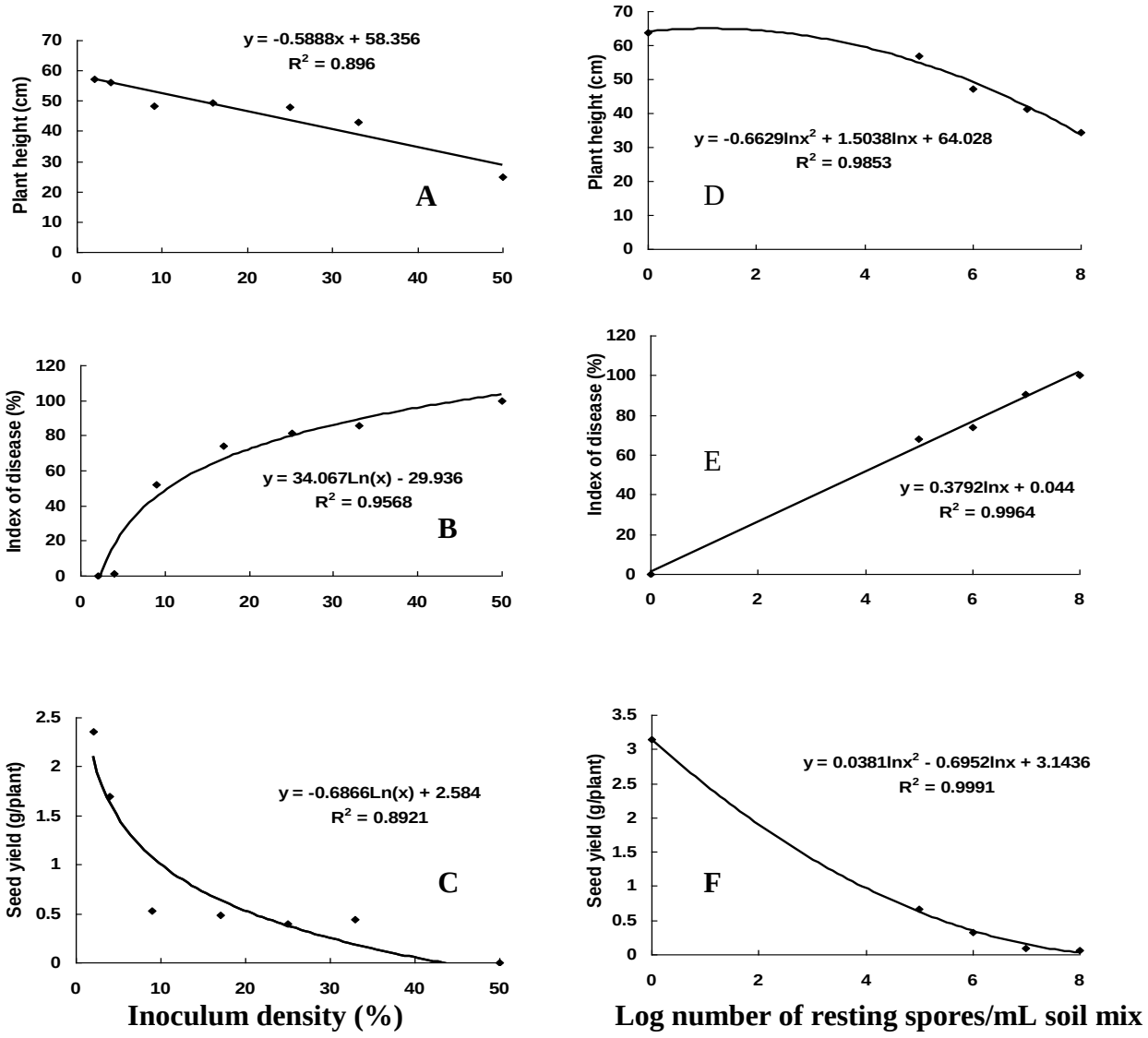

Fig 2. Effect of inoculum density on plant height, clubroot severity (index of disease), and seed yield of canola (A, B, C: dilution of naturally infested soil; D, E, F: soil inoculated with known concentrations of resting spores extracted from root galls).

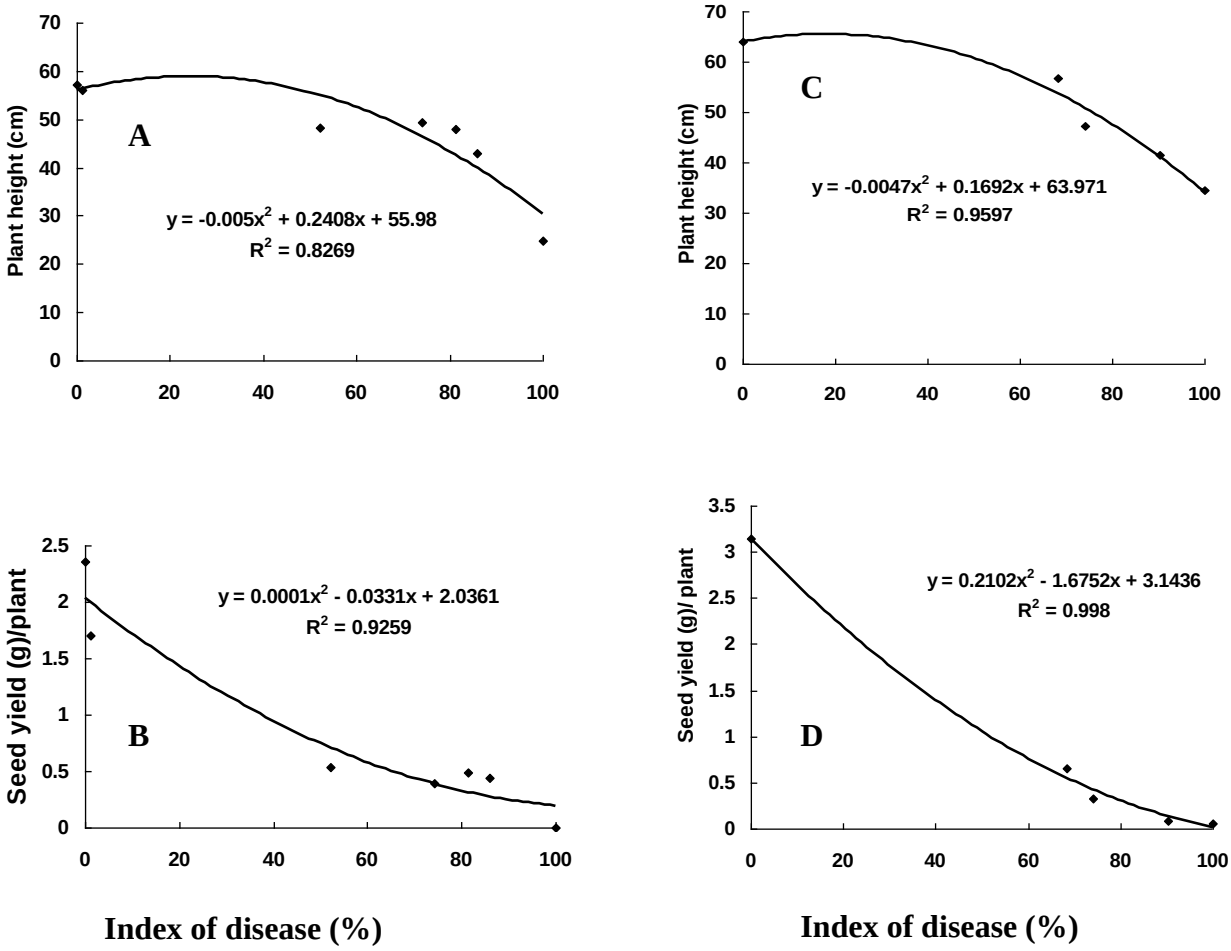

Fig 3. Relationship of plant height and seed yield of canola to clubroot severity (A, B: dilution of naturally infested soil; C, D: soil inoculated with known concentrations of resting spores extracted from root galls).

CHAPTER II

Manipulation of cultural practices for the management of clubroot (*Plasmodiophora brassicae*) of canola

Abstract

Clubroot, caused by *Plasmodiophora brassicae,* is a serious threat to canola (*Brassica napus, B. rapa*) production in western Canada because of its long-lived resting spores, high spore production potential, and negative impact on seed yield when inoculum pressure is high. The effect of baiting crop on resting spore and clubroot severity was studied under greenhouse and field conditions. Clubroot severity was reduced after baiting with cruciferous (canola and Chinese cabbage) followed by non-cruciferous (red clover, perennial rye grass and orchard grass) host and non-host (barley and wheat) crops compared to the control. This result suggests that the effect of the crops used for baiting was non-specific, although the magnitude of the effect of baiting was different on clubroot severity. No significant effect of baiting on clubroot severity was observed under field condition. The rotation of canola-fallow-canola was consistently reduced clubroot severity. The late seeding of canola reduced clubroot severity and the seed yield. Baiting or rotating crop may be effective for the management of management of clubroot of canola.

Introduction

While Brassica crops including broccoli, cabbage, cauliflower, kale, turnip, radish, canola, rapeseed, and various mustards have the potential as biofumigant to control multiple soilborne fungal pathogen and diseases (Larkin and Griffin, 2007) *Plasmodiophora brassicae* Woronin, the causal organism of clubroot, has become a

severe constrain to canola (*Brassica napus* L., *B. rapa* L.) industry worldwide, including Canadian prairie (Tewari, et al., 2005; Strelkov et al., 2006). Plasmodiophora brassicae forms gall on infected roots. Severe clubroot disrupt water and nutrient uptake and transport in affected plants (Dixon 2006), which cause wilting, stunting, and premature ripening that result in yield and quality losses (Wallenhammar et al. 1999; Strelkov et al. 2006). Each large gall contains millions of resting spores that can persist in the soil as resting spore for more than 15 years (Wallenhammar, 1996). Repeated cultivation of susceptible hosts favours the rapid build-up of spores in the soil. Therefore, the rotation breaks between susceptible crops that are required to reduce losses to acceptable levels are far longer than producers on the Canadian prairies deem acceptable (Strelkov et al. 2006).

A canola cultivar that is resistant to clubroot is now commercially available in Canada, and several others are in development. However, the number of genes for resistance that are available to breeders at the present time is limited (Hirai 2006), and substantial genetic and pathotype variation is present in the *P. brassicae* populations of western Canada (Strelkov et al. 2006; Xue et al. 2008; Cao et al. 2009). Production of a cultivar with single-gene resistance against a genetically diverse pathogen on a large acreage imposes a strong selection pressure for pathogen genotypes that are able to overcome the resistance. Breakdown in resistance to clubroot has occurred in winter canola and other crops (Kuginuki et al. 1999), so the cultivar resistance should be only one component of the solution for management of clubroot in canola on the Canadian prairies.

Several clubroot management strategies are used in high value horticultural crops, including drench application of fungicide (Shimotori et al., 1996; Donald et al., 2001) and amendment with lime (Mukarami et al., 2002) to increase the soil pH. However, these management options are not practical or cost effective for use in field crops. For example, several tons of lime per hectare would be required to increase soil pH to a level where clubroot severity is reduced (Campbell et al. 1985; Myers and Campbell 1985; Webster and Dixon 1991). These large tonnages are not only too expensive to be cost-effective for a field crop, but are also physically impractical to source, apply and incorporate over hundreds of fields each year. Reduction in clubroot severity is also possible with application of fungicides (Mitani et al. 2003) and soil amendment products (Hwang et al. 2008), but the rates used for horticultural crops are not cost-effective for canola. As a result, a range of alternative management strategies are being assessed for their usefulness for canola production, including timing of seeding (Gossen et al. 2009), biological control (Peng et al. 2009), and use of bait crops (Kroll et al. 1984; Ikegami 1985; Mukarami et al., 2001).

Besides host plants, non-host plants are known to enhance resting spore germination of *P. brassicae* (Friberg, 2006). Some studies indicated root hair infection of some plants, e.g. *Holcus lanatus*, *Lolium perenne* and *Tropaeolum majus* (Webb, 1949; Macfarlane, 1952), and cortical infection and the subsequent development of resting spores in non-crucifers e,g. *T. majus* and *Beta vulgaris* (Ludwig-Müller et al., 1999). Moreover, laboratory experiment revealed root exudates of *L. perenne*, *Allium porrum*, *Secale cereale* and *Trifolium pretense* has been shown to induce resting spore germination (Friberg, 2006).

In absence of host plant germinated spores are considered to survive only for short periods of time (Suzuki et al., 1992; Takahashi, 1994b). The possibility of inducing resting spore germination in absence of host plants is suggested to be effective to control clubroot (Friberg, 2006). Based on this principle crop that are sensitive to clubroot are allowed to grow as bait crop to stimulate resting spore germination, and then ploughed down before the pathogen completes it life cycle thus reducing the resting spore population in that field.

We often recommend cultural practices like baiting crop and crop sequences have effect on the clubroot of crucifers. However, empirical data in this aspect is conflicting and inadequate (Friberg, 2006). Therefore, the objectives of this study were to: i) determine the effect of baiting, and sequencing host and non-host crops on resting spore density and clubroot of canola, and ii) evaluate the effect of seeding dates on clubroot and seed yield of canola at field conditions.

Materials and methods

Effect of baiting crop on clubroot

Greenhouse experiment

Eight crops including cruciferous hosts such as Polish type canola (*Brassica napa* L.) and Chinese cabbage (*Brassica rapa* L.), non-cruciferous hosts bent grass (*Agrotis stolonifera* L.), orchard grass (*Dactylis glomerata L*, red clover (*Trifolium pratense* L), and perennial rye grass (*Lolium perenne* L.) and non-host crops barley (*Hordeum vulgare* L) and wheat (*Triticum aestivum* L.) (Table 1), were used. Four replicate plastic containers (size 35 x 23 x13.5 cm) for each treatment were filled with 6 litre of infested soil (collected from

Leduc, Alberta) which was diluted to 50% (v/v) with soil-less mix (Sunshine Mix 4; pH 6.5; SUN GRO® Horticulture Canada Ltd., Seba Beach, AB). As per treatment four rows of each crop was seeded (25 seeds/row) in each replicate container. The control treatment was left fallow. The trays were placed on the greenhouse bench following a randomized complete block (RCB). The study was conducted under greenhouse conditions (20 ± 2° C day/16 ± 2° C night) with a 16-h photoperiod. The plants were uprooted after two weeks, and each treatment unit was re-seeded with the same crop and uprooted again after two weeks. After two 2-week-cycles of seeding, all the treatments including the control were seeded with Argentine canola cv. 34 SS 65 (*Brassica napus L.*). After each seeding, the containers including control treatment were placed in water-filled trays to ensure high soil moisture for 12 days. Afterwards the plant was water with overhead sprinkler.

Six weeks after last seeding, the plants were harvested, and data on plant height, disease incidence and disease severity were recorded. The clubroot severity was assessed using a 0–3 scale; where 0 = no gall, 1 = a few small galls, 2 = moderate galling and 3 = severe galling (Kuginuki et al., 1999). The disease severity data were converted to index of disease (ID%) following Horiuchi and Hori (1980) and Strelkov et al. (2006).

Exploratory data analysis was performed to test homogeneity in the data set using normal probability plot. Analysis of variance (ANOVA) of the data was performed using SAS statistical software (SAS Institute Inc.). Pre-planned one degree of freedom (df) linear contrast were made to compare the effect of cruciferous host vs. other crops; cruciferous vs. non-cruciferous host ; cruciferous host vs. cereal; non-cruciferous host vs. cereal; cruciferous host vs. control; non-cruciferous host vs. control; and all crops vs. control on

the plant height, clubroot disease incidence and index of disease (%ID). The experiment was conducted twice.

Field experiment

The experiments were conducted in the clubroot infested fields at Leduc and St. Albert, Alberta during 2008. Polish canola cv. Reward was used as a baiting crop. The treatments were i) canola was seeded for the entire crop season, ii) grown canola for three weeks and killed the plants with roundup herbicide for four cycles in the season, iii) canola was grown for four weeks and killed the plants for three cycles, and iv) canola was grown for six weeks and killed the plants for 2 cycles. The experiments were laid out in a RCB design with four replications. At the end of each treatment operation the plants were assessed for disease severity using the (0-3) as described earlier.

Soil resting spore counting

To determine the impact of the crop cycling on P. brassicae resting spore population, soil samples were collected twice from each plot of the experiments at both sites described above. The soil samples were collected from Leduc on June 18, 2008 and April 29, 2009 and from St. Albert on June 19, 2008 and May 16, 2009. Each soil sample was processed by plot for spore counting. Five cores of soil samples were collected from each replicate plot and were mixed thoroughly. The soil samples were air dried and 10 g of soil samples were mixed in to 50 mL water, and thoroughly mixed with a stirrer bar, and sieved through eight layers of cheese cloth. Three 1-mL aliquot of soil suspensions were centrifuged at 500 rcf for 10 min., the supernatant was collected and the spores were counted using a haemocytometer (3 slides for each of the three 1-mL samples) after

staining with aceto-orecin red under a microscope (10 x40). The data were subjected to analysis of variance following General Linear Model procedure using SAS (SAS Institute Inc.)

Bioassay of soil samples

Four replicate cups of the same soil samples from each field plot were planted with Polish canola cv. Reward under greenhouse conditions. The plants were assessed for club root severity after three weeks of seeding using the 0-3 scale described earlier. The severity data was used to calculate index of disease (ID %), and the data were analysed following the procedure described above.

Effect of sequencing baiting crop on clubroot

Two trials were conducted under greenhouse conditions to determine the effect of baiting crop sequence on clubroot disease development. The experiment was set up with clubroot infested soil as described in the previous experiment. Five sequences of Polish canola cv. Reward and perennial ryegrass as: ryegrass-fallow-canola; canola-fallow-canola; ryegrass-canola-canola; canola-ryegrass-canola; and fallow-fallow-canola (as control) were used in the study. The first crop was grown for three weeks or remained fallow. Three weeks after first seeding the plants were uprooted and the trays were reseeded according to the predetermined crop sequence. Three weeks later, the crops were uprooted again and planted to canola cv. "34 SS 65" on same day. The treatments were laid out in a RCB design with four replications. High soil moisture was maintained after each seeding following the procedure described in the previous experiment. Six weeks after the last seeding, data on canola plant height, plant shoot dry weight and clubroot

disease severity were recorded. The disease severity data were used to calculate percent index of disease (%ID). The data were subjected to analysis of variance, and mean separation was done following Fishers' LSD test at $P \leq 0.05$ significant levels.

Effect of seeding date on clubroot

Field trials to assess the impact of seeding date on disease severity was conducted at two sites in naturally-infested soil. Small plots (6m x 1.5 m) of canola were seeded on May 11, May 19 and May 28 at St. Albert and May 16, May 29 and June 4, 2008 at Leduc. Seeding dates as treatment were replicated four times in a RCB design. Data on seedling emergence was noted after three weeks of seeding and clubroot disease severity (0-3 scale) and yield were recorded at maturity of the crop for both sites. The data were subjected to ANOVA and mean separation was done following LSD test.

Results

Effect of baiting crop under greenhouse conditions

The effect of baiting different cruciferous (canola and Chinese cabbage) and non-cruciferous host (bent grass, orchard grass, red clover and rye grass), and non-host (barley and wheat) on plant height, disease incidence and index of disease is presented in Table 2. Result revealed that plant height was significantly higher with cruciferous host compared to the non-crucifers host or non-host crops. In contrast the plant height was significantly higher ($P \leq 0.05$) with the control treatment than the treatments where cruciferous, non-cruciferous or non-host crops were used as a bait crop (Table 2). No significant difference in plant height was observed when non-cruciferous and non-host

cereal crop was compared. Disease incidence after baiting with cruciferous host was significantly lower with baiting non-cruciferous or non-host crops. No significant difference was evident when non-cruciferous and non-host cereal crops were compared. When compared to the control disease incidence was significantly ($P \leq 0.05$) lower with any baiting crops. Similar results were obtained with the %ID (Table 2).

Effect of baiting crops under field conditions

The soil samples from 2008 and 2009 were assessed for the presence of viable spores. The viable spore count in the Leduc soil collected in 2008 was significantly reduced when canola was grown for six weeks and reseeded for two cycles (Table 3). The effect of other treatments was not evident. No significant effect of baiting crop cycling was found in the soil from St. Albert collected in June 2008, and Leduc collected in 2009 (Table 3).However, the resting spore in the 2009 St. Albert soil was significantly reduced when canola was grown for 2, 3 or 4 cycles compared to the treatment where canola was grown for the entire season. No significant differences were observed among the treatments in the disease index from the original soil, the soils in which the baiting crops had been grown, or in the changes in disease index from one year to the next (Table 4). This data suggests no change in the population of resting spores due to any of the baiting crop sequence cycling.

Effect of baiting crop sequence on clubroot

Two trials of the experiments were conducted to determine the effect of baiting crop sequences on clubroot with infested soil under greenhouse conditions. In trial I, the results presented in Table 5 indicated, as expected, that clubroot disease severity and %ID were significantly higher ($P \leq 0.05$) when the soil was left for two three-week consecutive fallow periods compared to the canola-fallow-canola and ryegrass-canola-canola. The other two crop sequences ryegrass-fallow-canola and canola-ryegrass-canola had lower disease severity and %ID but the difference was not statistically significant. In Trial II, canola-fallow-canola had the lowest clubroot but was not significantly different from the control. The shoot dry weight was lower in the baiting crop rotation treatments compared to the control, where ryegrass-canola-canola and canola-ryegrass-canola had the lower shoot mass compared to ryegrass-fallow-canola and canola-fallow-canola in trial 1. Where as in trial 2 the least shoot mass was obtained with canola-ryegrass-canola followed by ryegrass-canola-canola, and canola-fallow-canola. In trial1, the plant height was significantly lower with canola-ryegrass-canola followed by ryegrass-canola-canola, canola-fallow-canola and ryegrass-fallow-canola although the plant height following ryegrass-fallow-canola was not significantly different from the control where the treatment unit left fallow for two times before the final crop canola. In the trial 2, the plant height was not almost similar including the control with the lowest with canola-ryegrass-canola. However, the results were not consistent over the trials.

Effect of seeding date on seedling emergence, clubroot and yield

Analysis of variance indicated a significant effect of seeding date on seedling emergence, clubroot severity and yield of canola in the St Albert trial. Seeding date had no effect on

clubroot disease severity in the Leduc trial. Seedling emergence was significantly higher in the late seeding (June 04) at Leduc and in the mid to late seeding (May 19 and May 28) at St. Albert (Table 6). Clubroot severity was significantly higher in the late seeding compare to the early seeding at St. Albert. The canola yield at both sites was significantly higher in the early-seeded plots.

Discussion

Plasmodiophora brassicae resting spore concentration in the field is a major epidemiological factor (Mukarami et al., (2002) for the development of clubroot of canola. Therefore, reducing inoculum density will reduce the impact on clubroot severity. We studied the impact of baiting cruciferous and non-cruciferous plant under greenhouse conditions. The clubroot disease incidence and severity was reduced when cruciferous and non-cruciferous host crops were baited suggesting that during two cycles of baiting root exudates from the plants might have stimulated the germination of the resting spore thereby reduced the resting spore load. The plant height was higher in the control treatment compared to the treatment where baiting crops were grown, and this was expected as soil nutrition was depleted with growing two cycles of baiting. We observed higher root mass leftover in the non-cruciferous host and cereal crops than the cruciferous host during uprooting the plants which also might have negative impact the plant height of canola. However, this result indicated that baiting cruciferous may be a potential way of reducing spore load in the soil. Although, use of a baiting host crop under field conditions had no significant effect on the resting spore populations and clubroot disease severity at either field site. This is due to the fact that these experiments

were conducted in a heavily clubroot infested soil. There might have been reduction in the spore load due to baiting but the reduction was that much that can impact in the reduction of clubroot severity. Therefore, Donald and Porter (2009) postulated that at high soil inoculum concentrations, extremely long rotations are needed to reduce soil inoculum to levels below a disease-causing threshold. For example, 17.3 years were required to reduce the level of infestation in soil from 100% infection to below that which could be detected using a plant bioassay (Wallenhammar, 1996). Moreover, another study revealed that a 5-year period of continuous fallow, or cultivation with a clubroot-resistant Japanese radish caused the greatest decrease in resting-spore viability (Donald and Porter, 2009). Considering these our study period was not too long enough to give conclusive evidence of the effect of baiting crop and crop rotations on clubroot.

Our study indicated that baiting crops canola and rye with three weeks of fallow reduced disease in canola-fallow-canola and rye-canola-canola compared to fallow-fallow-canola in one trial. However, the result was not conclusive as the canola-fallow-canola although had the lowest clubroot but was not significantly different from the control. Development of clubroot is highly dependent of the environmental conditions (Wallenhammar,1996) which likely contributed to the difference between the trials.

Early seeding date reduced clubroot severity on canola by 10-50% (although this difference was only significant at one of two sites) and increased yield by 30-58%. We observed a significant impact of seeding date on seedling emergence, clubroot severity and yield. Clubroot severity was higher where canola was seeded later, and yield was higher where the crop was seeded earlier. Similar results were obtained by manipulating seeding date to minimize clubroot (*Plasmodiophora brassicae*) damage in canola and

vegetable brassicas (Gossen, 2009). Therefore, growing baiting crop or manipulating seeding date may be a useful tool when used along with other crop management options including host plant resistance, fungicide and bio-control agent to control clubroot of canola.

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Table 1. List of baiting crop with used in the study.

Common name	Scientific name	Family	Clubroot reation
Cruciferous host			
Canola	*Brassica napus* L.	Brassicaceae	Severe clubroot
Chinese cabbage	*Brassica rapa* L.	Brassicaceae	Severe clubroot
Non-cruciferous host			
Bentgrass	*Agrotis stolonifera* L.	Poaceae	Root hair infection only
Perenial ryegrass	*Lolium perenne* L.	Poaceae	Root hair infection only
Orchardgrass	*Dactylis glomerata* L.	Poaceae	Root hair infection only
Red Clover	*Trifolium pratense* L.	Fabaceae	Root hair infection only
Non-host			
Barley	*Hordeum vulgare* L.	Poaceae	Not known
Wheat	*Triticum aestivum* L.	Poaceae	Not known

Table. 2. Baiting of cruciferous and non-cruciferous host on the plant height and clubroot severity under greenhouse conditions, 2010.

Contrasts	Plant height (cm)		Disease incidence		Index of Disease (%)	
Cruciferous host vs. other crops	**18.0**	**15.8**	**86.2**	**96.0**	**64.9**	**72.7**
Cruciferous vs. non-cruciferous host	**18.0**	**15.9**	**86.2**	**96.2**	**64.9**	**72.8**
Cruciferous host vs. cereal	**18.0**	**15.6**	**86.2**	**95.6**	**64.9**	**72.7**
Non-cruciferous host vs. cereal	15.9	15.6	96.2	95.6	72.8	72.7
Cruciferous host vs. control	**18.0**	**21.7**	**86.2**	**100.0**	**64.9**	**75.3**
Non-cruciferous host vs. control	**15.9**	**21.7**	**96.2**	**100.0**	**72.8**	**75.3**
All crops vs. control	**16.4**	**21.7**	**93.5**	**100.0**	**70.8**	**75.3**

Cruciferous hosts= Canola and Chinese cabbage; Non-cruciferous host= Bent grass, orchard grass and perennial rye grass; Cereal= Barley and wheat; and other crops= All non-cruciferous hosts and cereals.

Table 3. Effect of repeated cultivation of a baiting crop (*Brassica rapa* cv. Reward) on population of germinating resting spores in field plots.

	Resting spores/g soil			
	St. Albert		Leduc	
Treatment	June 2008	April 2009	June 2008	April 2009
Crop for entire season	7.80E+05	5.4E+06 a	2.50E+06 b	2.80E+06
Crop seeded for 4 cycles	5.10E+05	4.1E+06 b	3.00E+06 ab	2.50E+06
Crop seeded for 3 cycles	4.30E+05	3.7E+06 b	1.50E+06 bc	2.00E+06
Crop seeded for 2 cycles	3.70E+05	3.9E+06 b	1.30E+06 c	2.30E+06

Data are the mean of 4 replications x 3 slides. The means followed by the same letter are not significantly different at $P \leq 0.05$ level.

Table 4. Disease index of canola seedlings grown in soil collected from plots subjected to repeated cultivation of a baiting crop (*Brassica rapa* cv. Reward).

Treatment	Disease Index (ID%)			
	St. Albert		Leduc	
	Jun-08	May-09	Jun-08	Apr-09
Crop for entire season	42.5	60.4	49.1	60.9
Kill @ 3 wk, reseed for 4 cycles	33.9	60.5	49.0	54.8
Kill @ 4 wk, reseed for 3 cycles	34.5	64.8	53.4	61.0
Kill @ 6 wk, reseed for 2 cycles	45.2	70.0	59.6	59.4

Data are the mean of 4 replications. The means are not significantly different at $P \leq 0.05$ level.

Table. 5. Effect of baiting crop and crop sequence on clubroot, plant height and canola growth parameters

Trial I Treatment	% disease Index	Shoot dry weight (g)	Plant height (cm)
Ryegrass-Fallow-Canola	29.16 a	15.20 a	45.92 ab
Canola-Fallow-Canola	10.00 b	15.54a	38.84 bc
Ryegrass-Canola-Canola	8.76 b	6.85 b	32.54 c
Canola-Ryegrass-Canola	29.16 a	6.63 b	30.61 c
Fallow-Fallow-Canola	37.52 a	20.76 a	52.82 a
Trial II			
Ryegrass-Fallow-Canola	16.12 bc	12.94 a	40.07 a
Canola-Fallow-Canola	9.40 c	6.56 ab	35.99 ab
Ryegrass-Canola-Canola	38.55 a	4.34 b	30.46 b
Canola-Ryegrass-Canola	32.23 ab	3.40 b	23.58 b
Fallow-Fallow-Canola	20.43 abc	10.82 ab	37.10 ab

Data are the mean of 4 replications. Data followed by the same letters are not significantly different by Fisher's Protected Least Significant Difference test ($P \leq 0.05$).

Table 6. Effect of seeding date on emergence, clubroot severity and yield of canola seeded into clubroot-infested soils near Leduc and St. Albert, AB in 2008.

Treatment		Emergence		Clubroot severity		Yield (t/ha)	
		Leduc	St. Albert	Leduc	St. Albert	Leduc	St. Albert
Early	May 11	-	58.6 b	-	0.65 b	-	1.18 a
Mid	May 19	-	167.8 a	-	0.88 ab	-	0.78 b
Late	May 28	-	145.4 a	-	1.30 a	-	0.91 b
Early	May 16	50.0 b	-	2.05 a	-	0.81 a	-
Mid	May 29	78.1 b	-	2.45 a	-	0.42 b	-
Late	June 04	165.3 a	-	2.25 a	-	0.14 b	-

Data are the means of four replications. Data followed by the same letters are not significantly different at $P \leq 0.05$ level by Fishers' protected Least Significant Difference test.

GENERAL CONCLUSION

Clubroot severity increased with the increase inoculum density and plant height and seed yield decreased. The younger seedlings are more vulnerable to the disease, suggesting that effective seed treatment chemicals that have a long residual period of four or more weeks may be useful for the management of clubroot. The period of baiting preceding the crop needed to be reduced to less than three weeks. than the combination of management options like use of resistant cultivars, seed treatments and other cultural practices to minimize disease is required for sustainable management of clubroot of canola.

Technology transfer

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