PROJECT DETAILS

- **Title**: Mitigation of risk to canola from spring flea beetle injury
- **Funders:** Agriculture and Agri-Food Canada
- **Research program:** Growing Forward
- **Principal investigator:** Juliana Soroka, Agriculture and Agri-Food Canada (AAFC) Saskatoon
- **Collaborators/additional investigators:** Bob Elliot, Lloyd Dosdall, John Gavloski, Owen Olfert, Chrystel Olivier, and Jennifer Otani
- **Year completed:** 2013

Final report

Flea beetles are the most important chronic insect pest of canola on the Canadian prairies, with annual yield losses to the crop from flea beetle damage often exceeding $300 million. There is evidence of a shift in distribution of the two principal crucifer-feeding flea beetle species in the southern areas of prairie canola production, potentially troublesome because the two species may have biological differences that affect their potential impact on the crop. The objectives of the study were to quantify the risk of injury by flea beetles to prairie canola production by determining the factors that affect distribution, spring emergence, flight, and feeding levels of the two principal crucifer-feeding flea beetle species; and by investigating conditions and reasons for seed treatment failures as well as alternatives to seed treatments for flea beetle control. The study encompassed investigations in four geographic locations - Carman, MB, Saskatoon, SK, Edmonton and Beaverlodge, AB.

When the potential distribution and relative abundance of crucifer and striped flea beetles are mapped for western Canada, climate is a limiting factor for the occurrence of striped flea beetles in southern areas, particularly southern Saskatchewan and Alberta. Therefore, the recent increased presence of striped flea beetle in these areas appears not to be directly related to climate factors.

Tower trap data indicate that flea beetle flight height is dependent principally upon ambient temperatures, relative humidity, and wind speed. Fight occurs throughout the growing season, and flights can be longer and more sustained than previously thought. Crucifer flea beetles prefer to fly in some, but not excessive, wind, with flight activity greatest at wind speeds of approximately 10 km per hour.

Patterns of spring flea beetle emergence from Carman, MB and Saskatoon, SK, were similar in two of four site years, with the first *P. striolata* emerging 5-6 weeks earlier than *P. cruciferae*. However, data was inconsistent and factors other than temperature may also influence flea beetle emergence. High numbers of *P. cruciferae* were found as early as lower numbers of *P. striolata* at Carman in 2011, and analysis of ambient temperatures indicated that *P. cruciferae* emerged after only one day of maximum temperatures greater than 15 ˚C. An investigation of summer generation flea beetle emergence from early and late seeded canola at Saskatoon found much lower numbers of summer generation *P. striolata* than *P. cruciferae* emerging from canola in all three years of the study. The pattern of emergence of *P. cruciferae* varied with year; in 2010, greater numbers emerged from early than late seeded plots, while the reverse occurred in 2011 and 2012.

Populations of flea beetles were lower in the field at Beaverlodge in 2011 and 2012 than in 2010, and foliar damage was concomitantly lower in the latter two years. Interspecific competition between species appears to be limited, as caging of canola plants revealed that summer generation adults of both *P. cruciferae* and *P. striolata*, as well as the hop flea beetle *Psylliodes punctulata,* can emerge from the space around the same canola plant.

Infection of canola seedlings with a stress-reducing bacterium, *Burkholderia phytofirmans*, did not decrease levels of feeding by flea beetles, although treated seedlings had greater levels of germination at cooler temperatures than untreated seedlings.

This project investigated the effects of growing conditions and seed treatments on flea beetle feeding damage, mortality and canola seedling growth. When crucifer or striped flea beetles were allowed to feed on untreated open-pollinated canola, hybrid canola, or canola-quality mustard seedlings in growth chambers set at 5, 10, 15, 20 or 25°C, crucifer flea beetle damage to each canola type nearly doubled with each 5°C increase in temperature. Regression equations indicated that the economic threshold for crucifer flea beetle damage at densities of two beetles per seedling was reached after 91 degree days (DD) in open-pollinated canola, 81 DD in hybrid canola and 87 DD in canola mustard. Canola mustard was more susceptible to striped flea beetle damage at higher temperatures than open-pollinated or hybrid canola. At two striped flea beetles per seedling, the economic threshold was reached after 95 DD in open-pollinated canola, 94 DD in hybrid canola and 72 DD in canola mustard.

We investigated the effects of three temperatures, (10, 20 and 30°C), and two soil moisture levels,(25-30% and 90-100% soil saturation), on the toxicity of six formulations of neonicotinoid seed treatments to crucifer and striped flea beetles on open-pollinated and hybrid canola in the laboratory. The treatments included untreated seed and seeds treated with Tribune (fungicides only), Cruiser (200 g and 400 g thiamethoxam with no fungicide), Gaucho CS FL (400 g imidacloprid), Prosper FX (400 g clothianidin), Helix (200 g thiamethoxam) and

Helix XTra (400 g thiamethoxam). Mortality of crucifer flea beetles after 72 hours and feeding damage after 24, 48 and 72 hours varied significantly depending on the seed treatment, temperature and moisture regime. Neonicotinoid seed treatments caused higher mortality of crucifer flea beetles in dry soil than in wet soil and better control at 30°C than at 10°C. The seed treatments provided excellent protection against crucifer flea beetle feeding in both dry and wet soils.

Temperature, soil moisture and seed treatments likewise had a significant effect on mortality of striped flea beetles, with the neonicotinoid seed treatments providing poor mortality of striped flea beetles at all conditions tested. Striped flea beetle damage to the cotyledons after 24, 48 and 72 hours varied depending on the seed treatment, temperature and soil moisture regime. Neonicotinoid seed treatments provided better protection against striped flea beetles at 10 and 20°C than at 30°C. After 72 hours, damage with the neonicotinoid seed treatments at 10, 20 and 30°C averaged 4-6%, 9-13% and 13-26%, respectively, in dry soil and 5-6%, 14-16% and 25-40%, respectively, in wet soil. These laboratory bioassays suggested that seed treatment failures in canola may originate from several sources.

Neonicotinoid seed treatments provided substantially better control and protection against crucifer flea beetles than striped flea beetles. Therefore, seed treatment failures are more likely to occur when striped flea beetles are the most abundant species in commercial fields. Neonicotinoid seed treatments also provided better flea beetle control in dry soil than in wet soil. Consequently, seed treatment failures are more likely to occur when above-average rainfall causes saturated soil conditions during germination and seedling emergence. Temperature had little effect on the toxicity of neonicotinoid seed treatments to striped flea beetles. However, higher temperatures resulted in a rapid increase in feeding damage from striped flea beetles. Therefore, seed treatment failures against striped flea beetles are highly probably when saturated soil and high temperatures (20-30°C) occur during germination and seedling emergence. Clearly, new insecticides and seed treatments are needed to control striped flea beetles when these environmental conditions occur in commercial fields.

Field trials were conducted at AAFC-Saskatoon in 2010-2012 to determine the effect of neonicotinoid seed treatments on flea beetle damage and agronomic performance of hybrid canola and canola mustard in summer fallow and wheat stubble. The treatments were the same as in the laboratory studies. Above-average precipitation during the winter months of 2010-2012 and 21 days after seeding resulted in elevated moisture levels in most tests. Under these conditions, neonicotinoid seed treatments had limited effect on flea beetle damage and agronomic performance of hybrid canola. Damage varied depending on the year and seed treatment. In 2010, 2011 and 2012, damage to hybrid canola in all treatments averaged 3.1%, 10.9% and

19.6%, respectively, in summer fallow and 7.1%, 3.6% and 25.1%, respectively, in wheat stubble. The neonicotinoid treatments reduced flea beetle damage to canola in some years and soil stubble types but not in others. Prosper FX and Helix XTra provided the best flea beetle protection over three test years, reducing damage by 3-4% compared to untreated seed. Neonicotinoid seed treatments had no effect on the establishment of hybrid canola in 2010 and 2012 but improved establishment by 11-12% in 2011. The treatments improved the establishment of hybrid canola in summer fallow by 4-10% over three test years. However, the treatments had no effect on the seed yield of hybrid canola in summer fallow in any year. In wheat stubble, seed treatments containing a neonicotinoid insecticide reduced flea beetle damage after 20-22 days by 2-3% in 2010 but had no effect on damage in 2011 and 2012. The treatments improved the establishment of hybrid canola by 10-22% in 2010 but had no effect on establishment in 2011 and 2012. Compared to untreated seed, Prosper FX and Helix XTra improved the establishment of hybrid canola in wheat stubble by 7-9% over three test years. As in summerfallow, the neonicotinoid seed treatments had no effect on the seed yield of hybrid canola in wheat stubble. Consequently, the field trial suggests that more effective seed treatments are needed for protection of hybrid canola against flea beetles when cool wet conditions occur during seedling emergence and stand establishment.

Flea beetle damage to canola-quality mustard varied with the year and seed treatment. Field tests in summer fallow and wheat stubble in 2010-2012 indicated that cool wet conditions during seedling emergence reduced the efficacy of neonicotinoid seed treatments against flea beetles in canola mustard. The treatments had no effect on flea beetle damage after 20-21 days in summer fallow and wheat stubble in any year. Flea beetle damage exceeded 55% in all neonicotinoid seed treatments in summer fallow in 2012. Neonicotinoid seed treatments improved the establishment of canola mustard in some tests but not in others. In summer fallow, the treatments improved stand establishment by 7-15% in 2010 and by 6-14% in 2011. Prosper FX and Helix improved the establishment of canola mustard in summer fallow by 5% over three test years. In wheat stubble, seed treatments containing a neonicotinoid insecticide improved the establishment of canola mustard by 10-15% in 2010 but had no effect on establishment in 2011 and 2012. In all tests on summer fallow and wheat stubble, neonicotinoid seed treatments had no effect on the seed yield of canola mustard. Clearly, more efficacious seed treatments are needed to protect canola mustard from flea beetles when cool wet conditions occur during stand establishment.

Flea beetles are the most important chronic insect pest of canola on the Canadian prairies. Annual canola yield losses from flea beetle damage average 8-10%, usually exceeding $300 million (Lamb and Turnock 1988, Knodel and Olson 2002). Insecticidal seed treatment is the principal means of flea beetle control in canola production. Currently more than 90% of the 5-6 million ha seeded to canola in North America are treated with neonicotinoid seed treatments (Soroka et al. 2008), often in a prophylactic manner with no solid knowledge of the actual need for the insecticide in a particular area or season. Concerns have been raised about the effects of such large amounts of insecticide on the environment. If knowledge of risk of flea beetle feeding severity were known prior to seed preparation and seeding, producers in high risk areas could be better prepared for action, while producers in low risk areas could evaluate whether control is warranted at all.

Recent laboratory work has suggested that neonicotinoid seed dressings have differential mortality against the principal flea beetle pests *Phyllotreta striolata* and *P. cruciferae*, with the former species being less susceptible to these insecticides (Tansey et al. 2008). If this species-differential morality holds true on a wide geographic scale, it may in part explain recent reports of increased numbers of striped flea beetles occurring in areas where crucifer flea beetles were traditionally more common (Soroka 2012); our current knowledge of flea beetle control, based primarily on observations of *P. cruciferae*, may be in question. Further, dry conditions and low temperatures are known to reduce the efficacy of neonicotinoid seed treatments against the crucifer flea beetle. In field trials at AAFC-Saskatoon in 2003-2009, the efficacy of neonicotinoid seed treatments against crucifer flea beetles varied greatly from year to year. In some years, the seed treatments provided excellent protection against flea beetle damage. With this protection, the treatments improved seedling emergence, stand establishment, shoot weight, biomass accumulation and seed yield. However, in other years, the treatments provided poor protection against flea beetle damage and had little or no effect on agronomic performance (Elliott et al. 2008). Knowledge of specific conditions that decrease seed treatment efficacy can alert producers to situations when other management actions should be taken to optimize canola seed production and yield. In addition, some seed treatments are purported to have plant stimulating characteristics along with pesticidal properties. Research into plant-growth promoting factors has shown that they hold promise as alternatives to chemicals for pest control. One such factor, the naturally-occurring rhizobacterium *Burkholderia phytofirmans,* is known to increase plant resistance to biotic and abiotic stresses (Compant et al. 2005). If the bacterium can deter flea beetle feeding and/or render canola tolerant to flea beetles, it may be a valuable addition to the control arsenal.

Objective 1: Examining environmental factors that affect flea beetles

a) Bioclimate Modeling - Olfert, Weiss:

Climate is the principal factor governing the distribution and abundance of most insects. Bioclimatic simulation models, also known as bioclimate envelope or ecological niche models, have been applied successfully to predict the distribution and extent of insect pests and their natural enemies to establish in new environments. CLIMEX® facilitates the development of models that describe the potential distribution and relative abundance of a species, based on climate. CLIMEX® derives an Ecoclimatic Index (EI) that defines the climatic suitability of specific loctions for species survival and growth. In order to develop and apply a bioclimate simulation model for flea beetles, 538 data points (distribution records) for *P. cruciferae* and 570 data points for *P. striolata* were confirmed using *CABI, Fauna Europa* and *Global Biodiversity Information Facility* data bases. The data were formatted and incorporated into the CLIMEX® database. Numerous model iterations were conducted to rationalize parameter values (e.g. relevant temperature and moisture ranges) that influence flea beetle distribution and abundance. Models were run with the current climate dataset (CM10_1975H_V1).

Model results (e.g. distribution and abundance maps) correlate well with the known North American distribution of *P. cruciferae* and *P. striolata. P. cruciferae* is most abundant in the southern Canadian prairie regions, and *P. striolata* has traditionally been found in greatest numbers along the northern Parkland of the prairies and in the Peace River Region of Alberta. Model output agrees with observed distributions by showing that although the two species' distributions overlap, there are differences between them (Olfert Figs. 1 and 3). Projections agree with observed distributions that indicate that *P. striolata* abundance is greater in the Parkland region of the Canadian prairies and that *P. cruciferae* abundance is greater to the south of the *P. striolata* range (Olfert Figs. 2 and 4). Based on observed abundance patterns, it was determined that EI values >20 indicate geographical regions that are suitable for flea beetle distribution and population abundance. Our results indicate that the historic pattern of *P. cruciferae* and *P. striolata* flea beetle species distributions on the Canadian prairies correlate well with climate parameters of the region. Therefore, it is probable that if changes in distribution of the two species are occurring, the cause is not directly related to climate, or at least, not climate alone.

b) Flight Height of Flea Beetles - Dosdall, Soroka:

For each of the three years of this study, we investigated *Phyllotreta cruciferae* and *P. striolata* seasonal flight height using specialized traps at the University of Alberta, St. Albert Research Farm (2010), at a farmer's field of *B. napus* canola near Leduc, AB (2011 and 2012), and at the Agriculture and Agri-Food Canada Research Station in Saskatoon, SK (2010, 2011, and 2012). The study was designed to determine periods of greatest flight activity of the two beetle species, and how these related to environmental factors. Understanding how environmental parameters affect flight (dispersal) activity of the beetles can give canola producers improved information on the best timing for mitigating the feeding impact of the beetles in canola crops.

A new trapping apparatus, described in Tansey et al. (2012), was designed for the study. Traps consisted of yellow, 15 cm-wide, 9 cm-deep, plastic (polypropylene) bowls filled with diluted propylene glycol antifreeze (1:1 water: glycol). The propylene glycol functioned as a beetle killing agent, and its low evaporate rate allowed weekly emptying of he traps. Traps were set on 6 m metal poles at heights of 0.2, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 m to form an individual array. Each height (array) was replicated four times; replicated arrays were separated by 8 m. Beetles were collected from traps weekly from early May to late August in each year of the study.

Captured beetles were examined to determine species and sex.

Climate data, consisting of weekly mean, maximum and minimum air temperatures (°C), relative humidity (%), wind speed (km per h), wind direction (in 10 degree increments with 360° corresponding to true north), visibility (km), precipitation (mm) and atmospheric pressure (kPa) were obtained from the Environment Canada weather stations at Namaio, AB (53.6667°N, 113.4667°W) and Diefenbaker International Airport, Saskatoon (52.1667°N, 106.7167). These sites were approximately 8 and 10 km from the study sites, respectively. Because of relatively close proximity to study sites and great similarities in topography, elevation and vegetation, data from climate stations were considered to reasonably represent conditions at the study sites.

Evaluations of the effects of height and sampling date on beetle captures were made with a generalized linear model specifying a Poisson distribution. Comparisons of weekly counts and trapping heights were made using a Wald chi-square. A peak in capture numbers in July to early August indicates emergence of the summer generation of beetles; therefore, capture data were separated by generation and effects of climate factors on capture heights were evaluated for each generation. Relationships of capture heights and linear atmospheric parameters were assessed using stepwise multiple regression. Analysis included variables developed to represent quadratic relationships of capture heights and atmospheric factors. Comparisons of capture heights for weeks with and without precipitation were conducted with a t-test. Circular correlation analyses of relationships of mean capture heights and linear environmental parameters with wind direction were conducted using Oriana 3 software (Kovach Computing Services).

Data analyses for *P. cruciferae* are complete and are present here; analyses of *P. striolata* data are also finished and are in the process of summarization. Capture heights of *P. cruciferae* were related to increases in mean air temperature. Similar relationships of temperature and capture height were found for both the spring and summer generations. The flea beetle flight height model developed in this study indicated that capture heights would continue to increase as air temperature increased (Dosdall Fig. 1). Flight heights of the crucifer flea beetle were predicted to keep increasing as temperatures extended to maximum levels experienced at study sites in Alberta and Saskatchewan during the canola growing season. Capture heights decreased with increases in mean relative humidity. This relationship was consistent for overwintered and summer generations. Decreases in capture heights were also associated with maximum and minimum relative humidity. No relationship of mean wind speed and capture height was found. However, capture heights increased with weekly minimum wind speeds. Decreases in capture heights with increases in maximum wind speed were also detected. Mean wind direction was also correlated with capture height. Capture heights were significantly higher for weeks without rain. Decreases in capture heights were associated with increases in mean daily precipitation. Decreases in capture heights with increases in maximum daily precipitation were also detected. Our results support previous reports that flea beetle activity is influenced by ambient temperature and relative humidity. Our results also indicate that flights are longer than anecdotal accounts suggest of movement by walking and hopping or short flights, and we found that flight activity occurs throughout the growing season. Greatest numbers of flea beetles were captured in early August; these results are associated with emergence of the new, overwintering generation of adults and are consistent with results reported previously by several researchers. We found that both overwintered- and summer-generation beetle capture heights increased with mean temperatures. We also recorded captures at heights of 3 m during weeks with mean temperatures of approximately 12°C; beetles were captured at only the lowest heights when maximum air temperatures did not exceed 15°C. This suggests that 15°C is also a minimum temperature for flea beetle flight activity. Ulmer and Dosdall (2006) reported that emergence of spring generation flea beetles coincided with mean soil temperatures of 15°C, while Lamb (1983) reported that, in Manitoba, beetles flew only when temperatures reached 14°C or above.

Weekly minimum relative humidity was also found to be an important factor influencing flea beetle capture heights. Modeled functions associated with flea beetle capture heights and minimum relative humidity indicated great sensitivity of these beetles to this parameter. High relative humidity indicates the likelihood of a rain event; minimum relative humidity and mean precipitation were highly correlated in the current study. Because rain is associated with mortality of small insects, reduced activity in these conditions is adaptive. We found that rain events significantly reduced weekly flea beetle capture heights and that precipitation amounts were related to reductions in capture heights.

We found a marked increase in beetle capture heights with increases in minimum wind speed, and a reduction in capture heights with increases in maximum wind speeds. Intersection of these functions suggests that flight activity is greatest when wind speeds are approximately 10 km per h. Insect flight is generally stimulated by light winds and suppressed by faster wind speeds (McManus, 1988). One of the most important factors associated with capture heights was the range of atmospheric pressures (barometric flux).

Greater flea beetle damage to plants on field margins in cool, rainy springtime conditions have been attributed to concentration of beetles in these areas as they invade from overwintering sites. Correlation of capture heights and temperature, relative humidity and the range of atmospheric pressures indicate that environmental conditions in the spring may be effective predictors of the extent and speed of flea beetle invasion of canola crops. Flea beetle dispersal distances are likely proportional to flight behaviour. However, flight mill studies are required to determine flight duration and rigorous trapping studies are needed to accurately determine effects of temperature and relative humidity on field invasion and flea beetle spatial distribution.

Monitoring of temperature, relative humidity and atmospheric pressure coupled with flea beetle population monitoring should improve estimates of crop invasion under specific conditions and contribute to a better understanding of the risks to crops posed by flea beetle populations. These results should also allow for more judicious and efficacious application of foliar-applied chemical controls.

c) Assessing patterns of flea beetle emergence - Soroka, Gavloski:

Circular metal cone-shaped emergence traps, with a basal diameter of 36 cm, were set up at the University of Manitoba Farm near Carman in April of 2011 and 2012. Four traps were set in a canola stubble field, with another four traps set up in a wooded area nearby. Probes to measure daily temperatures (average, minimum and maximum) were placed just beneath the soil or debris layer in each emergence trap. In 2011 the field in which the traps were placed was cultivated on May 17th, and the four traps were removed and returned after cultivation. Traps remained in the field and wooded area until August. In Saskatoon, eight pyramidal traps with a base of 1 m square were set out in April 2011-2012 on field plots of the Saskatoon Research Centre farm that had been seeded to canola early and late in the previous year, and four traps were set out in a nearby grassy shelterbelt. Temperature probes were placed inside and outside of the traps.

At Carman in 2011 both *P. cruciferae* and *P. striolata* were first captured in emergence traps in the wooded area on April 26th, the first sapling period. *P. striolata* continued to emerge in traps in the wooded area until May 16th, whereas *P. cruciferae* continued to emerge until June 27th (Soroka Fig. 1). Only one trap recorded a high level of emergence of flea beetles. The maximum temperature under the debris in this trap reached 9.2°C on April 23, 12.8°C on April 24th and 15.2°C on April 25th, the 3 days prior to the trap being checked. When the traps were checked on April 26th there were 24 flea beetles in the vial at the top of one trap; the maximum temperature in the trap on April 26th was 13.1°C. There was only 1 flea beetle collected from this emergence trap after April 26th. It appears the flea beetles at this specific location received the cues to emerge over a very brief period and not over several weeks. Lamb (1983) concluded that *P. cruciferae* flew only when daily maximum temperatures exceeded 14°C, based on correlating air temperature with flea beetles caught in traps. According to the results from 2011, significant emergence of both species of flea beetles can occur when temperature in the overwintering habitat are at most 15°C. The results from 2012 were not consistent with those of 2011. In 2012, the first *P. striolata* adults to emerge were collected from March 31 to April 5, while the first *P. cruciferae* were not collected until May 11-18, which was the last period for collecting *P. striolata*. *P. cruciferae* continued to emerge until June 8. Maximum temperatures in the area first exceeded 15°C on March 15; the weather stayed warm for about a week, turned colder until March 31, then warmed. Mean daily air temperatures exceeded 15 °C for the first time on May 13, the time period when *P. cruciferae* were first collected.

At Saskatoon in 2011, *P. striolata* and hop flea beetles *Psylliodes punctulata* emerged by the first sampling period April 27, several weeks earlier than did *P. cruciferae* adults (Soroka Fig. 1). Average soil temperatures in cages were 9.0, 10.7, and 7.8°C on April 25th, 26th and 27th, respectively, coinciding with the first collection of *P. striolata* adults. The first *P. cruciferae* beetles were captured in cages in the shelterbelt in the May 17-24 sampling period; the maximum soil temperatures reached 16.1°C on May16, suggesting that *P. striolata* are

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active at temperatures lower than *P. cruciferae*. Numbers of *P. striolata* increased in the traps in the July 12-18 sampling period, indicating the beginning of the emergence of the summer generation. In 2012 the numbers of flea beetles collected from the emergence traps were very low throughout the spring, and no patterns of emergence could be discerned.

In an investigation monitoring emergence of summer generation flea beetle adults, 750 ml clear plastic containers with lids on but the base removed were placed over individual canola plants severed near the soil surface. A 5.5x5.4 cm yellow sticky trap was affixed to the underside of the lid of each container to catch emerging flea beetles. Six containers were placed in each plot while plots, each 6.1x30.5 m in dimension, consisted of hybrid canola cultivar 5020 seeded early (mid-May) or late (early June) in a randomized complete block pattern with five replicates. Greater numbers of summer generation *P. cruciferae* flea beetles emerged from the traps than *P. striolata* beetles in all three years (Soroka Fig. 2). Patterns of emergence varied with year. In 2010, greater numbers of both species emerged from traps on canola seeded early in the year. In 2011 and 2012, however, greater numbers of *P. cruciferae* emerged from later seeded plots, while the numbers of *P. striolata* beetles were similarly low in both years.

d) Assessing damage to early and late seeded crucifer seedlings and monitoring flea beetle species and emergence of summer generation beetles - Otani:

Field tests were conducted at Beaverlodge, AB, between 2010 and 2012. Five cruciferous cultivars, *Brassica juncea* cv. Duchess, *B. napus* cv. Banner, *B. napus* cv. 45H21, B. *juncea* cv. 8571 and *Sinapis alba* cv. Andante, were seeded into “early” and “late” seeding date blocks (Otani - Table 1 for annual seeding dates). All seed was treated with Helix Xtr® prior to seeding and a target seeding rate of 6 lbs/ac was used in all plots. Within each block, cruciferous varieties were seeded into cereal stubble using zero-till (Fabro double-disk seeder on 9” row spacing) using a randomized complete block design with four replicates. The objectives of the field study were to investigate the effect of seeding date on flea beetle damage, flea beetle emergence and crucifer agronomic performance.

Plant densities and flea beetle damage were assessed at 14, 21, and 28 days after seeding (DAS) (Refer to Otani - Table 1 for mean damage and plant densities). The entire south Peace River region, including field plots at Beaverlodge, was affected by drought in 2010. The adverse growing conditions resulted in short, thin plots of all test varieties. Flea beetles were obvious in both early and late seeded plots in 2010. More flea beetle damage was observed in late seeded plots (means of 34.5% defoliation at 14 days after seeding (DAS), 43.8% at 17 DAS, and 37.1% at 21 DAS) compared to early seeded plots (means of 3.9% at 14 DAS, 3.4% at 17 DAS, and 6.8% at 21 DAS) (Otani Table 1). By 21 DAS, mean plant seedling densities of 137.5 seedlings per 1-metre-row in early seeded versus 64.0 seedlings per 1-metre-row in late seeded plots was observed in 2010. When plant dry weight sampling was performed 28 DAS, smaller plants were observed in early seeded plots compared to

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late seeded plots (Otani - Table 1). The spring of 2011 was again very dry at Beaverlodge. Seedling emergence and growth suffered while flea beetle feeding was obvious. In 2011, similar levels of flea beetle damage were observed in early (mean=20.2% defoliation) compared to late seeded plots (mean=21.9%), in contrast to 2010 when the reverse was observed. Plots of Andante suffered the lowest levels of flea beetle damage during the 28 DAS monitoring period (5.7% defoliation in early seeded and 7.5% in late seeded plots) followed by 45H21 (11.9% in early and 17.4% in late plots), Banner (18.8% in early and 15.5% in late), Duchess (23.8% in early and 23.3% in late) whereas the highest levels of flea beetle damage were observed in plots of 8571 in 2011 (38.7% in early and 39.8% in late) (Otani Table 1). When comparing plant growth for 10 plants per plot harvested at 28 DAS in 2011, early seeded plants were slightly larger (mean=0.482 g) compared to those seeded late (mean=0.366 g). In early seeded plots, plant dry weights at 28 DAS were heaviest in plots of Andante (mean=0.758 g), followed by Banner (mean=0.576 g), 45H21 (mean=0.517 g), Duchess (mean=0.312 g), with the smallest plants observed in plots of 8571 (0.248 g) (Otani Table 1). Amongst the late seeded plots, the heaviest plants at 28 DAS occurred in Andante plots (mean=0.570 g) compared to 45H21 plants (mean=0.562 g), Duchess (mean=0.263 g), or Banner (mean=0.231 g) with the smallest plants were present in plots of 8571 (mean=0.200 g) in 2011 (Otani Table 1). The spring of 2012 was again characterized by dry seeding conditions with low soil moisture extending into early June. The third dry spring affected germination and plant stands in field plots at Beaverlodge. Low soil moisture and little precipitation from May to mid-June were accompanied by low levels of flea beetle activity (Otani Table 1).

At full flower, two plants per plot in the Banner and 45H21 treatments were cut at ground level and a 750 ml cage was immediately placed over the remaining root and surrounding soil surface to collect newly eclosed flea beetles weekly. The species of adult flea beetles and date of emergence was determined weekly for each caged root from full flower (July) until hard frost in the fall (Otani Table 2). Three species of newly eclosed adult flea beetles (*Phyllotreta striolata, P. cruciferae, Psylliodes punctulata*) were collected from the cages containing early and late seeded Banner and 45H21 roots in plots at Beaverlodge between 2010 and 2012 (Otani Table 2). In total, 501 newly eclosed flea beetles were collected in this study with 457 collected in 2010, 40 collected in 2011, but a mere 4 collected in 2012 (Otani Table 2). The dominant species emerging in each year was *P. striolata,* followed by *P. cruciferae*, then *Psy. punctulata* (Otani Table 2). In 2011, most of the 40 newly emerged flea beetles arose from late seeded plots of Banner and 45H21. Three species of flea beetles were collected in 2011, *P. striolata* (80%), *P. cruciferae* (15%) and *Ps. punctulata* (5%). Two *P. striolata* and one *P. cruciferae* adult emerged from cages enclosing early seeded 45H21, with three *P. striolata*, two *P. cruciferae*, and one *Ps*. *punctulata* adult emerging from cages of early seeded Banner plants. Seven *P. striolata* and two *P. cruciferae* adults were collected from late seeded 45H21 plots, while 19 *P. striolata*, one *P. cruciferae*, and one *Ps. punctulata* adults emerged from late seeded Banner plots. In 2012, flea beetle damage was very low and a mere four newly eclosed flea beetles were trapped (Otani Table 2). Overall, low levels of flea beetle damage

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were observed in 2012 in early (mean=1.02% defoliation) and late seeded plots (mean=0.12%) (Otani Table 2) compared to damage observed in 2010 and 2011.

Most importantly, the field data clearly indicates that multiple flea beetle species will feed and develop upon the same *B. napus* root. Multiple species of flea beetles were repeatedly collected from a caged root in both early and late seeded plots of either 45H21 or Banner in 2010. For example, during the collection period of August 23-25 in 2010, *P. striolata* (N=8), *P. cruciferae* (N=13), plus *Ps. punctulata* (N=1) were simultaneously collected from a Late seeded 45H21 root (Cage B in Plot 201). Also in 2010, there were 46 instances where two species were collected on the same root during the same collection period; at least one *P. striolata* plus one *P. cruciferae* were collected on 39 different instances from the same Banner or 45H21 root, at least one *P. striolata* plus one *Ps. punctulata* were collected on seven different instances from the same root, and at least one *P. cruciferae* plus one *Ps. punctulata* were collected 10 different instances from the same root.

At maturity, a 1m x 2m area in each plot was hand-harvested by clipping plants at ground level then placing them inside cotton sacks. Sacks were hung outdoors, then placed in a forced-air dryer to obtain plant samples of a uniform, dry moisture content, then weighed to obtain an above-ground dry biomass measurement. The contents of each sack were threshed and cleaned to obtain yield data (Otani Table 1). Yields were relatively low due to the continuous lack of precipitation from May until September in 2010 (Otani Table 1). Early seeded plots generated relatively low biomass values starting at a mean of 150.8 g/m^2 in plots of 8571, then increased to 160.3 g/m^2 in plots of Andante, 260.6 g/m^2 in plots of Duchess, 261.4 g/m^2 in plots of Banner, and 329.8 g/m^2 in plots of 45H21 (Otani Table 1). Seed yields in these same early seeded plots started at a mean of 29.2 g/m^2 in plots of 8571, then increased to 34.2 g/m^2 in plots of Andante, 67.6 g/m^2 in plots of Banner, 75.3 g/m^2 in plots of Duchess, and 75.4 g/m^2 in plots of in plots of 45H21. In comparison, higher biomass values were observed in late seeded plots and ranged from 290.9 g/m^2 in plots of Andante, 434.4 g/m^2 in plots of Banner, 442.1 g/m^2 in plots of Duchess, 467.1 g/m^2 in plots of 8571, up to 497.2 g/m^2 in plots of 45H21. The correspondingly higher seed yields in these same late seeded plots started at 68.5 g/m^2 in plots of Andante, and increased to 111.1 g/m^2 in plots of 8571, 129.1 g/m^2 in plots of Duchess, 136.2 g/m^2 in plots of 45H21, and 137.8 g/m^2 in plots of Banner. In 2011, biomass values in early seeded plots ranged from 394 g/m^2 in plots of Andante to 801 g/m^2 in plots of 45H21 (Otani Table 1). Preliminary seed yields in early seeded plots ranged from a mean of 32.5 g/m^2 in plots of Andante, 92.4 g/m^2 in plots of Duchess, 133 g/m^2 in plots of 8571, 228 g/m^2 in plots of 45H21, to 258 g/m^2 in plots of Banner in 2011 (Otani Table 1). Late seeded plots were lower in above-ground biomass and yield compared to early seeded plots. Biomass values ranged from 425 g/m^2 in plots of Andante to 693 g/m^2 in plots of Banner in 2011 (Otani Table 1). Seed yields in these same late seeded plots ranged from a mean of 37.1 g/m^2 in plots of Andante, 87.8 g/m^2 in plots of 8571, 109 g/m^2 in plots of Duchess, 218 g/m^2 in plots of Banner, to 226 g/m^2 in plots of 45H21 in 2011 (Otani Table 1). In 2012, biomass values in early seeded plots ranged from 292 g/m^2 in plots of Andante to 393 g/m^2 in plots of 45H21 (Otani

Table 1). Preliminary seed yields in early seeded plots ranged from a mean of 48.9 g/m^2 in plots of 8571, 60 g/m^2 in plots of Andante, 99 g/m^2 in plots of Banner, 101 g/m^2 in plots of Duchess, to 105 g/m^2 in plots of 45H21 in 2012 (Otani Table 1). Late seeded plots were lower in above-ground biomass and yield compared to early seeded plots again in 2012. Biomass values ranged from 285 g/m^2 in plots of 45H21 up to 327 g/m^2 in plots of Andante in 2012 (Otani Table 1). Seed yields in these same late seeded plots ranged from a mean of 53 g/m^2 in plots of 8571, 66 g/m^2 in plots of Andante, 67 g/m^2 in plots of Duchess, 70 g/m^2 in plots of Banner, to 76 g/m^2 in plots of 45H21 in 2012 (Otani Table 1).

e) Inoculation of canola with *Burkholderia phytofirmans* and its effects on flea beetle feeding - Olivier:
Burkholderia phytofirmans strain PsJN is a rhizobacterium that colonizes plant tissues and increases growth rate and resistance to plants under various forms of stress. The goals of this project were to develop a protocol to inoculate canola with *B. phytofirmans*, observe bacterial multiplication and localization in canola seedlings, and investigate the effects of PsJN on flea beetle feeding. If bacteria-infected canola can deter flea beetle feeding and/or render canola tolerant to flea beetles, it may be a valuable addition to our control arsenal against them.

Goal 1 was achieved in 2010-2011. Surface-sterilized seeds were inoculated for 4 hr with a bacterial suspension and placed on sterile soil in sterile Magenta boxes. Inoculated seedlings were grown under sterile conditions for 1 month and transplanted in potted soil for further experiments. Bacterial colonization was more successful when seeds were dipped into the bacterial solution as compared to bacterial solution deposited at the crown level of seedlings.

Goal 2 was achieved in 2011-2012. Cultures of *B. phytofirmans* obtained from AAFC-Kentville Nova Scotia, were maintained at AAFC-Saskatoon. PCR tests on inoculated canola plants revealed that *B. phytofirmans* can colonize cotyledon/leaf and hypocotyl/stem tissue of canola after seed or seedling inoculation and that tissue from cotyledon/leaf and hypocotyl/stems were more often colonized than root tissue.

Goal 3 was achieved in 2012. Canola seeds were coated with PsJN in a bacterial solution (25g seed + 1ml PsJN solution, 10^{3-5} cfu/seed) using two different types of media - clay or microencapsulation using a gelatin-gum arabic mixture. One week old seedlings were exposed to flea beetles for 1 week, with daily damage assessments. No significant differences were observed in damage levels among the treatments regardless of the temperature (Olivier Fig. 1). In fact, the microencapsulation treatment tended to increase flea beetle feeding and damage, possibly because of the vigorous appearance of these seedlings. A complementary experiment aimed at estimating the effect of *B. phytofirmans* on seed germination at lower temperatures was done in collaboration with B. Elliott (AAFC-Saskatoon). Canola seeds coated with low and high rates of B. *phytofirmans* PsJN showed higher germination at low temperature (+5ºC) compared to non-treated seeds (Olivier Fig. 2).

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f) Effects of temperature on flea beetle damage and seedling vigour - Elliott:
Growth chamber experiments were conducted annually to determine the effects of temperature on flea beetle damage and seedling vigour of open-pollinated (op) canola, hybrid canola and canola mustard. Crucifer and striped flea beetles were collected from commercial fields near Saskatoon beginning in late April. Beetles were collected with sweep nets along swaths where volunteer seedlings were present. Beetles of each species were placed in separate cages (20/15°C; 16L/8D photoperiod) and reared on canola and cabbage leaves for at least 7 days before testing. Untreated seeds of op canola, hybrid canola and canola mustard were planted individually in plastic cones containing a soil-less mix and placed in the greenhouse for 7 days. After 7 days, seedlings at the cotyledon stage were transferred into bioassay cages. Each cage contained two rows of five seedlings of op canola and a corresponding number of seedlings of either hybrid canola or canola mustard. The cages were placed in growth cabinets at 5, 10, 15, 20 and 25°C. Each cabinet contained a cage with no beetles and a cage with 40 crucifer or striped flea beetles (n = 2 beetles/seedling).

Crucifer flea beetles

Crucifer flea beetle damage to cotyledons of op canola, hybrid canola and canola mustard increased daily at each temperature (Elliott Fig. 1). In most instances, the increase in feeding damage over time followed a linear trend ($P \leq 0.001$) at each temperature. Feeding damage to each canola type increased exponentially (linear trend $P \leq 0.001$; quadratic trend $P \leq 0.001$) at higher temperatures. With two crucifer flea beetles per seedling, damage after 7 days at 5, 10, 15, 20 and 25°C averaged 2, 5, 10, 28 and 47%, respectively, in op canola; 3, 7, 16, 32 and 48%, respectively, in hybrid canola and 2, 5, 12, 28 and 42%, respectively, in canola mustard. Damage after 7 days indicated that crucifer flea beetle damage to each canola type nearly doubled with each 5°C increase in temperature. With two beetles per seedling, feeding damage at 15°C was below the economic threshold (25% damage) in all canola types after 7 days. In contrast, feeding damage to each canola type exceeded the threshold after 5-6 days at 20°C and after 3-4 days at 25°C. With the rapid increase in damage at higher temperatures, canola and canola mustard should be inspected daily when temperatures reach 20-25°C.

Feeding damage to cotyledons of each canola type was assessed in relation to accumulated degree-days (DD) above 5°C when damage was minimal (Elliott Fig. 2). The relationship between DD accumulation and crucifer flea beetle damage was significant ($P \leq 0.001$; $R^2 = 0.92$-0.96) in each canola type. Damage increased linearly as accumulated DD increased. With two crucifer flea beetles per seedling, the slope of the regression equation indicated that the increase in damage with higher DD was similar in op canola ($b = 0.29$), hybrid canola ($b = 0.31$) and canola mustard ($b = 0.29$). The regression equations also indicated that the economic threshold for crucifer flea beetle damage (n = 2 beetles/seedling) was reached after 91 DD in op canola, 81 DD in hybrid

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canola and 87 DD in canola mustard.

The effect of feeding damage on shoot fresh weight at different temperatures was evaluated in each canola type (Elliott Fig. 3). Compared to seedlings grown without beetles, damage from crucifer flea beetles at 5, 10, 15, 20 and 25°C reduced the shoot fresh weight by 11, 12, 21, 44 and 59%, respectively, in op canola; by 2, 15, 25, 44 and 62%, respectively, in hybrid canola; and by 0, 1, 16, 29 and 48%, respectively, in canola mustard. The results indicate that crucifer flea beetle damage at 15, 20 and 25°C had a greater effect on the shoot fresh weight of op canola (21, 44 and 59% reduction, respectively) and hybrid canola (25, 44 and 62% reduction, respectively) than canola mustard (16, 29 and 48% reduction, respectively). The impact of feeding damage on dry matter content was also greater in op canola and hybrid canola than in canola mustard (Elliott Fig. 4). At 5, 10, 15, 20 and 25°C, feeding damage by crucifer flea beetles reduced dry matter by 14, 15, 19, 28 and 41%, respectively, in op canola; by 8, 14, 18, 29 and 42%, respectively, in hybrid canola; and by 3, 0, 8, 8 and 23%, respectively, in canola mustard. The results suggest that canola mustard is more tolerant to crucifer flea beetle damage than op and hybrid canola. Feeding damage had little effect on the water content of each canola type at 5 and 10°C (Elliott Fig. 5). However, damage at 15, 20 and 25°C reduced water content by 0.1, 1.2 and 2.4%, respectively, in op canola; by 0.5, 1.2 and 2.7%, respectively, in hybrid canola; and by 0.4, 1.4 and 2.7%, respectively, in canola mustard. Collectively, the results indicate that crucifer flea beetle damage has a substantial negative effect on seedling vigour of the three canola types at 20 and 25°C. The negative effect of feeding damage on seedling vigour emphasizes the importance of inspecting commercial fields daily when temperatures reach 20-25°C.

Striped flea beetles

Striped flea beetle damage to cotyledons of the three canola types increased daily over 7 days at each temperature (Elliott Fig. 6). The increase in damage over time followed a linear trend ($P \leq 0.001$) at each temperature in each canola type. With two beetles per seedling, damage increased exponentially (linear trend $P \leq 0.001$; quadratic trend $P \leq 0.05$) with higher temperatures in each canola type. Damage after 7 days at 5, 10, 15, 20 and 25°C, averaged 2, 6, 12, 24 and 36%, respectively, in op canola; 3, 7, 15, 25 and 35%, respectively, in hybrid canola; and 2, 4, 13, 36 and 57%, respectively, in canola mustard. Damage was similar in the three canola types at 5, 10 and 15°C. However, damage at 20 and 25°C was higher in canola mustard (36 and 57%, respectively), than in op canola (24 and 36%, respectively) and hybrid canola (25 and 35%, respectively). In op and hybrid canola, the economic threshold with two striped flea beetles/seedling was reached after 7 days at 20°C and after 4-5 days at 25°C. In canola mustard, the threshold was reached after 4-5 days at 20°C and 3-4 days at 25°C. The results indicate that canola mustard is more susceptible to striped flea beetle damage at higher temperatures than op and hybrid canola.

Striped flea beetle damage to each canola type was assessed in relation to accumulated DD above 5°C (Elliott Fig. 7). The relationship between DD accumulation and feeding damage was significant ($P \leq 0.001$, $R^2 = 0.93$-0.98) in each canola type. Damage from striped flea beetles increased linearly as accumulated DD increased. The slope of the regression equations indicate that feeding damage increased more rapidly in canola mustard ($b = 0.38$) than in op canola ($b = 0.27$) and hybrid canola ($b = 0.26$). At an infestation rate of two beetles/seedling, the economic threshold was reached after 95 DD in op canola, 94 DD in hybrid canola and 72 DD in canola mustard.

The effect of feeding damage at each temperature on shoot fresh weight was evaluated in the three canola types (Elliott Fig. 8). Compared to seedlings grown without beetles, damage from striped flea beetles at 5, 10, 15, 20 and 25°C reduced shoot fresh weight by 9, 21, 34, 37 and 53%, respectively, in op canola; by 9, 19, 32, 44 and 52%, respectively, in hybrid canola; and by 5, 6, 25, 45 and 65%, respectively, in canola mustard. The results indicate that striped flea beetle damage at 5, 10 and 15°C has a greater effect on the shoot fresh weight of op and hybrid canola than canola mustard. The opposite was true at 25°C. Damage reduced shoot fresh weight by 65% in canola mustard compared to 52-53% in op and hybrid canola. The impact of striped flea beetle damage on dry matter content also differed among canola types (Elliott Fig. 9). Damage at 5, 10, 15, 20 and 25°C reduced dry matter by 11, 19, 30, 22 and 36%, respectively, in op canola; by 13, 16, 23, 30 and 37%, respectively, in hybrid canola and by 7, 3, 18, 22 and 32%, respectively, in canola mustard. Damage at each temperature had a greater effect on the dry matter content of op and hybrid canola than canola mustard. Damage by striped flea beetles had little effect on the water content of seedlings at 5 and 10°C (Elliott Fig. 10). Damage reduced the water content of seedlings of each canola type by 0.3-0.6% at 15°C, by 1.0-2.2% at 20°C and by 1.7-7.6% at 25°C. Damage by striped flea beetles at 20 and 25°C had a greater effect on the water content of canola mustard (2.2 and 7.6%, respectively) than op canola (1.0 and 1.7%, respectively) and hybrid canola (1.1 and 1.7%, respectively).

Objective 2: Potential origin of seed treatment failures - Elliott

In field trials at AAFC-Saskatoon in 2003-2009, the efficacy of neonicotinoid seed treatments against crucifer flea beetles varied greatly from year to year. In some years, neonicotinoid seed treatments provided excellent protection against flea beetle damage. With this protection, the treatments improved seedling emergence, stand establishment, shoot weight, biomass accumulation and seed yield. However, in other years, the treatments provided poor protection against flea beetle damage and had little or no effect on agronomic performance. Differences in the efficacy of seed treatments from year to year appeared to be related to environmental conditions during germination and seedling emergence. Protection against flea beetle damage

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was better in warm dry years than in cool wet years. Therefore, experiments were conducted to determine the effect of temperature and soil moisture on the efficacy of neonicotinoid seed treatments against crucifer and striped flea beetles.

a) Seed treatment evaluations in hybrid canola and canola mustard - Laboratory trials

Seeds of hybrid canola were treated with Tribune (fungicides only), Gaucho CS FL (400 g imidacloprid plus fungicides), Prosper FX (400 g clothianidin plus fungicides), Helix (200 g thiamethoxam plus fungicides) and Helix XTra (400 g thiamethoxam plus fungicides). Untreated and treated seeds were planted individual in plastic cones containing a soil-less mix. Seeds were grown under dry conditions (2 ml water/cone/day; 20-30% soil moisture) and wet conditions (6 ml water/cone/day; >90% soil moisture) at 20/15°C and corresponding 16 L/8 D photoperiod. After 7 days, the seedlings were transferred into bioassay cages. Each cage contained two rows of five seedlings from a particular seed treatment. Cages were placed in growth chambers set at 10, 20 or 30°C. Seedlings from each treatment were provided with a constant water supply (wet conditions) or watered daily (2 ml water/vial/dry; dry conditions). Twenty crucifer flea beetles that had been starved for 16-18 hours were added to each cage (n = 2 flea beetles/seedling). Flea beetle damage to the cotyledons was assessed after 24, 48 and 72 hours using a 0- to 10-point scale that corresponded to the percentage of the cotyledon surface that was damaged by flea beetles. Mortality of the beetles was assessed after 72 hours. Moribund beetles were placed in water to ascertain if they were dead or alive. The seedlings were harvested at soil level after 72 hours and weighed to determine the shoot fresh weight. Seedlings were dried at 60°C for 5 days to determine the dry matter content. The moisture content of the seedlings was calculated from the fresh and dry weights. The bioassays were replicated four times in 2011 and four times in 2012. Data were analyzed as a split-split plot design with temperature as main plots, seed treatments as subplots and soil moisture as sub subplots. In most instances, temperature, seed treatments and soil moisture had a significant effect ($P \leq 0.001$) on mortality of crucifer flea beetles, feeding damage, shoot weight and water content (Elliot Table 1). However, seed treatment, temperature and/or soil moisture interactions were also significant ($P = 0.05$-0.001) on most variables so seed treatment data from each temperature and moisture regime were analyzed separately.

Mortality of crucifer flea beetles after 72 hours varied significantly depending on the seed treatment, temperature and moisture regime (Elliott Table 2, Fig. 11). Mortality in the untreated check and fungicide check averaged less than 4% at each temperature and moisture regime. Mortality from neonicotinoid seed treatments at 10, 20 and 30 °C averaged 19-50%, 31-85% and 46-96%, respectively, in dry soil and 11-22%, 22-50% and 24-81%, respectively, in wet soil. Mortality from each neonicotinoid seed treatment was higher in dry soil than in wet soil and higher at 20 and 30°C than at 10°C. The results indicated that neonicotinoid seed treatments provide better control of crucifer flea beetles in dry soil than in wet soil and better control at 30°C

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than at 10°C. Helix XTra and Prosper FX or Helix provided the best control of crucifer flea beetles in dry and wet soil at 10, 20 and 30°C.

Crucifer flea beetle damage to the cotyledons after 24, 48 and 72 hours varied depending on the seed treatment, temperature and soil moisture (Elliott Table 3, Fig. 12). Damage in most treatments increased the longer seedlings were exposed to crucifer flea beetles. Damage increased more rapidly in the untreated check and fungicide check than in the neonicotinoid seed treatments. After 72-hour exposure, damage to seedlings grown from untreated seed at 10, 20 and 30°C averaged 5, 14 and 37%, respectively, in dry soil and 5, 12 and 26%, respectively, in wet soil. Seedlings grown from seed treated with only fungicide had similar damage except in dry soil at 30°C when damage was higher. Neonicotinoid seed treatments provided excellent protection against crucifer flea beetles. After 72 hours, damage in the neonicotinoid seed treatments at 10, 20 and 30 °C averaged 3-4%, 4-5% and 4-5%, respectively, in dry soil and 3-4%, 4-5% and 5-9%, respectively, in wet soil. Gaucho CS FL, Prosper FX, Helix and Helix XTra provided similar protection against crucifer flea beetles in dry soil at 10, 20 and 30°C. Prosper FX, followed by Helix XTra and Gaucho CS FL, provided the best protection in wet soil at 10, 20 and 30°C.

The shoot fresh weight of hybrid canola seedlings after exposure to crucifer flea beetles for 72 hours varied depending on soil moisture, temperature and seed treatment (Elliott Table 4, Fig. 13). Shoot weights in all treatments were consistently higher in wet soil than in dry soil and higher at 20 and 30 than at 10 °C. Compared to untreated seed, neonicotinoid seed treatments had no effect on shoot weight in dry and wet soil at 10°C. In dry soil, Gaucho CS FL, Prosper FX, Helix and Helix XTra improved shoot fresh weight by 15-26% at 20°C and by 67-72% at 30°C. In wet soil, Prosper FX, Helix and Helix XTra improved fresh weight by 11-13% at 20°C and by 14-38% at 30°C.

The dry matter content of hybrid canola seedlings varied depending on soil moisture, temperature and seed treatment (Elliott Table 5, Fig. 14). Dry matter content in all treatments was higher in wet soil than in dry soil and higher at 30°C than at 10 or 20°C. Neonicotinoid seed treatments had no effect on dry matter in dry soil at 10°C. Compared to untreated seed, Helix and Helix XTra improved dry matter by 12% in dry soil at 20°C. Gaucho CS FL, Prosper FX, Helix and Helix XTra improved dry matter by 25-42% in dry soil at 30°C. Neonicotinoid seed treatments had no effect on the dry matter content of the seedlings in wet soil at 10 and 20°C. At 30°C, Gaucho CS FL, Prosper FX, Helix and Helix XTra improved dry matter by 14-28% compared to untreated seed.

The water content of hybrid canola seedlings after exposure to crucifer flea beetles for 72 hours varied

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depending on temperature, soil moisture and seed treatment (Elliott Table 6, Fig. 15). Water content of seedlings in all treatments was higher in wet soil than in dry soil. At each moisture regime, water content was highest at 10°C and lowest at 30°C. At 10, 20 and 30°C, the water content of the untreated check averaged 93.8%, 92.9% and 88.8%, respectively, in dry soil and 94.4%, 93.8% and 92.2%, respectively, in wet soil. Neonicotinoid seed treatments had no effect on water content in dry and wet soil at 10°C. Compared to untreated seed, Gaucho CS FL, Prosper FX, Helix and Helix XTra improved water content in dry soil by 0.7-1.0% at 20°C and 2.3-3.1% at 30°C, Gaucho CS FL, Prosper and Helix improved water content by 0.4-0.6% in wet soil at 20°C. Helix and Helix XTra improved water content by 0.3-0.7% in wet soil at 30°C.

Temperature, soil moisture and seed treatments had a significant effect ($P = 0.05$-0.001) on mortality of striped flea beetles, feeding damage and vigour of hybrid canola seedlings (Elliott Table 7). Seed treatment, temperature and soil moisture interactions were significant ($P \le 0.05$) on most variables so seed treatment data from each temperature and moisture level were analyzed separately.

Mortality of striped flea beetles after 72 hours varied depending on the seed treatment, temperature and moisture regime (Elliott Table 8, Fig. 16). Mortality in the untreated and fungicide checks was less than 4% at each temperature and soil moisture. Mortality from neonicotinoid seed treatments was very low. At 10, 20 and 30°C, mortality averaged 6-11%, 5-11% and 22-45%, respectively, in dry soil and 1-6%, 3-12% and 3-12% respectively, in wet soil. Mortality in each neonicotinoid seed treatment was marginally higher in dry soil than in wet soil and higher at 30°C than at 10 and 20°C. The results indicated that neonicotinoid seed treatments provide poor control of striped flea beetles at most conditions. Helix XTra and Prosper FX provided the best control of striped flea beetles in dry and wet soil at 30°C.

Striped flea beetle damage to the cotyledons after 24, 48 and 72 hours varied depending on the seed treatment, temperature and soil moisture (Elliott Table 9, Fig. 17). Damage in most treatments increased over time. Damage increased more rapidly in the untreated and fungicide checks than in the neonicotinoid seed treatments. After 72 hours, damage to seedlings grown from untreated seed at 10, 20 and 30°C averaged 6, 22 and 71%, respectively, in dry soil and 6, 15 and 43%, respectively, in wet soil. Seedlings grown from seed treated with only fungicide had similar damage. Neonicotinoid seed treatments provided better protection against striped flea beetles at 10 and 20°C than at 30°C. After 72 hours, damage with the neonicotinoid seed treatments at 10, 20 and 30°C averaged 4-6%, 9-13% and 13-26%, respectively, in dry soil and 5-6%, 14-16% and 25-40%, respectively, in wet soil. Helix XTra, Prosper FX and Gaucho CS FL provided the best protection against striped flea beetles in dry soil at 10°C (4-5% damage) and 20°C (9-11% damage). Prosper FX and Helix XTra provided the best protection in dry soil at 30°C (13-14% damage) and wet soil at 30°C (25-28% damage).

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The shoot fresh weight of hybrid canola after exposure to striped flea beetles for 72 hours varied depending on soil moisture, temperature and seed treatment (Elliott Table 10, Fig. 18). Shoot weights in all treatments were consistently higher in wet soil than in dry soil and higher at 10 and 20°C than at 30°C. Compared to untreated seed, neonicotinoid seed treatments had no effect on shoot weight in dry and wet soil at 10°C and wet soil at 20°C. Neonicotinoid seed treatments improved shoot weight in dry soil by 11-21% at 20°C and by 130-187% at 30°C. Compared to untreated seed, Gaucho CS FL, Prosper FX and Helix XTra improved shoot weight by 16-26% in wet soil at 30°C.

The dry matter content of seedlings exposed to striped flea beetles for 72 hours varied depending on soil moisture, temperature and seed treatment (Elliott Table 11, Fig. 19). Dry matter content in all treatments was higher in wet soil than in dry soil and higher at 20 and 30°C than at 10°C. Neonicotinoid seed treatments had no effect on dry matter in dry and wet soil at 10 and 20°C. Compared to untreated seed, Helix XTra improved dry matter by 24% in dry soil at 30°C. Prosper FX, Helix and Helix XTra improved dry matter by 14-17% in wet soil at 30°C.

The water content of seedlings exposed to striped flea beetles for 72 hours varied depending on soil moisture, temperature and seed treatment (Table 12, Fig. 20). Water content was higher in wet soil than in dry soil. In dry and wet soil, water content was highest at 10°C and lowest at 30°C. At 10, 20 and 30°C, water content in the untreated check averaged 93.4%, 92.0% and 71.1%, respectively, in dry soil and 94.2%, 93.9% and 90.4%, respectively, in wet soil. Compared to untreated seed, neonicotinoid seed treatments had no effect on water content in wet soil at 10, 20 and 30°C. In dry soil, Prosper FX improved water content by 0.3% at 10°C. Gaucho CS FL, Prosper FX and Helix XTra improved water content by 0.8-1.1% in dry soil at 20°C. Gaucho CS FL, Prosper FX, Helix and Helix XTra improved water content by 18.9-20.1% in dry soil at 30°C.

Laboratory bioassays suggested that seed treatment failures in canola may originate from several sources. Neonicotinoid seed treatments provided substantially better control and protection against crucifer flea beetles than striped flea beetles. Therefore, seed treatment failures are more likely to occur when striped flea beetles are the most abundant species in commercial fields. Neonicotinoid seed treatments also provided better flea beetle control in dry soil than in wet soil. Consequently, seed treatment failures are more likely to occur when above-average rainfall causes saturated soil conditions during germination and seedling emergence. Temperature had little effect on the toxicity of neonicotinoid seed treatments to striped flea beetles. However, higher temperatures resulted in a rapid increase in feeding damage from striped flea beetles. Therefore, seed treatment failures against striped flea beetles are highly probably when saturated soil and high temperatures (20-30°C) occur during germination and seedling emergence. Clearly, new insecticides and seed treatments are needed to control striped flea beetles when these environmental conditions occur in commercial fields.

b) Seed treatment evaluations in hybrid canola and canola mustard – Field trials

Field trials were conducted at AAFC-Saskatoon in 2010-2012 to determine the effect of neonicotinoid seed treatments on flea beetle damage and agronomic performance of hybrid canola and canola mustard in summer fallow and wheat stubble. The treatments included untreated seed and seeds treated with Tribune (fungicides only), Cruiser (200 g and 400 g thiamethoxam), Gaucho CS FL (400 g imidacloprid), Prosper FX (400 g clothianidin), Helix (200 g thiamethoxam) and Helix XTra (400 g thiamethoxam). Cruiser contained only insecticide whereas the last four treatments contained a neonicotinoid insecticide and several fungicides. Seeds were planted in six-row plots at 200 seeds per 6.1 m row, 0.30 m row-spacing and 1.5-2.0 cm depth. Tests in summer fallow were seeded May 16-19 with a four-cone double-disc drill equipped with on-row packers. Tests in wheat stubble were seeded May 17-26 with a six-cone hoe drill equipped with on-row packers. Plots were fertilized based on annual soil-test recommendations for canola production. Each test was replicated four times using a randomized complete block design.

Flea beetle damage to the cotyledons (n = 20 samples/plot) was assessed 13-14, 16-18 and 20-22 days after seeding (DAS) using a 0 to 10-point scale that corresponded to the percentage of the cotyledon surface that was damaged by flea beetles. Agronomic assessments focused on seedling emergence, shoot growth and seed yield. Numbers of seedlings along a centre row of each plot were assessed 13-14, 20-22 and 27-28 DAS. To evaluate shoot growth, 10 seedlings or plants were collected randomly from the outer rows of each plot 13-14, 20-22, 27-29 and 34-36 DAS. Samples were cleaned and weighed to determine shoot fresh weight then dried at 60°C for 3-7 days to assess dry matter content. Shoot biomass was calculated from the number of seedlings per m-row and shoot fresh weight on the four sampling dates. The four centre rows of each plot were swathed and harvested at maturity with a small-plot combine to determine seed yield. This report will focus on flea beetle damage (16-17 and 20-22 DAS), stand establishment (20-22 DAS), shoot fresh weight and shoot biomass (20-22 DAS) and seed yield.

Hybrid canola

Cumulative precipitation during the preceding winter months before planting (Oct. - April) was above-average in 2010 (126 mm) and 2012 (114 mm) and below-average in 2011 (50 mm). Mean air and soil temperatures during the first 21 days after seeding were higher in 2011 (12.3°C and 15.5°C, respectively) than in 2010 (11.6°C and 14.5°C, respectively) and 2012 (12.0°C and 13.6°C, respectively). Soil temperatures during germination and stand establishment ranged from 2.9-37.2°C in 2010, from 6.4-26.7°C in 2011 and from 4.2-28.2°C in 2012. Rainfall during this period was higher in 2010 (130 mm) than in 2011 (24 mm) and 2012 (53 mm).

With cool wet conditions, flea beetle damage to hybrid canola after 16-17 days was below the economic threshold in most tests (Elliott Table 13). Damage varied depending on the year and seed treatment. In 2010, 2011 and 2012, damage in all treatments averaged 3.1%, 10.9% and 19.6%, respectively, in summer fallow and 7.1%, 3.6% and 25.1%, respectively, in wheat stubble. In summer fallow, neonicotinoid seed treatments reduced flea beetle damage in 2010 and 2011 but not in 2012. Prosper FX and low or high rate of Cruiser provided the best protection in 2010. The treatments reduced damage by 3-4% compared to untreated seed. All treatments containing a neonicotinoid insecticide reduced damage by 5-7% in 2011. The high rate of Cruiser, Prosper FX and Helix XTra provided the best flea beetle protection in summer fallow over three test years. The treatments reduced damage by 2-3% compared to untreated seed. In wheat stubble, neonicotinoid seed treatments had no effect on flea beetle damage to hybrid canola after 16-17 days in 2010, 2011 and 2012. Damage exceeded the economic threshold in most treatments in 2012. The low or high rate of Cruiser, Prosper FX and Helix XTra provided the best flea beetle protection in wheat stubble over three test years. The treatments reduced damage by 2-3% compared to untreated seed.

Neonicotinoid seed treatments had limited effect on flea beetle damage to hybrid canola after 20-22 days in summer fallow and wheat stubble (Elliott Table 14). In 2010, 2011 and 2012, overall damage averaged 10.6%, 15.1% and 28.1%, respectively, in summer fallow and 9.6%, 9.4% and 28.8%, respectively, in wheat stubble. Damage over three test years averaged 17.9% in summer fallow and 15.9% in wheat stubble. Neonicotinoid seed treatments had no effect on flea beetle damage to hybrid canola in summer fallow in 2010, 2011 and 2012. Prosper FX and Helix XTra provided the best protection over three test years. The treatments reduced damage by 3-4% compared to untreated seed. In wheat stubble, seed treatments containing a neonicotinoid insecticide reduced damage by 2-3% in 2010 but had no effect on damage in 2011 and 2012. Flea beetle damage exceeded the economic threshold on most treatments in summer fallow and wheat stubble in 2012.

Establishment of hybrid canola after 20-22 days varied depending on the year and seed treatment (Elliott Table 15). In 2010, 2011 and 2012, stand establishment averaged 85%, 80% and 75%, respectively, in summer fallow and 63%, 44% and 51%, respectively, in wheat stubble. Establishment over three test years was 27% higher in summer fallow (80%) than in wheat stubble (53%). Neonicotinoid seed treatments had no effect on the establishment of hybrid canola in summer fallow in 2010 and 2012. In 2011, Gaucho CS FL, Prosper FX and Helix improved establishment by 11-12% compared to untreated seed. Seed treatments containing a neonicotinoid insecticide improved the establishment of hybrid canola in summer fallow by 4-10% over three test years. In

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wheat stubble, neonicotinoid seed treatments improved the establishment of hybrid canola by 10-22% in 2010 but had no effect on establishment in 2011 and 2012. Compared to untreated seed, Prosper FX and Helix XTra improved the establishment of hybrid canola in wheat stubble by 7-9% over three test years.

Neonicotinoid seed treatments had little effect on the shoot fresh weight of hybrid canola in most tests (Elliott Table 16). Shoot weight after 20-22 days in 2010, 2011 and 2012 averaged 203, 659 and 275 mg/plant, respectively, in summer fallow and 126, 376 and 205 mg/plant, respectively, in wheat stubble. Shoot weight over three test years was 1.6 times higher in summer fallow (379 mg/plant) than in wheat stubble (235 mg/plant). Neonicotinoid seed treatments had no effect on the shoot weight of hybrid canola in summer fallow in 2010 and 2012. In 2011, the high rate of Cruiser improved shoot weight 1.4 times compared to untreated seed. Neonicotinoid seed treatments had no effect on the shoot fresh weight of hybrid canola in wheat stubble in 2010, 2011 and 2012.

Neonicotinoid seed treatments had no effect on the shoot biomass of hybrid canola in most tests (Elliott Table 17). Shoot biomass after 20-22 days in 2010, 2011 and 2012 averaged 5.7, 17.4 and 6.8 g/m-row, respectively, in summer fallow and 2.6, 5.4 and 3.5 g/m-row, respectively, in wheat stubble. Shoot weight over three test years was 2.6 times higher in summer fallow (10.0 g/m-row) than in wheat stubble (3.8 g/m-row). Neonicotinoid seed treatments had no effect on the shoot biomass of hybrid canola in summer fallow in 2010 and 2012. The high rate of Cruiser, Gaucho CS FL and Helix improved shoot biomass by 1.4-1.6 times in 2011. The high rate of Cruiser, Helix and Helix XTra improved the shoot biomass of hybrid canola in summer fallow by 1.2-1.3 times over three test years. In wheat stubble, the low rate of Cruiser, Gaucho CS FL, Prosper FX and Helix XTra improved the shoot biomass of hybrid canola by 1.6-2.0 times in 2010. Neonicotinoid seed treatments had no effect on the shoot biomass of hybrid canola in wheat stubble in 2011 and 2012.

Neonicotinoid seed treatments had no effect on the seed yield of hybrid canola in all tests on summer fallow and wheat stubble (Elliott Table 18). Overall yields in 2010, 2011 and 2012 averaged 256, 422 and 284 g/m^2, respectively, in summer fallow and 322, 254 and 275 g/m^2, respectively, in wheat stubble. Seed yield over three test years was 13% higher in summer fallow (320.9 g/m^2) than in wheat stubble (283.5 g/m^2).

Previous investigations at AAFC-Saskatoon have shown that neonicotinoid seed treatments (Gaucho, Prosper, Helix and Helix XTra) provide excellent protection against flea beetle damage when warm dry conditions occur during germination and seedling emergence. With this protection, the treatments improved stand establishment, shoot growth, biomass accumulation and ultimately seed yield. In the current study, above-average precipitation during winter months and 21 days after seeding resulted in elevated moisture levels in

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most tests. Under these conditions, neonicotinoid seed treatments had limited effect on flea beetle damage and agronomic performance of hybrid canola. In tests on summer fallow, neonicotinoid seed treatments had no effect on flea beetle damage after 20-22 days in 2010, 2011 and 2012. Prosper FX and Helix XTra provided the best flea beetle protection over three test years, reducing damage by 3-4% compared to untreated seed. Neonicotinoid seed treatments had no effect on the establishment of hybrid canola in 2010 and 2012 but improved establishment by 11-12% in 2011. The treatments improved the establishment of hybrid canola in summer fallow by 4-10% over three test years. Neonicotinoid seed treatments had no effect on the fresh weight and shoot biomass of hybrid canola in 2010 and 2012 but improved shoot weight and shoot biomass by 1.4-1.6 times in 2011. Over three test years, the high rate of Cruiser, Helix and Helix XTra improved the shoot biomass of hybrid canola after 20-22 days by 1.2-1.3 times over untreated seed. Despite the improvement in biomass, neonicotinoid seed treatments had no effect on the seed yield of hybrid canola in summer fallow in 2010, 2011 and 2012. In wheat stubble, seed treatments containing a neonicotinoid insecticide reduced flea beetle damage after 20-22 days by 2-3% in 2010 but had no effect on damage in 2011 and 2012. The treatments improved the establishment of hybrid canola by 10-22% in 2010 but had no effect on establishment in 2011 and 2012. Compared to untreated seed, Prosper FX and Helix XTra improved the establishment of hybrid canola in wheat stubble by 7-9% over three test years. In most instances, neonicotinoid seed treatments had no effect on the fresh weight, shoot biomass and seed yield of hybrid canola in wheat stubble in 2010, 2011 and 2012. Consequently, field trials in 2010-2012 suggest that more effective seed treatments are needed for protection of hybrid canola against flea beetles when cool wet conditions occur during seedling emergence and stand establishment.

Canola mustard

Flea beetle damage to canola mustard after 15-19 days was below the economic threshold in most tests (Elliott Table 19). Damage varied depending on the year and seed treatment. In 2010, 2011 and 2012, overall damage averaged 6.8%, 12.8% and 24.1%, respectively, in summer fallow and 3.6%, 3.2% and 16.3%, respectively, in wheat stubble. Neonicotinoid seed treatments had no effect on flea beetle damage to canola mustard in summer fallow in 2010 and 2012. However, the treatments reduced damage by 9-13% in 2011. The low rate of Cruiser and Helix XTra provided the best protection in 2011. Over three test years, neonicotinoid seed treatments reduced flea beetle damage to canola mustard in summer fallow by 5-7%. In wheat stubble, neonicotinoid seed treatments had no effect on flea beetle damage after 15-19 days in 2010, 2011 and 2012.

Neonicotinoid seed treatments had no effect on flea beetle damage to canola mustard after 20-21 days in most tests (Elliott Table 20). In 2010, 2011 and 2012, overall damage averaged 12.9%, 23.9% and 64.4%, respectively, in summer fallow and 5.2%, 7.7% and 23.0%, respectively, in wheat stubble. Damage over three test years

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averaged 33.7% in summer fallow and 12.0% in wheat stubble. Neonicotinoid seed treatments had no effect on flea beetle damage to canola mustard in summer fallow in 2010 and 2011. Damage was exceptionally high in 2012, ranging from 58% in the untreated check to 72% in the Helix XTra treatment. In wheat stubble, neonicotinoid seed treatments had no effect on flea beetle damage to canola mustard after 20-21 days in 2010, 2011 and 2012.

Establishment of canola mustard after 20-21 days varied greatly depending on the year and seed treatment (Elliott Table 21). Stand establishment in 2010, 2011 and 2012 averaged 76%, 71% and 37%, respectively, in summer fallow and 48%, 28% and 24%, respectively, in wheat stubble. Establishment over three test years was 28% higher in summer fallow (61.6%) than in wheat stubble (33.6%). Compared to untreated seed, neonicotinoid seed treatments improved the establishment of canola mustard in summer fallow by 7-15% in 2010 and by 6-14% in 2011. The high rate of Cruiser, Prosper FX and Helix provided the best stand establishment in 2010. Helix provided the best establishment in 2011. Prosper FX and Helix improved the establishment of canola mustard in summer fallow by 5% over three test years. With the exception of the low rate of Cruiser, neonicotinoid seed treatments improved the establishment of canola mustard in wheat stubble by 10-15% in 2010. Neonictoinoid seed treatments had no effect on establishment in 2011 and 2012.

The shoot fresh weight of canola mustard varied from year to year (Elliott Table 22). Shoot weights after 20-21 days in 2010, 2011 and 2012 averaged 98, 469 and 84 mg/plant, respectively, in summer fallow and 108, 302 and 99 mg/plant, respectively, in wheat stubble. Shoot weight over three test years was 1.2-1.3 times higher in summer fallow (217 mg/plant) than in wheat stubble (170 mg/plant). Neonicotinoid seed treatments had no effect on the shoot weight of canola mustard in every test on summer fallow and wheat stubble.

The shoot biomass of canola mustard varied yearly (Elliott Table 23). Biomass after 20-21 days in 2010, 2011 and 2012 averaged 2.5, 10.9 and 1.0 g/m-row, respectively, in summer fallow and 1.7, 2.7 and 0.8 g/m-row, respectively, in wheat stubble. Shoot biomass over three test years was 2.8 times higher in summer fallow (4.8 g/m-row) than in wheat stubble (1.7 g/m-row). Neonicotinoid seed treatments had no effect on the shoot biomass of canola mustard in all tests.

Seed yield of canola mustard varied yearly (Elliott Table 24). In 2010, 2011 and 2012, yields averaged 253, 372 and 259 g/m^2, respectively, in summer fallow and 305, 304 and 293 g/m^2, respectively, in wheat stubble. Seed yield over three test years was similar in summer fallow (295 g/m^2) and wheat stubble (301 g/m^2). Neonicotinoid seed treatments had no effect on seed yield in every test.

Field tests in summer fallow and wheat stubble in 2010-2012 suggest that cool wet conditions during seedling emergence reduced the efficacy of neonicotinoid seed treatments against flea beetles in canola mustard. The treatments had no effect on flea beetle damage after 20-21 days in summer fallow and wheat stubble in 2010, 2011 and 2012. Flea beetle damage exceeded 55% in all neonicotinoid seed treatments in summer fallow in 2012. Neonicotinoid seed treatments improved the establishment of canola mustard in some tests but not in others. In summer fallow, the treatments improved stand establishment by 7-15% in 2010 and by 6-14% in 2011. Prosper FX and Helix improved the establishment of canola mustard in summer fallow by 5% over three test years. In wheat stubble, seed treatments containing a neonicotinoid insecticide improved the establishment of canola mustard by 10-15% in 2010 but had no effect on establishment in 2011 and 2012. In all tests on summer fallow and wheat stubble, neonicotinoid seed treatments had no effect on the shoot weight, shoot biomass and seed yield of canola mustard. Clearly, more efficacious seed treatments are needed to protect canola mustard from flea beetles when cool wet conditions occur during stand establishment.

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