Developing nominal threshold levels for *Phyllotreta cruciferae* (Coleoptera: Chrysomelidae) damage on canola in Montana, USA

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**A B S T R A C T**

The flea beetles *Phyllotreta cruciferae* (Goeze) and *Phyllotreta striolata* (F.) (Coleoptera: Chrysomelidae) are serious pests infesting canola (*Brassica napus* L.; Brassicales: Brassicaceae) in the Northern Great Plains of the United States. In Montana, *P. cruciferae* is the only flea beetle species that attacks canola during the crop growing stage. Management of *P. cruciferae* is usually focused on treating adults feeding on canola seedlings, which is the stage most vulnerable to flea beetle damage. In the Golden Triangle area in Montana, canola growers traditionally use seed treatments or calendar based spraying to control *P. cruciferae*. Here, we compared calendar-based spraying with seed treatment and threshold-based treatment. The experiment treatments included threshold levels (15–20, 25, 45% of leaf area damaged), calendar based sprays (15, 30 and 45 day intervals after plant emergence), seed treatments (imidacloprid), and untreated controls. The trials were done at two locations (Conrad and Western Triangle Agricultural Research Center). We found that calendar-based spraying at a 15-day interval did not differ significantly in yields from threshold-based treatment at 15–20% leaf damage. Also, the seed treatment did not give significantly higher yields compared to calendar-based sprays. A negative correlation was detected between leaf damage and yield in each treatment. Overall, calendar-based and threshold-based treatments were most effective in improving yields. However, treatment made at the threshold of 15–20% leaf area damage is recommended in order to reduce the number of chemical applications and also to reduce the possibility of selecting for resistance in flea beetles.

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**1. Introduction**

Canola (*Brassica napus* L.; Brassicales: Brassicaceae) is an important oilseed crop in North America, where it is grown mostly in western Canada and the northern central United States, especially the northern Great Plains, including Montana (Knodel and Olson, 2002). Flea beetles (Coleoptera: Chrysomelidae) are major insect pests infesting canola in North America. Each year, yield losses due to flea beetle damage have been estimated to be tens of millions of U.S. dollars (Burgess, 1977; Lamb and Turnock, 1982; Madder and Stermeroff, 1988). In the Golden Triangle area in Montana (an area known for its ideal climatic conditions for growing wheat of exceptionally high quality; the three points of the Golden Triangle in north-central Montana are Havre, Conrad, and Great Falls), the crucifer flea beetle *Phyllotreta cruciferae* (Goeze) is the most important flea beetle species attacking canola crops (Reddy et al., 2014). The insects survive throughout winter as adults, primarily in the leaf litter and turf of shelterbelts, and emerge in the spring to injure canola seedlings (Burgess, 1977, 1981). Adult *P. cruciferae* feed on cotyledons and developing leaves and stems of seedlings, leading to loss of photosynthetic capability and finally plant death (Westdal and Romanow, 1972). Feeding starts at the first 2 weeks after beetle emergence, and produces a shot-hole appearance and necrosis (Knodel and Olson, 2002).

Insecticide application is the main approach for *P. cruciferae* management in canola (Lamb, 1988), and more than 90% of the 5 million ha of canola in North America are treated with insecticides (Waite et al., 2001). Typically, insecticide applications are made targeting adults in early spring when the canola crop is at the seedling stage, which is the most vulnerable to *P. cruciferae* injury (Thomas, 2003). While foliar sprays of chemical insecticides are effective in...
controlling flea beetles, there is only a narrow time window for application. Furthermore, there is no method available for predicting the occurrence of economically significant spring flea beetle densities, therefore, seed treatment with insecticides is commonly used for the management of the beetles (Turnock and Turnbull, 1994; Glogoza et al., 2002; Thomas, 2003). In the Golden Triangle area in Montana, most canola growers rely on seed treatment and calendar-based spraying for insecticide applications (Reddy et al., 2014). However, sometimes this might lead to unnecessary chemical exposure. Frequent and repeated use of insecticides may hasten the development of insecticide resistance and is more likely to affect non-target organisms (pollinators, natural enemies, etc.) to a greater extent (Hassan et al., 1998; Newstrom-Lloyd, 2013).

The objective of the current study was to explore alternative treatment schedules to the current practices for the control of *P. cruciferae*. The efficacies of treatments made at different leaf injury levels were evaluated, and compared to calendar-based sprays and seed treatment in both damage reduction and yield production.

2. Materials and methods

2.1. Field location and agronomic practices

Field trials were conducted in May 2013 at 2 locations in Conrad, Montana at the Western Triangle Agricultural Research Center (N 48° 18.627’ W 111° 55.402’) and in a grower’s field (N 48° 11.633’ W 111° 48.290’) near Conrad. The canola variety ‘Nexera 1012’, commonly grown in the region, was used. Treatment plots were 8 m x 4 m and separated from each other by a 1 m buffer to avoid cross contamination from spray drift. Each plot was comprised of 12 rows, spaced 15.2 cm apart. Canola plants were seeded at the rate of 12 seeds per 30 cm using a 4 row plot drill. The plant density was 72 plants m⁻², or approximately 576 plants per plot. Roundup® Powermax (glyphosate) formulation was applied before seeding at 2.5 L/ha to control weeds. Weeds, *Kochia scoparia* (L) Schrad (Caryophyllales: Amaranthaceae), and *Amaranthus retroflexus* (L) (Caryophyllales: Amaranthaceae) were removed manually as needed during the growing season. Fertilizer was applied at 134.5 kg/ha of nitrogen, 2.5 kg/ha of phosphorus, 61.6 kg/ha of potassium and 22.4 kg/ha of sulfur.

The time and number of applications are given in the Table 1. Data on air temperature, relative humidity, wind velocity, and rainfall prevailing during the experimental period were obtained.

2.2. Experimental design and treatments

Each trial had 8 treatments and 3 blocks, arranged in a randomized complete block design. For treatments 1, 2, and 3, an application of Warrior-II® (lambda cyhalothrin, Syngenta, Greenboro, NC) at the rate of 15 g ai/ha was sprayed within 12 h after the plot reached mean threshold levels of 15–20, 25 and 45% of leaf area damaged by *P. cruciferae*, respectively. Applications were repeated when leaf area damage in weekly sampling reached the threshold in each of the 3 treatments. For treatments 4, 5, and 6, an application of the same chemical insecticide was applied at 15, 30, and 45 day intervals after plant emergence, respectively. Lambda-cyhalothrin was used in this study because it is one of the most commonly used insecticides by canola growers in the Golden Triangle area. Treatment 7 was a seed treatment using Gauch® (imidacloprid, Bayer Crop Science) at a concentration of 190 mL of the product/45 kg of seed.

2.3. Damage assessment

Leaf area damaged in each plot was determined weekly. In each plot, 1 m² was randomly selected (approximately 72 plants in 1 m²³). To assess the feeding injuries caused by *P. cruciferae*, all plants and leaves within the chosen area (1 m²) were determined by measuring the amount of leaf area injured by *P. cruciferae* and comparing with the total leaf surface area in order to calculate the percentage of leaf area damaged on each leaf. The leaf area injured by *P. cruciferae* was measured by the 5-grade visual scale as defined in OEPP/EPPO (2004) with a slight modification. The plants were assessed on a scale from 1= (no damage); 2= 15–20% leaf area eaten; 3= 25% leaf area eaten; and 4= 45% leaf area eaten. We did not assess the number of *P. cruciferae* per plant because the adults are highly mobile. For the 15–20%, 25%, and 45% leaf area damage treatments and the calendar-based sprays at 15, 30, and 45-day intervals, a Hudson Never Pump Bak-Pak DC Pump sprayer- 4 gallon, 60 PSI, Model # 13854, cone nozzle, 739.34 mL/min flow was used to apply Lambda-cyhalothrin. The spray volume of 60–100 L/ha were applied at both the locations.

The crop was thinned in late Sept 2013, when 50% of the canola seeds in the pods were very dark in color. The cut canola was left to air dry for 7–10 days to allow the seeds to finish ripening. Windrows were harvested using a Heg® 140 combine. Yield was calculated using the plot weight divided by plot area.

2.4. Statistical analysis

All the data were analyzed using PROC GLIMMIX in SAS version 9.3 (SAS Institute 2011). Percentage data (% leaf area damage) were subjected to arc-sine transformation prior to analysis. Analysis of
variance (Two-way ANOVA) was used to detect differences among treatments. Means were compared using the least significant square difference (LSD) tests. Values of \( P \leq 0.05 \) were considered significant. Linear regression was analyzed using PROC REG to investigate the correlation between yield production and percentage of leaf damage.

3. Results

During the crop growing period, the average temperature was 16.1 °C, the average relative humidity was 57.9%, the average wind speed was 12.0 km/h, and rainfall was 192 mm.

3.1. Percentage of leaf area damaged

Significant differences in percentage of leaf area damage were found among various treatments \( (F = 10.07; df = 7, 14; P < 0.0001) \) at both locations. The untreated control had the highest leaf area damage the treatment made at the threshold of 15–20% leaf area damage had the lowest damage, followed by the calendar-based spray program at 15-day intervals after sowing. Both of these two treatments had significantly lower leaf area damage than the untreated control \( (P < 0.05) \), whereas the other treatments did not reduce damage by \( P. cruciferae \) significantly \( (P > 0.05) \) (Multiple comparison LSD test) (Fig. 1).

3.2. Correlation between yield and percentage of leaf area damage

A negative correlation \( (t = 16.97; df = 1; P < 0.001; R^2 = 0.5482) \) was detected between yield and percentage of leaf area damage (Fig. 2).

3.3. Effect of treatments on yield

There were significant differences among treatments in yield \( (F = 6.37; df = 7, 14; P = 0.0091) \), and all chemical treatments resulted in significantly higher yields than the untreated control (Fig. 3) at both the locations. Calendar-based applications made at 15 day intervals after sowing had the highest yield; the application made at the threshold of 15–20% leaf area damage gave the second highest yield (Fig. 3). However, the difference between treatments at the 15–20% threshold and the calendar-based spray at 15 day intervals was not significant \( (F = 0.67; df = 1, 4; P > 0.05) \). Applications made at 25% and 45% leaf injuries had equal effects to those made at 30 and 45 days intervals and seed treatment in yield \( (P > 0.05) \), Multiple comparison LSD test (Fig. 3).

4. Discussion

Insecticides have traditionally been used to control the important pests attacking \( B. napus \) crops such as \( Mamestra configurata \) Walker (Lepidoptera: Noctuidae) (Turnock and Phillip, 1977; Finlayson, 1979; Bracken and Bucher, 1984), \( Psylliodes chrysocophala \) (L) (Coleoptera: Chrysomelidae) (Alford, 1977; Coll, 1991; Winfield, 1992; Büch, 1993), \( Meligethes aeneus \) F. (Coleoptera, Nitidulidae) (Nilsson, 1987; Tulisalo and Wuori, 1986; Sivtsev et al., 2012; Ahmed et al., 2013), and \( Chiasmia assimilis \) (Warren) (Lepidoptera: Geometridae) (Tulisalo et al., 1976; Free et al., 1983). Economic thresholds, in conjunction with pest monitoring have

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Fig. 1. Cumulative percentage leaf area damage on canola attacked by \( P. cruciferae \) in various treatments \( (\text{Mean} \pm \text{SE}) \) at two locations (Conrad and WTARC - Western Triangle Agricultural Research Center). Different letters above the bars indicate significant differences (Two-way ANOVA, LSD test, \( a = 0.05 \)). 15–20% = spray at 15–20% leaf area damage (Treatment1); 25% = spray at 25% leaf area damage (Treatment2); 45% = spray at 45% leaf area damage (Treatment3); 15-day = calendar-based spray at 15-day intervals (Treatment4); 30-day = calendar-based spray at 30-day intervals (Treatment 5); 45-day = calendar-based spray at 45 days intervals (Treatment 6); Seed Treatment = seed treatment only (Treatment 7); Control = untreated control (Treatment 8).

Fig. 2. Regression between canola yield and percentage of leaf area damage from \( P. cruciferae \).

Fig. 3. Yield of canola in various treatments against \( P. cruciferae \) \( (\text{mean} \pm \text{SE}) \) Conrad and WTARC - Western Triangle Agricultural Research Center. Different letters above the bars indicate significant differences (Two-way ANOVA, LSD test, \( a = 0.05 \)). 15–20% = spray at 15–20% leaf area damage (Treatment1); 25% = spray at 25% leaf area damage (Treatment2); 45% = spray at 45% leaf area damage (Treatment3); 15-day = calendar-based spray at 15-day intervals (Treatment4); 30-day = calendar-based spray at 30-day intervals (Treatment 5); 45-day = calendar-based spray at 45 days intervals (Treatment 6); Seed Treatment = seed treatment only (Treatment 7); Control = untreated control (Treatment 8).
been used to minimize the use of insecticides in *Brassica* crops, especially for the control of *M. aeneus* (Nilsson, 1987), *C. assimilis* (Tulisalo et al., 1976; Free et al., 1983), and *P. cruciferae* in Finland (Augustin et al., 1986).

From an agronomic point of view, the return to the producer depends not only on the yield, but also on the harvestability and quality of the seed (Lamb, 1989). Carbaryl was reported to be effective in controlling the flea beetles in canola (Weiss et al., 1991). However, carbaryl has a 14 day harvest interval, which could preclude its use on crops that are being harvested frequently or at a young age, as are many brassicas (Andersen et al., 2006). Canola was harvested for its seeds. Repeated applications of carbaryl are often needed in order to keep flea beetles below economic injury levels, leading to the development of resistance by flea beetles to this chemical (Turnock and Turnbull, 1994). In Montana, growers often use synthetic pyrethroids to control flea beetles, especially *P. cruciferae* (Desneux et al., 2007). Lambda cyhalothrin, a commonly used pyrethroid insecticide, disrupts the normal functioning of the nervous system in an organism, causing paralysis or death (He et al., 2008). In addition, it has a repellent property against insects (He et al., 2008) including predators (trungu, 2007) and parasitoids (Tillman, 2008) while the response of entomopathogenic nematodes to this agrochemical is species and strain specific (Laznik and Trdan, 2014).

Seed treatment with or without fungicides is a more targeted way of controlling flea beetles, providing a significant increase in potential yield (Canola Council of Canada (2007)). Seed treatments that provide the longest flea beetle protection usually ensure the best seedling establishment, highest plant weight, and highest seed yield. Differences among insecticidal seed treatments were greater when flea beetle infestations were higher than when infestations were low (Elliot et al., 2004). Imidacloprid is one of the risk-reduced compounds that has very low toxicity to mammals and little impact on non-target organisms (Andersen et al., 2006). This reduced risk insecticide has long been used for seed treatment of canola and has been successfully used to control flea beetles (Doyle et al., 2001; Kuhar et al., 2002). However, there are concerns of potential adverse effects of imidacloprid on honey bees, *Apis mellifera* (L.) (Hymenoptera: Apidae). Several studies indicated that chronic exposure to concentrations of imidacloprid at the same amount of those found in seed treatments cause insignificant risks to honey bees (Schmuck et al., 2001; Maus et al., 2003; Schmuck, 2004; Faucon et al., 2005; Nguyen et al., 2009). Contrarily, the laboratory studies showed that honey bees rejected imidacloprid contaminated food at 20 ppb (Kirchner, 1999). Suchail et al. (2001) reported high chronic toxicity in honey bees fed low concentrations of imidacloprid.

The amount of defoliation is often used as a guide to determine the need to take action for flea beetle control (Lyseng, 2013). Flea beetles that attack the early growth stages of canola are usually controlled with systemic insecticides such as imidacloprid applied as a seed dressing or as in-furrow granules. Contrarily, in our study, the seed treatment did not provide as a high yield of canola as the foliar insecticide treatments (Fig. 3). While the foliar application of lambda cyhalothrin provided better control of flea beetles by reducing the number of feeding wounds in the leaf surface more than the seed treatment with imidacloprid, the seed treatment shows better yield than the untreated control. Lambda cyhalothrin is also compatible with most other insecticides and fungicides and could be applied together with other pesticides while still maintaining its efficacy (Gough and Wilkinson, 1984). The advantage of lambda cyhalothrin is that it has been found to be effective at low application rates against insect pests on many different crops. It may also moderately persist in the soil environment. The field half-life of this insecticide ranges from 4 to 12 weeks (Wauchope et al., 1992). Agnihotri et al. (1997) stated that residues of lambda cyhalothrin become non-detectable on the 60th day after application and there is no leaching of residues beyond a depth of 15 cm when soil was continually irrigated. However, for aquatic ecosystems, lambda cyhalothrin was still found to exceed the standard level, which may cause the adverse health effects on people using the water and on aquatic environments (Elftman et al., 2011).

Imidacloprid is a systemic insecticide which has been used as a seed treatment for controlling many insect pests including wireworms (Oregon Pesticide Applicator Training Manual, 2001). Lensen et al. (2007) reported that canola fields without seed treatments showed more damage than those with imidacloprid seed treatment, which was similar to our observations. Imidacloprid seed treatment has been used for pest control in many crops, including corn and potato. Lamb and Turnock (1982) reported that systemic seed treatments were more effective than foliar sprays against sudden and unpredictable invasions of flea beetles, especially in spring. There are some limitations to insecticidal seed treatments, such as the limited dose capacity, limited duration of protection, and possible phytotoxicity to treated seeds. The duration of protection is usually determined by how much of the active ingredient is steadily available to the seed, and the extent of diffusion and speed of breakdown of the chemical as the plant grows.

Moreover, because seed treatments must have high concentrations on the tender tissues of germinating seeds and seedlings, they must have very low phytotoxicity (Oregon Pesticide Applicator Training Manual, 2001). Even so, some insecticidal seed treatments may reduce the length of the sprout (for example corn), thereby influencing planting depth (Oregon Pesticide Applicator Training Manual, 2001). Furlan et al. (2006) found that imidacloprid seed treatment was ineffective in controlling the Western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), in maize. In the current study, seed treatment with imidacloprid did not significantly reduce leaf injuries by *P. cruciferae* (Fig. 1); however, it gave better yields than the untreated controls, although not significantly higher than those from the foliar applications with lambda cyhalothrin (Fig. 3).

Possibly the imidacloprid seed treatment did not perform as well as expected in controlling the flea beetles because its effectiveness was not maximized due to inadequate farming practice. To enhance seed treatment effectiveness, seed canola should be placed into warm soil (5 °C or higher). The proper depth of seed should be 1–2 cm to ensure rapid emergence (Canola Council of Canada (2007)). Plants were seeded 0.635 cm in depth in this study, because in the Golden Triangle area, soil temperature in May ranged from 1 to 4 °C, and the soil was hard when the canola was seeded. The cool soil temperature, combined with the shallow sowing, was likely to have prolonged the time required for the crop to grow beyond the vulnerable early-seeding stage. If canola germinates but stays below ground for 14 days or longer before emerging due to cool soil, the likelihood that seed treatment protection will diminish before the canola crop advances beyond the 4-leaf stage is greatly increased (Canola Council of Canada (2007)).

Another factor which may contribute to the low effectiveness of seed treatment in our experiment was that the rate of insecticide used for seed treatment was too low. Knodel et al. (2008) demonstrated that flea beetle (*Phyllotreta spp.*) injury ratings declined when a high rate of insecticide for seed treatment was used. From their experiment, the rate of 8 g/1 kg of imidacloprid seed treatment lowered the *P. cruciferae* damage significantly compared to the rate of 4 g/1 kg of seeds. Seed treatments typically have an effective residue of 21 days against *P. cruciferae* feeding injury (Knodel and Olson, 2002). Because of that, the canola crop might be vulnerable when crop emergence or growth is delayed or peak
emergence and invasion of flea beetles are later than the 21 days window of protection (Knodel et al., 2008). However, our study was in agreement with Knodel et al. (2008) and Dosdall and Stevenson (2005), in which less flea beetle damage was found on plants treated with insecticide seed treatment than on plants without an insecticide seed treatment.

Our study showed that a calendar-based program at 15-day intervals resulted in significantly higher yields compared to other treatments, except for the threshold-based spray at 15–20% leaf damage (Fig. 1). Interestingly, this calendar-based program (15-day interval) had significantly more leaf damage than 15–20% threshold-based treatment though not a significantly greater yield. This may be explained by various factors. For example, the canola plants in plots treated on a calendar based might have had better ability to outgrow damage by P. cruciferae after bolting than plots treated based on threshold levels. In general, however, a negative correlation was indicated between yield level and leaf damage (Fig. 2). On the other hand, Trdan et al. (2005) reported that statistically significant and positive correlation between leaf damage and number of flea beetles (Phyllotreta spp.) on white and Chinese cabbage. The authors also reported that Phyllotreta spp were less favorable for the crop with regards to the weather conditions (drought and high air temperatures) (Toshova et al., 2006) reported that air temperature and humidity strongly influenced the allyl-isothiocyanate-baited trap catches of flea beetles on cabbage and horse-radish crops. Studies by Gao et al. (2005) showed the temperature and wind orientation had significantly positive correlations with the dispersion of Phyllotreta striolata and humidity weakly influenced their activity. The negative correlation between yield and leaf damage found in our study could be due to low temperatures having a negative effect on populations. Our results agree with Çağak et al. (2006) and Gao et al. (2005) who reported that low temperatures in the winter and high temperatures in the warm season had a negative effect on populations of flea beetles. Additionally, Shukla and Kumar (2003) demonstrated that P. cruciferae populations were negatively correlated with mean temperature and positively correlated with mean relative humidity.

Although calendar-based application at 15-day intervals showed lower damage and higher yield, it did not differ significantly from the treatment made at 15–20% leaf damage. This indicates that there was no necessity to spray every 15 days. It is thus advisable to spray when leaf area damage reaches 15–20%, to reduce numbers of chemical applications. Knodel and Olson (2002) proposed that the threshold for foliar application should be at 25% leaf damage. However, the economic injury level proposed by them was a nominal threshold injury level, and no experiment was conducted to test on that nominal threshold.

The information generated on the nominal threshold level for P. cruciferae from the current study is important and timely as the management of flea beetles has become more challenging. Research on alternative possible methods for controlling/determing flea beetles has been underway for many years but no effective control method has been identified to date. Our previous studies (Reddy et al., 2014) revealed that combined use of the entomopathogens such as Beauveria bassiana (Bals.-Criv.) Vuill. GHA and Metarthizium brunneum (Metcchnikoff) Sorokin FS2 in two repeated applications was effective in reducing feeding injuries by P. cruciferae and improving yields of canola. However, the combined use here of two commercialized fungal preparations from differing manufacturers may present some sort of impediment to the ready adoption of this recommended treatment. It is possible that a concerted screening of a range of isolates of B. bassiana and M. brunneum from established culture collections might yield a pairing of fungal isolates that could be at least as effective as those tested here, and that could then be produced locally or even commercially as a new biocontrol product after appropriate. The applications of entomopathogenic nematodes (Steinernema carpocapsae Stannuszek All and Heterorhabditis indica Poinar, Karunakar & David LN2) were capable of controlling P. striolata populations when applied at least at 0.75 × 10^6 IJs ha^-1 (Yan et al., 2013). Nevertheless, in the context of an integrated approach the cost benefit ratio for the control of flea beetles needs further field studies. While, azadirachtin was reported to control adult populations of P. striolata (He and Xu, 2005), the results by Yan et al. (2013) indicated that azadirachtin alone was not effective for preventing crop injury by P. striolata. There have been some studies on the use of trap crops for flea beetles (Bohinc and Trdan, 2013) but no single ideal trap crop has been effective to date (Bohinc et al., 2013).

In summary, this study has established a threshold for control of P. cruciferae on canola, especially in Montana, i.e., an average of 15–20% leaf area damaged. This study may help canola growers decide when to apply insecticides, and if control is justified. Using this threshold, canola growers can minimize the numbers of spray applications for crucifer flea beetles, representing a step forward in timing insecticide applications compared to calendar or preventive conventional spray schedules. Not only will this save growers money, it may slow down the development of resistance that might occur when flea beetles are exposed to frequent insecticide applications.

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