Quantification of flea beetle injury Phyllotreta cruciferae (Coleoptera: Chrysomelidae) on canola in Montana
by Elizabeth Lee Nance

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Entomology
Montana State University
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Abstract:
Canola, an oilseed crop, is a potential alternative to small grain production in Montana. A major deterrent to the success of the crop is the crucifer flea beetle, Phyllotreta cruciferae (Goeze), which feeds on the emerging seedlings causing direct and indirect damage that results in seedling mortality, reduced number of surviving plants, and delayed plant development. All contribute to reduced yield. Field and greenhouse studies were designed to quantify losses by this pest species. The objectives of field studies were 1) determine the early plant growth stage that is most vulnerable to flea beetle attack in terms of final pod yield, and 2) determine how many beetles cause the yield loss. Greenhouse objectives were 1) corroborate field experiment results and 2) simulate flea beetle feeding by mechanically defoliating canola plants to determine effects on final pod yield. In the field studies, canola was seeded in a split plot, completely randomized design in a factorial arrangement.

Yield, assessed as total numbers of pods per plants, and biomass dry weights were measured after plants were subjected to four levels of flea beetle density feeding through three canola growth stages at two planting dates. Of the three growth stages tested (cotyledon, first true leaf and rosette), the cotyledon growth stage was most susceptible to herbivory by flea beetles. One beetle feeding per plant was sufficient to cause a significant decrease in yield. Mean yield per plant reached a maximum of 971 pods for control plants. When plants were fed on by one, two and five beetles per plant, yield decreased in a significant linear fashion from 542, 396 and 233 pods per plant respectively. Results from the same study performed in the greenhouse corroborated the field study for the main effect of flea beetle density. One flea beetle feeding per plant was sufficient to cause a yield reduction. However, there was a significant decrease in yield for the growth stages cotyledon and true leaf, compared to rosette, in the greenhouse which was not detected in the field. Greenhouse studies were performed that simulated flea beetle feeding by mechanically defoliating canola cotyledons. Canola was seeded in pots in a completely randomized design in a factorial arrangement. Results indicated that seedlings can lose as little as 25 % of their surface area and show a significant yield decrease. Yield was further reduced when mechanical damage to seedlings was performed in combination with meristematic damage.

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ABSTRACT

Canola, an oilseed crop, is a potential alternative to small grain production in Montana. A major deterrent to the success of the crop is the crucifer flea beetle, Phyllotreta cruciferae (Goeze), which feeds on the emerging seedlings causing direct and indirect damage that results in seedling mortality, reduced number of surviving plants, and delayed plant development. All contribute to reduced yield. Field and greenhouse studies were designed to quantify losses by this pest species. The objectives of field studies were 1) determine the early plant growth stage that is most vulnerable to flea beetle attack in terms of final pod yield, and 2) determine how many beetles cause the yield loss. Greenhouse objectives were 1) corroborate field experiment results and 2) simulate flea beetle feeding by mechanically defoliating canola plants to determine effects on final pod yield. In the field studies, canola was seeded in a split plot, completely randomized design in a factorial arrangement. Yield, assessed as total numbers of pods per plants, and biomass dry weights were measured after plants were subjected to four levels of flea beetle density feeding through three canola growth stages at two planting dates. Of the three growth stages tested (cotyledon, first true leaf and rosette), the cotyledon growth stage was most susceptible to herbivory by flea beetles. One beetle feeding per plant was sufficient to cause a significant decrease in yield. Mean yield per plant reached a maximum of 971 pods for control plants. When plants were fed on by one, two and five beetles per plant, yield decreased in a significant linear fashion from 542, 396 and 233 pods per plant respectively. Results from the same study performed in the greenhouse corroborated the field study for the main effect of flea beetle density. One flea beetle feeding per plant was sufficient to cause a yield reduction. However, there was a significant decrease in yield for the growth stages cotyledon and true leaf, compared to rosette, in the greenhouse which was not detected in the field. Greenhouse studies were performed that simulated flea beetle feeding by mechanically defoliating canola cotyledons. Canola was seeded in pots in a completely randomized design in a factorial arrangement. Results indicated that seedlings can lose as little as 25% of their surface area and show a significant yield decrease. Yield was further reduced when mechanical damage to seedlings was performed in combination with meristematic damage.

CHAPTER 1

INTRODUCTION

Approximately 6 million acres of wheat and barley are seeded annually in Montana (MT Ag Statistics 1993). Farmers have encountered some problems by exclusively growing small grains. For example, weeds can build up to persistent levels, insect life cycles can correspond to established production schemes and pesticides can lose their efficacy through repeated use. Rotating to another crop can modify problems associated with continuous cropping. To be successful, an alternative crop should have similar requirements as small grains. Requirements needed for small grain production are similar to those needed for production of an oilseed crop, canola, Brassica napus L.

Canola has many advantages that make it an acceptable alternative crop. It allows for more diversity in dry-land or irrigated systems; it breaks insect and disease cycles associated with continuous cultivation of a single crop; and it utilizes the same farm machinery used for grain production. Canola has similar fertilizer requirements as small grains and has a net dollar return equivalent to malting barley.

Under the 1985 Food, Agriculture, Conservation and Trade Act (Farm Bill), Montana farmers were paid not to grow small grains on a percentage of their acreage. They could plant a certain percentage of base acres to grains, but to be eligible for federal subsidies, could not grow anything of marketable value on acres that were set-

aside. However, due to the minor oilseeds provision in the 1990 Farm Bill, canola could be grown on those acres.

Canola acreage in North America is expanding. Canadian acreage has risen steadily since 1981 so that it is now the second largest crop produced behind wheat. As of 1992, Canadian acreage represented approximately 16% of the world market. Canada produced 10.3 million acres in 1993 and estimates 12.4 million acres in 1994 (Nick Underwood, Canola Council of Canada, personal communication). In contrast to Canada, U.S. production is minor. It was only because of the 1990 Farm Bill that the U.S. Department of Agriculture began collecting statistics on canola. U.S.D.A. production estimates began in 1991 with 155,000 acres. The same acreage was reported in 1992, and increased to 199,000 acres in 1993. Because the crop is relatively new to Montana, little production data are available. The U.S. Canola Association has been compiling acreage estimates by state since 1989. Acreage estimates for Montana began in 1989 with 10,000 acres and rose to approximately 30,000 in 1993.

Of all the vegetable oils on the market, canola oil is one of the highest in unsaturated fats, the lowest in saturated fats and has no cholesterol. Its use as a salad oil, cooking oil and margarine is growing in popularity among health conscious consumers. For these reasons, it is anticipated that U.S. canola oil demand will reach one million tons by 1995, which could require up to eight million acres of production, most of which will be contributed by Canadian farmers (Cash & Baquet 1993).

Despite its many advantages, Montana farmers may be reluctant to rotate

canola into their small grain production systems. Because it is a relatively new crop in the U.S., there is not yet an effective data base in place for crop management. Furthermore, there is little known about the pest complex that attacks the crop. Johnson et al. (1993) surveyed the state during the summer of 1991 and found the crucifer flea beetle, Phyllotreta cruciferae (Goeze), to be the most threatening insect pest to canola production.

The following studies represent an effort to understand canola crop responses due to crucifer flea beetle feeding. Studies were conducted in the field and in the greenhouse. The objectives of the field study were 1) to determine, under constant flea beetle feeding pressure, the early plant growth stage that is most vulnerable to flea beetle attack in terms of final pod yield, and 2) to determine how many beetles cause the yield loss. The objectives of the greenhouse studies were to 1) corroborate the field experiment and to 2) simulate flea beetle feeding by mechanically defoliating canola plants in experiments to determine effects on final pod yield.

CHAPTER 2

REVIEW OF LITERATURE

Canola

Canola is a generic name for all edible rapeseed. When crushed, the seed yields two products: oil and rapeseed meal. Rapeseed oil was originally used as an illuminating oil in Asian countries 3000-4000 years ago (Boulter 1983), but has only reached economic importance during the last 50 years. Its industrial applications include use as a rubber additive, a marine lubricant and a hardening agent in the paint and plastic industries (Ohlson 1979). Rapeseed meal has been used as an organic fertilizer (Lamb 1989), and as a high protein feed supplement for livestock and poultry in Canada and Europe (Downey 1983).

Both agriculture and the food industries interchange the use of the words rapeseed and canola. In North America, rapeseed in general refers to the seeds of two plants in the family Cruciferae: common, or Argentinean rapeseed, Brassica napus (L.) and turnip, or Polish rapeseed, B. campestris (L.). Both are the source of winter and spring varieties as well as edible and industrial varieties.

Prior to the development of canola varieties, rapeseed contained two major components, erucic acid and glucosinolates, that restricted its use as an edible oil or meal (Sharp et al. 1990). The expanded use of the crop, and the consequent increase in production, occurred when Canadian plant breeders minimized these two

compounds in the seed (Anstey 1986).

Erucic acid is a long chain fatty acid ($C_{22:1}$) which comprises from 1% to 60% of the total fatty acids occurring in the seed oil (Thompson 1980). Oils rich in erucic acid have been shown to cause myocardial lipidosis and necrosis in experimental animals when fed at a large proportion of the diet (Sauer & Kramer 1983).

Glucosinolates are sulphur containing antinutritional compounds which impart the characteristic tangy flavor in condiment mustards (Salunkhe et al. 1992). When plant tissues are crushed, glucosinolates are hydrolysed by the enzyme myrosinase to yield glucose and isothiocyanate (mustard oil) as the major product (Underhill 1980). The presence of myrosinase is responsible for the undesirable properties of rapeseed meal and the products formed by the enzymatic reaction, thiocyanate ions, exhibit the harmful effects that have been implicated in several physiological disorders in animals (Salunkhe 1992).

The term canola now identifies new quality characteristics of edible rapeseed that enable the oil to be used for human consumption ($\leq$ 2% erucic acid) and the meal as a protein supplement for animal feed ($\leq$ 30 μm of glucosinolates per gram of meal). Erucic acid at this level is classified as GRAS (generally recognized as safe) by the FDA, which makes it marketable for human consumption.

Flea beetles

Historical

The crucifer flea beetle, Phyllotreta cruciferae (Goeze) (Coleoptera:Chrysomelidae), is an introduced pest species from Eurasia which was first collected at Agassiz, British Columbia in 1923 (Milliron 1953). By the 1950's, it achieved economic status in Quebec and Ontario suggesting a rapid eastward spread (Goble 1965). In the late 1970's, Burgess (1977) reported the species as a common and serious pest on oilseed rape through Manitoba, Saskatchewan and south and central Alberta. Of the five principle species of flea beetles found on rape, P. cruciferae is the most numerous (Osgood 1975). Weiss et al. (1991) report it to be a severe threat to rape production areas of the northern Great Plains.

Description of life stages (Westdal & Romanow 1972)

Egg

Eggs are laid singly, or in groups of three or four in moist soil near the roots of the host plant. The egg is smooth, yellow, elongate oval, up to 0.5 mm long and requires an average of 12 days for incubation.

Larva

There are 3 larval instars. The newly-emerged larva is dirty-white with the head and anal plate turning brown soon after emergence. The third instar has an active feeding stage and a quiescent prepupal stage. In rearing experiments, Westdal & Romanov (1972) occasionally found P. cruciferae larvae which had burrowed into the host plant near the juncture of the root and stem. The larval period, including the

prepupal stage, can range from 25 to 34 days.

Prepupa

This stage begins when the third instar larva stops feeding. The larva thickens and shortens in earthen cells where it pupates in 3-6 days.

Pupa

Completion of the pupal stage occurs in 7-9 days. Newly emerged adults are white and remain in the soil for 1-2 days until the cuticle has sclerotized to the blue-black adult coloration.

Adult

The adults are black with a blue-black sheen, elongate oval and approximately 2-3 mm long. The elytra are densely punctate with no distinct patterns; the punctation is finer and less pronounced on the head, thorax and ventral surface of the body than on the elytra.

Flea beetles have enlarged femora on the hind legs enabling them to jump quickly when disturbed. They have been reported as univoltine in Manitoba, Saskatchewan (Burgess 1977), and in New York state (Feeny et al. 1970).

Biology

In late April to early May, P. cruciferae adults emerge from overwintering sites and begin feeding on volunteer rape or cruciferous weed seedlings. Burgess (1977) noted two ways in which flea beetles move into newly-emerged rape crops. Movement took the form of a creeping infestation, moving from plant to plant from

adjacent overwintering sites, or a more rapid and even infestation involving flight, especially in hot, sunny weather.

Females oviposit in moist soil under the dry surface around host plants (Westdal & Romanov 1972). Adults begin to die off in late June, and larvae begin emerging from the soil in early August and feed on green tissues of any cruciferous plant. By mid October, the adults enter diapause under leaf litter, hedgerows and woodlands until their re-appearance the following spring.

Host preference

Bonnemaison (1965) found the genus Phyllotreta to be specific to Cruciferae, while Feeny et al. (1970) found it restricted among the plant families they tested to broccoli and black mustard (Cruciferae), clammy weed and spider plant (Capparidaceae), nasturtium (Tropaeolaceae) and meadow-foam (Limnanthaceae). In addition to canola and garden crucifers, P. cruciferae also feeds on weed species such as wild mustard (Sinapis arvensis L.), horseradish (Armoracia rusticana (Lam.) Gaertn., Mey. & Scherb.), flixweed (Descurainia sophia (L.) Webb), field pennycress (Thlaspi arvense L.), and lamb's-quarters (Chenopodium album L.).

Putnam (1977) studied the susceptibility of four brassica species to attack by flea beetles; Brassica campestris, B. napus, B. juncea (Oriental mustard), and B. hirta (= S. alba, yellow mustard). Of the four, only B. hirta suffered the least flea beetle damage, which suggests preference within the Cruciferae. Palaniswamy and Lamb (1992b) also concluded that P. cruciferae show host preferences in choice and no-choice tests among nine species they tested in the family Cruciferae.

Plant damage/symptoms

Flea beetle adults feed on the cotyledons and first true leaves of the newly emerging plants (Burgess 1977). Damaged plants typically have a "shot-holed" appearance due to tissue desiccation surrounding feeding pits. Losses from flea beetle feeding includes seedling mortality (Burgess 1977, Lamb 1984), reduced number of surviving plants (Lamb & Turnock 1982, Bracken & Bucher 1986), and delayed plant development (Putnam 1977). All contribute to reduced yield. The level of flea beetle infestation has been found to increase with hot, dry weather, that is also associated with poor plant growth (Lamb & Turnock 1982). Feeding by newly emerged adults does not usually cause a yield loss, unless beetles congregate in late-planted fields and feed on the pods and stems (Lamb & Turnock 1982).

The effects of larval feeding in roots on yield remains to be accurately quantified. In the laboratory, evidence of feeding by second- and third-instar larvae was observed on both the root hairs and tap root of crucifer seedlings (Westdal & Romanow 1972). Bracken and Bucher (1986) estimated a 5% yield loss at larval densities of $0.16/cm^2$ though they felt their larval numbers to be underestimated.

Host plant biochemistry

Cruciferous plants are thought to have evolved glucosinolates as primary defensive chemicals against herbivory; insect specialists such as P. cruciferae have been able to overcome this evolutionary barrier by detoxifying the glucosinolates and then using them as long distance olfactory cues in the identification of their hosts (Feeny 1977).

In the mustard plant, B. juncea, and the broccoli plant, B. oleraceae, the major glucosinolate is allyl-glucosinolate (sinigrin) which breaks down to allyl-isothiocyanate. Allyl-isothiocyanate has been shown to be a powerful attractant for P. cruciferae (Burgess & Weins 1980), but it is not found in either species of canola (Sang et al. 1984).

In canola, B. napus, the major glucosinolate is 3-indolylmethyl glucosinolate (glucobrassicin) which has no volatile breakdown products. It is presumed that other secondary plant compounds produced by canola serve as attractants and feeding stimulants, though they have not yet been identified (Lamb 1989).

Myrosinase, the enzyme responsible for the hydrolysis of these glucosinolates, is compartmentalized within cruciferous plants so that the reaction occurs mainly when plant tissues are bruised or damaged (Underhill 1980). However, very small quantities of glucosinolates are released during normal plant growth (Cole 1980).

Although they presented no data to verify their hypothesis, Feeny et al. (1970), suspected that injury to the leaves of cruciferous plants increased their attractiveness to P. cruciferae . If flea beetles are attracted to allyl-isothiocyanate, and uninjured plants release only small quantities during plant development, injury may increase the plants' attractiveness to flea beetles. However, Peng et al. (1992) and Anderson et al. (1992) detected no difference in the amount of flea beetle feeding in choice tests between damaged and undamaged canola seedlings. Their results verify Lamb's hypothesis that some other compound must be responsible for the attractancy (Lamb 1989).

To determine which were more or less susceptible to flea beetles, Lamb (1988) tested 16 cultivars which had high and low levels of glucosinolates and erucic acid in their seed. He found no difference in susceptibility to damage by flea beetles between the older rapeseed cultivars and the newer, canola cultivars. He concluded that reduced glucosinolate and erucic acid levels in canola seed have not affected the susceptibility of the crop to flea beetle damage. These findings however, were based on the glucosinolate levels in the seeds, which are not eaten by flea beetles.

When the glucosinolates were minimized in the transformation of oilseed rape into canola (Daun 1983), it was thought that this reduction in the amount of glucosinolates might make the plants less desirable to flea beetles. Using a sulphur restriction method, Bodnaryk and Palaniswamy (1990) were able to remove foliar glucosinolates in canola (B. napus cv. Westar) seedlings and yet still found that flea beetles fed on the glucosinolate free leaves. They concluded a glucosinolate feeding stimulant is not essential for P. cruciferae and that a glucosinolate-derived mustard oil attractant also appears unnecessary for feeding to occur. Brassica napus cv. Westar is eaten by P. cruciferae because it contains no deterrent to feeding, indicating that elimination of foliar glucosinolates is probably not a useful control strategy.

Peng and Weiss (1992) offer a new mechanism to explain the attractancy of flea beetles to oilseed rape. They suggest a flea beetle-produced aggregation pheromone based upon: 1) canola damaged mechanically, by P. cruciferae, or by diamondback moth, Plutella xylostella (L.), did not attract P. cruciferae; 2) contact with the plants or feeding was required for the production of aggregation pheromone

because canola alone was not attractive when separated from flea beetles by a screen; and 3) equal numbers of males and females were attracted. Their findings suggest the possibility of a synthetic pheromone to be used as a flea beetle lure for population monitoring and for subsequent control in combination with pesticides.

Canola resistance to flea beetles

Antixenosis (non-preference) and tolerance are the two mechanisms of resistance in seedlings of yellow mustard, Sinapis alba L., that account for the high level of resistance this plant shows to flea beetle damage in the field (Bodnaryk and Lamb 1991). Bodnaryk (1991) subsequently identified sinalbin as the glucosinolate responsible for this resistance. Current research to hybridize B. napus and S. alba via embryo rescue is underway (Ripley & Arnison 1990).

Palaniswamy and Lamb (1992a) devised an efficient laboratory screening method for antibiosis resistance but found no significance among the 19 cultivars of B. napus L. and B. campestris L. they tested. Bodnaryk (1992) found that the wax layer on leaves of brassicas provides significant, but incomplete resistance to flea beetles by affecting the rate and pattern of their feeding. Conventional breeding methods or genetic engineering may enhance this form of resistance in canola.

Insecticidal management

In Canada, insecticides have been widely used to manage adult flea beetles (Kinoshita et al. 1978). Application methods include seed treatments, granules applied at planting, or post-emergence foliar sprays (Lamb & Turnock 1982). In-

furrow treatments of systemic insecticides are preferred because they protect the crop during the first two weeks after seedling emergence when damage is highest and plants are most susceptible (Lamb 1984).

Due to increasing environmental concerns caused by soil-applied systemic insecticides, Weiss et al. (1991) evaluated five foliar insecticides in the laboratory. They found carbaryl to be the most effective followed by permethrin, esfenvalerate, endosulfan and malathion. These compared favorably with in-furrow field treatments when flea beetle feeding was low in combination with cool temperatures during flowering. However, in years with high flea beetle stress, carbaryl as a foliar insecticide did not provide adequate control.

In the U.S., the general lack of knowledge of flea beetles causing economic damage is coupled with a lack of registered insecticides to suppress potential outbreaks. There are no current means of effective control, either insecticidal, cultural or biological. A crisis exemption (Section 18 FIFRA, amended) can be issued by the Environmental Protection Agency or by state regulatory agencies for an insecticide with established tolerances.

Economic thresholds

Conventional approaches that estimate yield loss due to a single pest attack include establishment of artificial populations and damage simulation (Poston et al. 1983). Artificial populations help in determining injury per individual pest and damage simulation allows for precise control of the degree of injury to assess crop loss. Both techniques are useful in providing the data necessary to calculate a direct

index of injury for an economic injury level.

Stern et al. (1959) set forth the terminology defining the decision process that determines how many insects cause significant damage. They defined economic damage as "the amount of injury which will justify the cost of artificial control measures" and the economic injury level (EIL) as "the lowest population density that will cause economic damage." The economic threshold (ET) was defined as "the density at which control measures should be initiated to prevent an increasing pest population from reaching the economic injury level."

To date, the extensive literature that documents flea beetles causing economic damage in Canada (Putnam 1977, Lamb 1984, Bracken and Bucher 1986, Lamb 1987) makes no mention of developing Sterns' concepts. Augustin et al. (1986) did provide an "action threshold" for Phyllotreta spp. attacking rapeseed in Finland. According to Pedigo et al. (1986), the use terminology such as "action threshold" or "control threshold" is little more than semantic wheel-spinning by authors in an attempt to correct deficiencies in the original definitions of ET and EIL. They feel that terminology such as "action threshold" is used to indicate entirely subjective levels for control that do not relate to an EIL. Therefore, the action threshold of one beetle per seedling provided by Augustin et al. (1986) is questionable in light of the definitions by Stern et al. (1959).

This becomes even more questionable when considering the factors necessary for developing an EIL. According to Pedigo et al. (1986), the concept has been applied primarily where management tactics are responsive rather than preventative.

Consequently, EILs have mostly been developed for occasional and perennial pests where scouting, evaluation, and subsequent therapy are possible (Stern et al. 1959).

To date, a definitive scouting and evaluation program for flea beetles attacking canola remain elusive. In the United States, this is compounded with the lack of effective insecticides needed to provide control in the event that a population reaches an economic level. However, quantification of these losses could be useful in providing a foundation for eventual pesticide registration and the development of realistic economic injury levels. It is with this in mind, that my research objectives were formulated.

CHAPTER 3

MATERIALS AND METHODS

Field Threshold Study

Study Site

Field plots were established on May 6 and May 20 at the Montana State University Horticultural Farm. The experiments were performed on a summer fallow site that had previously been planted with winter wheat. Prior to implementation, boll weevil traps (PMS Division, Story Chemical Corp.) baited with ally-isothiocyanate (Sigma Chemical Co., St. Louis, MO) were placed at ten random points at the farm to determine if there was an adequate flea beetle population. Trapping began on April 16 and beetles started appearing on April 22 with an abundant population present by the end of the month.

Soil samples were taken for analysis to ascertain fertilizer requirements. Afterwards, the field was shallow tilled and rolled to establish a firm seed bed. Trifluron, (DowElanco, Indianapolis, IN), a broadleaf herbicide, was broadcast at 8.40 kg/ha and ammonium sulfate (21-0-0-24; N-P-K-S) was broadcast at 140 kg/ha and incorporated into the top two inches of soil with a hand rake.

Experimental Design

The field experimental design was a split plot, completely randomized design

in a factorial arrangement. The factors consisted of two planting dates, four flea beetle densities and three canola plant growth stages. The two planting dates were treated as main plots with flea beetle density and plant growth stage as sub-plots. The 6.10 x 21.33 m blocks were staked out side by side separated by a 1.82 m buffer.

Treatments

Twelve treatments with ten replicates each were represented in both planting dates. Individual treatments consisted of numbers of flea beetles per cage: 0, 1, 2 and 5, and canola plant growth stages: cotyledon, first true leaf and rosette. Each treatment was randomly assigned to a cage prior to the start of the experiment. Flea beetles were introduced into the cages at the same time and allowed to feed until the completion of the growth stage to which they were assigned.

Confinement cages were constructed from 15.24 x 45.72 cm strips of hardware cloth formed into cylinders and secured with industrial staples. Organdy cloth was stapled to the outside of the cylinder to prevent beetles from escaping and still allow air movement through the cage. The top of the cage was formed by applying a bead of silicon to the inside edge of a 150 x 15 mm plastic petri dish and attaching it to one end of the cylinder. A hole was punched through the petri dish with a heated 7 mm diameter copper tube which allowed access to thin canola plants and introduce flea beetles.

Canola (B. napus) var.#IMC-01, treated with benomyl and captan (E.I. du Pont, Wilmington, DE), was drilled into prepared plots using a plot drill (Hege Co., Colwich, KS). Prior to seeding, germination was checked and found to be 99%. For

each planting date, twenty 21.33 m rows were planted on 30.48 cm centers at 7.84 kg/ha. Three days after seeding, the confinement cages were placed at 120 random locations in the center five rows of a plot, 24 cages per row, and secured with wire stakes. Washed quartzite sand was applied around the outside of the cages to prevent either confined flea beetles from escaping or beetles from the natural population from entering the cages. Once cages were secured in this manner, they were not moved until their respective treatment had ended.

Treatment Techniques

Canola seedlings began to emerge into the secured cages approximately seven days after planting. At that time, plants were mechanically thinned through the hole in the top of the cage using a stiff wire rod. A single canola seedling was left in the center of each cage for the study. The experiment was initiated 24 hours after seedlings formed their cotyledons and had been thinned to one per cage.

Unsexed P. cruciferae adults were captured on site from oilseed rape using a hand aspirator. Beetles were introduced into the cages through entrance holes which were covered with a piece of laboratory tape to prevent escape. They were allowed to feed until the plants in the buffer rows reached the first true leaf stage, approximately seven days. At that time, the 40 cages representing the four flea beetle densities feeding through the cotyledon stage were removed and the canola seedlings were flagged and sprayed with carbaryl, (Rhone-Poulenc, Research Triangle Park, NC) at 1.16 l/ha to prevent further injury. Plants were sprayed with carbaryl as necessary, usually once every week to two weeks after cages were removed if

herbivory persisted.

Seven to eight days later, when plants in the buffer rows grew their second set of true leaves, the next group of 40 cages representing flea beetle feeding through the first true leaf stage were removed and plants were flagged and sprayed. An experiment ended when the plants in the buffer rows reached the rosette stage. The last group of 40 cages representing flea beetle feeding through the rosette stage were removed, and those plants flagged and sprayed to prevent further feeding by the natural flea beetle population.

During the course of the experiment, some cages were lost due to either inclement weather, or flea beetle numbers fluctuating inside the cages. For example, a wind storm swept through the first planting date on day eight after planting, uprooting cages and exposing the plants to flea beetle feeding. Cages were checked daily to ensure that the number of flea beetles per cage was consistent with the beetle number that was intended for a particular treatment. A cage was discarded if, for example, a cage was supposed to have two beetles per cage, but increased to five, or if as a control cage, it was found to be contaminated with beetles. As a result, the experiment had unequal numbers of observations per treatment.

All experimental plants were left to grow until maturity in the 6.10 x 21.33 m plots. No attempt was made to thin the canola plants around each caged plant though there were more gaps in the rows where the cages were placed than would be found in a non-experimental canola field. Plots were irrigated twice during the growing season.

Individual plants were harvested when approximately 25% of the seeds in pods on the middle third of the main stem started to change color from green to brown. However, on August 23, a light snowfall together with a killing frost occurred, making it impossible to determine seed test weight. Plants were cut at the soil line, tagged and hung upside down to dry. Yield was assessed by removing all of the pods from the dried plants and recording total numbers of pods. Both the canola pods and the remaining plant biomass were oven dried in a 49°C drying oven for one week and then weighed.

Data analysis

A preliminary analysis of variance allowed examination of main effects and interaction effects. Because the data set failed to meet the requirements for balance, the GLMODEL procedure of MSUSTAT 5.22 (Lund 1994) was performed. The original model tested was a fixed factor model with three main effects; planting date (PD), plant growth stage (GS) and flea beetle density (FB), three first-order interactions; PD x GS, PD x FB and GS x FB, and a second-order interaction, PD x GS x FB. The model was reduced to an additive model (PD + GS + FB) because there were no three or two-factor interactions. Furthermore, only main effects due to flea beetle density were present, $P < 0.001$.

The response to flea beetle density was determined by regressing the dependent variable yield (total pods per plant) on the independent variable, flea beetle density, using a polynomial model. Contrasts to detect the level of the polynomial needed to explain the flea beetle response were utilized to compare the group means

and found to be linear, $p = 0.0001$ and quadratic, $p = 0.0001$.

Mechanical Damage Studies

Study site

Two greenhouse studies on foliar mechanical damage were performed at the Montana State University Plant Growth Center. For both studies, one hundred and twenty 19.05 cm pots were filled with M.S.U. soil mix (1/3 washed concrete sand, 1/3 Canadian sphagnum peat moss, 1/3 Bozeman silt loam topsoil) and arranged on a 1.83 x 7.32m greenhouse bench in 15 rows of eight pots each. Pots were watered and each planted with four B. napus canola seeds (var.#IMC-01). Plants were maintained in the greenhouse at 18-22°C, 40-80% RH and a 15:9 (L:D) photoperiod. Plants were watered as needed and fertilized at seven and 14 days with 15.98g/l 20-10-20 (N-P-K) Peatlite (Peters Fertilizer Products, W.R. Grace, Fogelsville, PA) diluted 1:16 and once with 0.15g/l 21-0-0-24 (N-P-K-NH_4SO_4), diluted 1:16 at day seven. Experimental treatments were initiated 24 hours after plant emergence.

Experimental Design

The first experiment studied the effect of four foliar mechanical damage treatments imposed at two separate times, on the response of canola seedlings. The experiment was a completely randomized design in a factorial arrangement. There were 15 replicates per treatment. The second experiment was a repeat of the first experiment, but in addition to the foliar damage, the meristems of the seedlings were

damaged.

Treatments

The treatments in the first experiment consisted of four levels of mechanical damage to the cotyledon surface area: 0%, 25%, 50% and 75% of the surface area removed with microdissecting scissors. All 120 pots were seeded at the same time with treatments imposed at two separate times to two separate groups of seedlings. Twenty-four hours after seedlings emerged, treatments were imposed to half of the experimental units. The remaining experimental units were left untreated to grow for four more days. At that time, the treatments were imposed on the remaining half of the experimental units. All four seedlings in each pot received the same treatment. Pots were randomized on the greenhouse bench after treatments were imposed.

The second experiment was a repeat of the first experiment except that after the cotyledons were removed, the apical meristems were sliced approximately 3 cm in a vertical direction with a hand scalpel. The remaining discussion refers to both experiments.

Sampling

At the first true leaf growth stage, 16 days after the seeds were planted, one seedling from each pot was randomly sampled and harvested by snipping the seedling off at the soil line. Seedling heights were measured. Plants were dried in a 49°C drying oven for one week and then weighed. Plants were then randomly thinned to two per pot and allowed to grow to the rosette growth stage. One plant from each

pot was again randomly sampled, harvested and weighed as described above, leaving one canola plant in each pot to grow to maturity. In this manner, the effects of leaf area removal could be studied as the plant matured.

One month after planting, canola plants were manually pollinated with a camel hair paintbrush. This procedure was performed daily for one week during peak bloom. At maturity, canola plants were cut at the soil line and tagged with their respective treatment. Plant height, prior to cutting, was measured. Plants were spread on the greenhouse bench for two weeks to allow pods to ripen. Yield was assessed by removing all pods from the dried plants and recording numbers of pods per plant. Both the canola pods and the remaining plant biomass were oven dried in a 49°C oven for one week and then weighed.

Data analysis

Data were analyzed via analysis of variance which allowed examination of main effects for percent leaf area removal, the time the treatment was imposed, and interaction effects. Simple linear regression was performed using averages of yield (total pods per plant) as the dependent variable and the percent cotyledon area removal as the independent variable. Contrasts to detect the level of the polynomial needed to explain the treatment response were utilized to compare the group means.

Greenhouse Threshold Study

Study site

The final greenhouse experiment was performed at the M.S.U. Plant Growth Center. One hundred and twenty 19.05 cm pots were filled with M.S.U. soil mix and arranged on the greenhouse bench in 20 rows of 8 pots each. Each pot was planted with two B. napus seeds (var.#IMC-01), and thinned to one after emergence. Plants were maintained in the greenhouse as previously described. Twenty-four hours after emergence, the experiment was initiated.

Experimental Design

The experimental design was a completely randomized design in a factorial arrangement. The factors were flea beetle density plant growth stages at four and three levels respectively. There were ten replicates per treatment.

Treatments

Treatments consisted of four levels of flea beetle density: 0, 1, 2 and 5 beetles per plant, which were allowed to feed through three canola plant growth stages: cotyledon, first true leaf and rosette. Each treatment was randomly assigned to a pot prior to the start of the experiment.

Flea beetles were collected from untreated canola plantings near the M.S.U. campus. Adults were maintained in a 61 cu. cm. holding cage on canola seedlings for 48 hours prior to the start of the experiment. Confinement cages, previously described, were placed on top of the pots and pressed into the soil approximately one

inch deep.

Treatment techniques

Five days after canola seeds were planted, seedlings were at the cotyledon stage. Unsexed flea beetles were transferred by aspiration directly from the holding cage into the confinement cages. Four days after beetles were introduced into the cages, the seedlings grew their first set of true leaves. The beetles in the 40 cages representing the four flea beetle densities feeding through the cotyledon growth stage were removed and seedlings maintained until maturity. At day six, (the canola seedlings grew their second set of true leaves), beetles in the 40 cages representing the four flea beetle densities feeding through the first true leaf growth stage were removed and the plants maintained until maturity. At day 16, (seedlings at the rosette stage), beetles in the remaining 40 cages were removed and plants maintained until maturity. After six weeks, plants were manually pollinated with a camel hair paintbrush. This procedure was performed daily for one week during peak bloom.

At maturity, canola plants were cut at the soil line and tagged with their respective treatment. Data were collected on final plant height. Plants were spread on the greenhouse bench to allow pods to ripen. Yield was assessed by removing all pods from the dried plants and recording numbers of canola pods per plant. Both the canola pods and the remaining plant biomass were oven dried for one week in a 49°C drying oven and then weighed.

Data analysis

Data were analyzed via analysis of variance which allowed examination of main effects and interaction effects (Lund, 1993). Multiple linear regression was performed using averages of yield (total pods per plant) as the dependent variable and plant growth stage and flea beetle density as the independent variables.

CHAPTER 4

RESULTS

Field threshold study

The GLM procedure of the analysis of variance for yield (total pods per plant) indicated significant differences only for the main effect of flea beetle density (Table 1). Main effects for planting date, growth stage and all first and second order interactions were not significant. Yield data for main effects due to flea beetle density were highly significant (Table 1, Table 2).

Table 1. Analysis of variance for total pods per plant.

SOURCE OF VARIATION	df	MEAN SQUARE x 1000	P-VALUE
Planting Date (PD)	1	5.55	-
Growth Stage (GS)	2	211.88	-
PD x GS	2	42.98	-
Flea Beetle Density (FB)	3	3943.10	**
PD x FB	3	45.61	-
GS x FB	6	117.63	-
PD x GS x FB	6	164.53	-
Error	162	149.72	
Total	168		

** denotes significance at the 0.05 level.

Table 2. Main effect mean yield and total plant oven dry weight (ODW) for flea beetle density across the three plant growth stages, 1992 field study.

FLEA BEETLE DENSITY	n	‡YIELD (pods/plant)	‡TOTAL ODW (g)
0	39	971.40	157.10
1	39	542.00	88.50
2	44	395.50	64.25
5	47	233.00	39.48
*LSD		171.90	20.36
†MSE		383.01	63.50

* calculated using the n that gives the maximum SE of the difference
† root estimated mean square
‡ significant decreasing response, $P < 0.001$

All experiments performed compared several levels of a quantitative factor. Because their regressions were significant, ($P < 0.001$), a multiple comparison treatment such as LSD is unnecessary because all treatments (including intermediate ones not used in the experiment) are significantly different in their effects. However, the LSD is presented and discussed herein as an alternate way of analyzing treatment means.

Mean yield per plant reached a maximum of 971 pods for control plants which decreased in a significant linear fashion from the yield from either one, two or five beetles per plant (Table 2). There was no statistical difference in yield when one or two beetles fed on plants, 542 and 395, respectively (Table 2). However, the mean yield from both one and two beetles per plant was significantly higher from the mean yield 233 per plant when five beetles fed on plants.

Biomass decreased significantly as flea beetle density increased. The plants that were not fed upon weighed significantly more than plants with flea beetle feeding. Biomass of plants with one and two beetles feeding were statistically similar, as were the biomass from plants with two and five beetles per plant (Table 2). Main effect means for yield and biomass due to growth stage across the four levels of flea beetle density were not significant (Table 3).

Table 3. Main effect mean yield and total plant oven dry weight (ODW) for growth stage across the 4 levels of flea beetle density, 1992 field study.

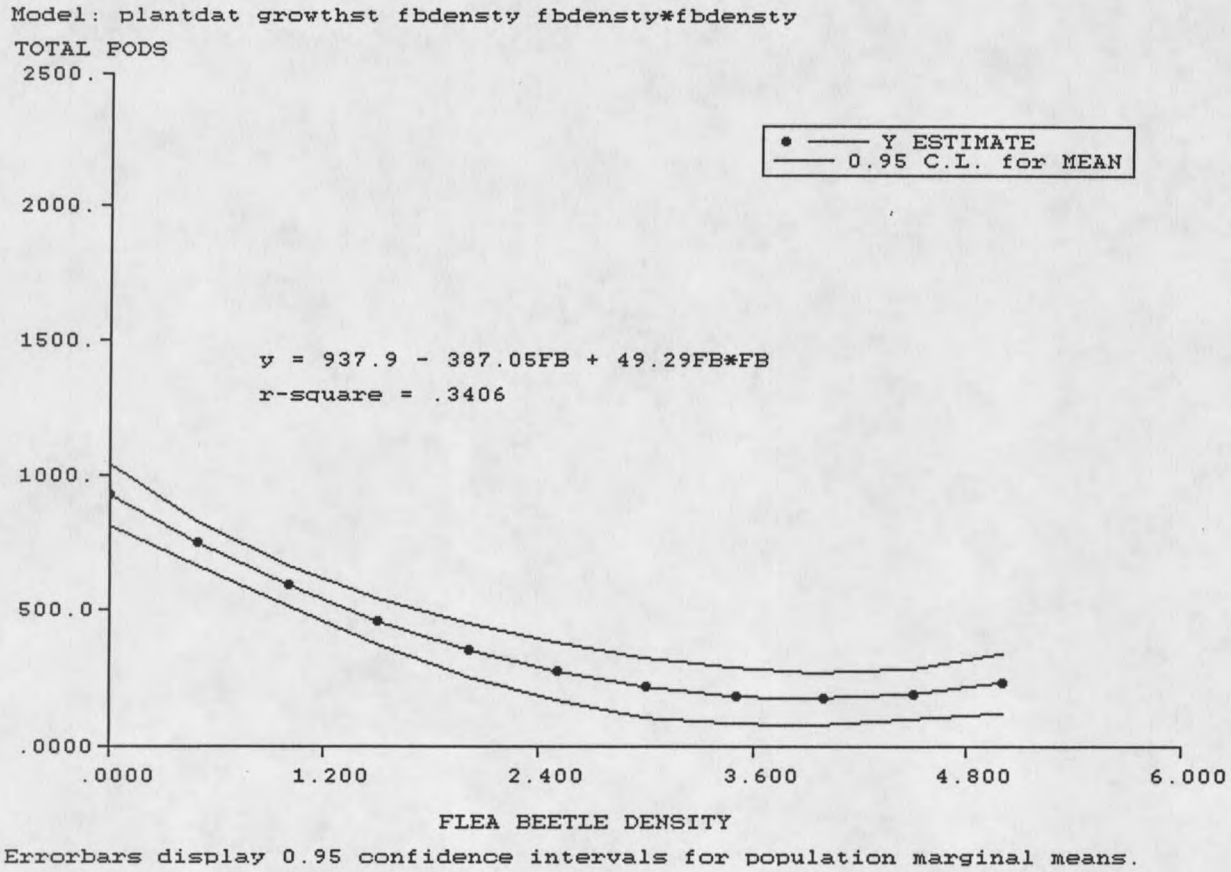
GROWTH STAGE	n	YIELD (pods/plant)	TOTAL ODW (g)
Cotyledon	58	457.90	78.70
True Leaf	56	607.20	96.91
Rosette	55	541.30	86.38
*LSD		72.32	11.99
†MSE		383.06	63.50

* calculated using the n that gives the maximum SE of the difference
† root error mean square

Yield data were submitted to regression analysis to determine the response curve that best described the relationship between yield and flea beetle density. The contrasts to detect the level of the polynomial needed to explain the relationship were found to be both linear, $P = 0.0001$ and quadratic, $P = 0.0001$. A cubic response was tested and not found to be significant. The quadratic regression, $y = 937.9 - 387.05(FB) + 49.29(FB)^2$, shows that increasing flea beetle density from zero through 5 flea beetles per plant, decreases canola pod yield per plant (Fig 1). The r^2

value of 0.3406 indicates that 34% of the variation in yield is attributed to flea beetle density.

Figure 1. Relationship of yield (total pods per plant) to flea beetle density.



Mechanical Damage Studies

In the first mechanical damage study, significant differences were not detected in the interactions of growth stage and percent defoliation, therefore, the main effects were additive (Table 4). Furthermore, there were no significant main effects due to growth stage (Table 5). Significant differences occurred only for the different levels of percent cotyledon removal (Table 6). The following abbreviations are used to interpret tables 4-9: HT 1 = plant height at the first true leaf growth stage, ODW 1 = plant oven dry weight at first true leaf growth stage, HT 2 = plant height at the rosette growth stage, ODW 2 = plant oven dry weight at rosette growth stage, HT 3 = plant height at harvest, YIELD = total number of pods per plant, TTL ODW = biomass oven dry weight.

Table 4. Analysis of variance for first mechanical foliage removal study.

SOURCE OF VARIATION	df	HT 1	ODW 1	HT 2	ODW 2	HT 3	YIELD	TTL ODW
Growth Stage (GS)	1	-	-	-	-	-	-	-
% Defoliation (PD)	3	*	*	*	*	-	*	-
GS x PD	3	-	-	-	-	-	-	-
Error	112							

* denotes significant at the .05 level.

Table 5. Main effect means for growth stage across the four levels of percent cotyledon removal (plus controls); first mechanical foliage removal study.

GROWTH STAGE	n	HT 1 (cm)	ODW 1 (g)	HT 2 (cm)	ODW 2 (g)	HT 3 (cm)	YIELD (#pods)	TTL ODW (g)
24 hours	60	8.025	0.021	16.98	0.43	141.12	93.53	12.85
5 days	60	7.983	0.191	16.80	0.40	145.27	94.87	12.64
LSD (0.05)		0.480	0.004	0.85	0.08	9.48	6.93	0.73
SEM		0.171	0.001	0.31	0.02	3.38	2.47	0.26

Table 6. Main effect means for percent cotyledon removal across the two levels of growth stage, first mechanical foliage removal study.

PCT DEFOL	n	†HT 1 (cm)	†ODW 1 (g)	†HT 2 (cm)	†ODW 2 (g)	HT 3 (cm)	†YIELD (#pods)	TTL ODW (g)
Control	30	9.850	0.033	17.62	0.54	145.77	106.60	13.53
25%	30	8.600	0.026	17.55	0.45	143.40	96.50	12.63
50%	30	7.500	0.011	16.45	0.36	140.50	87.36	12.45
75%	30	6.067	0.010	15.95	0.31	143.10	86.33	12.37
LSD (0.05)		0.680	0.006	1.21	0.09	13.40	9.80	1.04
SEM		0.243	0.002	0.43	0.03	4.78	3.50	0.37

† significant decreasing response, $P < 0.001$

Plant height decreased with increasing levels of cotyledon defoliation from 9.85 cm for control plants to 6.07 cm for plants which had 75% of their cotyledons removed. Control plant dry weights were significantly higher from the plants with foliar removal. Dry weights from plants that had 25% cotyledon removal were significantly different from plants with either 50% or 75% cotyledon removal. Plants

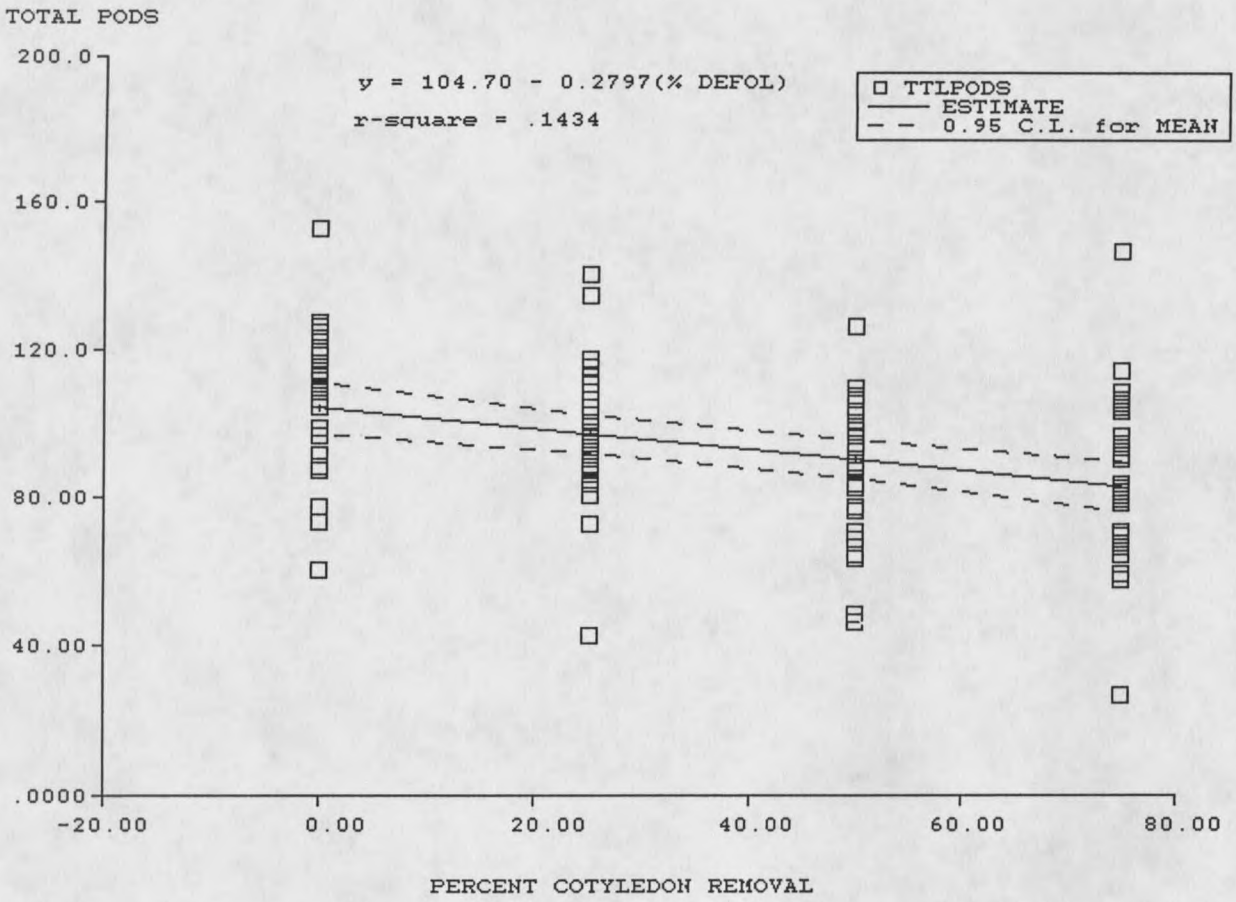
with 50% and 75% cotyledon removal had similar dry weights.

Plant height decreased with increasing levels of cotyledon defoliation but control plants and plants with 25% cotyledon removal were only significantly different from plants with 75% defoliation. Plants with 50% and 75% cotyledon removal had similar heights. Dry weights at this growth stage also decreased with increasing levels of cotyledon removal though control plants and plants with 25% cotyledon removal had similar weights which were significantly different from plants with 75% cotyledon removal. Plants with 25% and 50% cotyledon removal had similar weights, as did plants with 50% and 75% cotyledon removal.

At harvest, no significant differences in plant height could be detected across the four treatment levels (Table 6). Yield decreased significantly with increased levels of percent cotyledon removal. Biomass decreased with increasing percent defoliation. Significant differences occurred only among control plants as compared to plants with 50% and 75% cotyledon removal. Plants with 25%, 50% and 75% cotyledon removal all had similar biomass oven dry weights.

Figure 2 shows the estimated negative linear response ($y = 104.70 - 0.2797$[% defoliation], $r^2 = 0.1434$, $P < 0.0001$) of total pods per plant versus percent cotyledon removal. This relationship suggests that increasing cotyledon removal reduces total plant yield. The r^2 value, 0.1434, indicates that 14% of the total variation in yield is explained by the level of percent cotyledon removal.

Figure 2. Relationship of yield (total pods per plant) to percent cotyledon removal.



In the second mechanical damage study, significant differences were not detected in the interactions of growth stage and percent defoliation in combination with meristem damage, therefore, the main effects were additive (Table 7). As in the first greenhouse study, there were no significant effects due to growth stage (Table 8). Significant differences occurred only for the different levels of percent cotyledon removal and meristem damage (Table 9).

Table 7. Analysis of variance for second mechanical foliage removal study.

SOURCE OF VARIATION	df	HT 1	ODW 1	HT 2	ODW 2	HT 3	YIELD	TTL ODW
Growth Stage(GS)	1	-	-	-	-	-	-	-
% Defoliation (PD)	3	*	*	*	*	-	*	*
GS x PD	3	-	-	-	-	-	-	-
Error	112							

* denotes significance at the 0.05 level.

Table 8. Main effect means for growth stage across the three levels of percent cotyledon removal (plus controls); second mechanical foliage removal study.

GROWTH STAGE	n	HT 1 (cm)	ODW 1 (g)	HT 2 (cm)	ODW 2 (g)	HT 3 (cm)	YIELD	TTL ODW (g)
24 hours	60	8.075	0.028	19.11	0.31	131.80	81.93	8.69
5 days	60	7.825	0.024	18.24	0.28	130.60	79.18	7.98
LSD (0.05)		0.502	0.004	1.17	0.03	5.00	6.98	0.65
SEM		0.179	0.002	0.42	0.01	1.79	2.49	0.23

Table 9. Main effect means for percent cotyledon removal across the two levels of growth stage, second mechanical foliage removal study.

PCT DEFOL	n	†HT 1 (cm)	†ODW 1 (g)	†HT 2 (cm)	†ODW 2 (g)	HT 3 (cm)	†YIELD	†TTL ODW (g)
Control	30	9.533	0.051	20.37	0.37	134.30	96.13	11.22
25%	30	8.567	0.032	18.43	0.32	129.50	78.00	7.26
50%	30	7.483	0.013	18.22	0.27	132.10	75.93	7.37
75%	30	6.217	0.008	17.68	0.21	128.80	72.43	7.49
LSD (0.05)		0.710	0.006	1.65	0.05	7.08	9.87	0.92
SEM		0.253	0.002	0.60	0.02	2.53	3.52	0.33

† significant decreasing response, $P < 0.001$

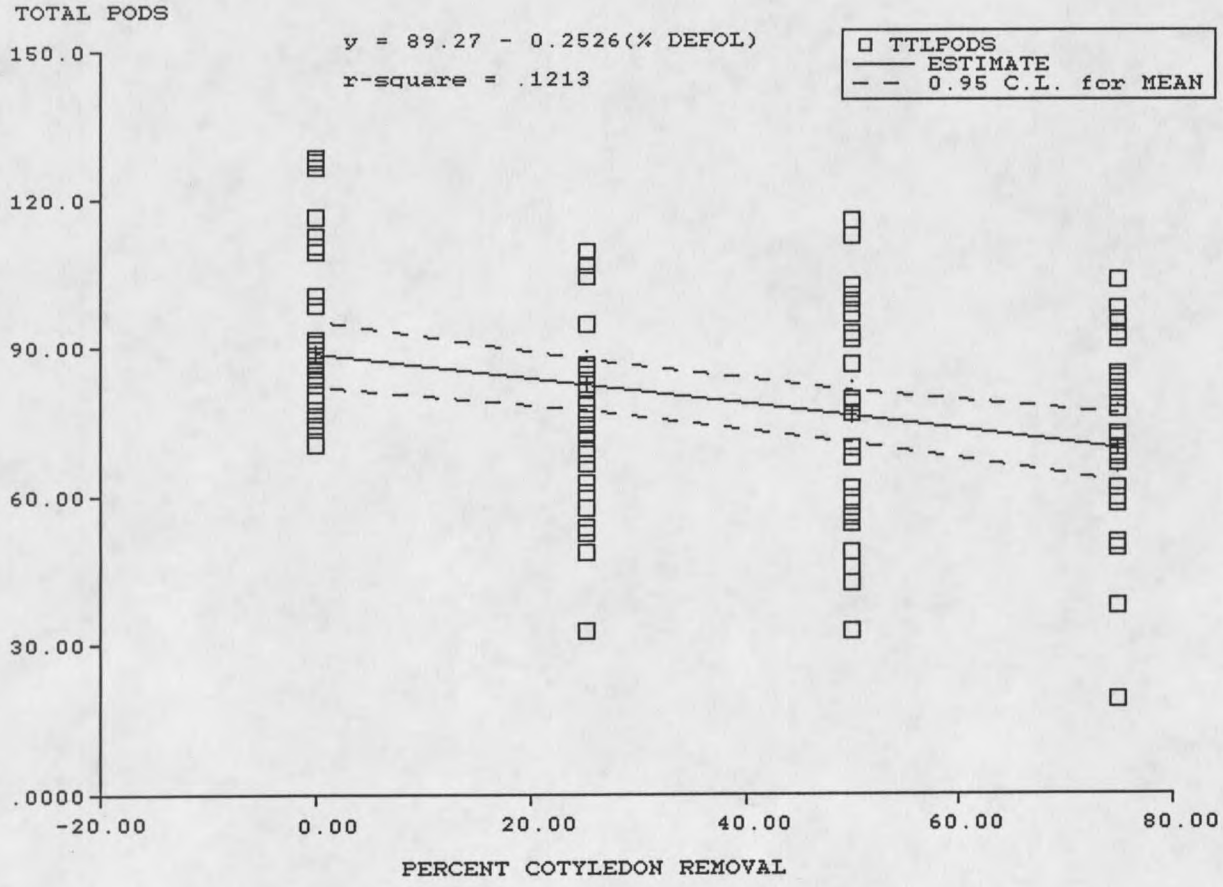
Plant height at the first harvest decreased with increasing levels of cotyledon damage from 9.53 cm for control plants to 6.22 cm for plants with 75% cotyledon removal and meristem damage. Control plant dry weights were also significantly different from plants with foliar removal and meristem damage. Plant height at the second harvest also decreased with increasing damage. Significant differences occurred only between control plants and plants with foliar removal and meristem damage. There were no differences among the remainder of the treatments. Dry weights at this second growth stage also decreased significantly with increasing levels of cotyledon removal and meristem damage. The final plant height at harvest showed no significant differences among the four treatments.

Yield decreased significantly with increased levels of percent cotyledon removal and meristem damage. The average yield for undamaged plants was 93 pods per plant. There were no significant yield differences among any of the damaged

plants. Biomass dry weights were statistically higher for control plants.

Figure 3 shows the estimated negative linear response ($y = 89.27 - 0.2526$[% defoliation], $r^2 = 0.1213$, $P < 0.0001$, from fitting total pods per plant to percent cotyledon removal and apical damage. This linear response suggests that increasing cotyledon removal and apical meristem damage reduces plant yield. The r^2 value, 0.1213, indicates that 12% of the total variation in yield is explained by the level of percent cotyledon removal.

Figure 3. Relationship of yield (total pods per plant) to percent cotyledon removal.



Greenhouse Threshold Study

Soon after the experiment began, all pots with five beetles per plant were completely consumed. Because there were no yield data, the treatments with five beetles per plant were excluded from the analysis. Significant differences were not detected in the interactions of growth stage and flea beetle density, therefore, the main effects were additive (Table 10).

Means for plant growth stage depict the influence of growth stage across the three levels of flea beetle density (Table 11). Response variable values decreased as the canola plants grew through the three plant growth stages. There was a significant decrease for all variables measured when comparing plants at the cotyledon stage to the rosette stage, although plants at the cotyledon and first true leaf growth stage responded to flea beetle herbivory in a similar manner. Yield for plants confined with flea beetles feeding through the cotyledon and first true leaf growth stage were not statistically different. Significant differences in yield occurred only when beetles remained feeding through the rosette growth stage. This same trend was observed for the other two variables measured. Plants grew to approximately the same height and had similar biomass dry weights when beetles were confined through the cotyledon and first true leaf growth stages. Significant differences only occurred when these two growth stages were compared with plant heights and biomass dry weights at the rosette growth stage.

Table 10. Analysis of variance for greenhouse threshold study.

SOURCE OF VARIATION	df	PLANT HEIGHT	YIELD	BIOMASS ODW
Growthstage (GS)	2	*	*	*
Flea Beetle Density (FB)	2	*	*	*
GS x FB	4	-	-	-
Error	81			

* denotes significance at the .05 level.

Table 11. Main effect means for growth stage across the three levels of flea beetle density treatments, greenhouse threshold study.

GROWTHSTAGE	n	†PLANT HEIGHT (cm)	†YIELD (# pods)	†BIOMASS ODW (g)
Cotyledon	30	131.80	134.03	14.21
True Leaf	30	121.40	108.03	11.97
Rosette	30	100.80	86.57	9.40
LSD (0.05)		25.52	29.34	2.87
SEM		9.07	10.43	1.02

† significant decreasing response, $P < 0.001$

Means for flea beetle density depict the influence of numbers of flea beetles per plant across the three levels of plant growth stage (Table 12). For each dependent variable, the mean response decreased with increasing flea beetles per plant. There were no significant difference between control plant height and plant height with one beetle feeding on plants. Plant height decreased significantly, however, when two beetles fed upon plants.

There were significant yield differences between control plants and plants with one beetle feeding per plant. These were both significantly higher than the yield from plants which were fed upon by two beetles per plant.

Biomass dry weight also decreased significantly though there was no significant difference between control plants and plants with one beetle feeding per plant. Biomass averaged 16 g per plant for control plants, 14 g per plant when one beetle fed on plants, and five g per plant when two beetles fed on plants.

Table 12. Main effect means for flea beetle density across the three levels of plant growth stage, greenhouse threshold study.

FLEA BEETLE DENSITY	n	†PLANT HEIGHT (cm)	†YIELD (# pods)	†BIOMASS ODW (g)
0	30	155.60	159.00	16.10
1	30	141.23	123.50	14.04
2	30	57.17	46.10	5.40
LSD (0.05)		25.52	29.34	2.87
SEM		9.07	10.43	1.01

† significant decreasing response, $P < 0.001$

After differences were detected in the analysis of variance, yield (i.e., dependent variable) was regressed on flea beetle density and plant growth stage (i.e., independent variables). The regression determined the response surface that best described the relationship between these three variables. Contrasts to detect the level of the polynomial needed to explain the treatment response were utilized to compare the group means and found only to be linear, $P= 0.0001$. The full model for the response surface was $Y = \beta 0 + \beta 1X1 + \beta 2X2$; where Y = yield expressed as total

pods per plant, X1 = canola growth stage (cotyledon, first true leaf or rosette) and X2 = flea beetle density. The canola plant's yield response to injury changed over physiological age as seen in a contour diagram (Figure 4). Because the two independent variables do not interact in this response function, the contour lines are parallel. The generalized plane is a three-dimensional representation of the response of plants to insect injury through the early physiological lifetime of the plant (Figure 5). The absence of interactions can be seen by considering the linear response surface for yield for a given flea beetle density as a function of plant growth stage. All points on the plane have the same trend and differ only by a constant. Therefore, each ordinate of the yield response plane when the mean flea beetle density is two individuals per plant is a constant number of units lower than the corresponding ordinate for the yield response plane when the mean flea beetle density is one individual per plant. The r^2 value, 0.4434, indicates that when the two independent variables, flea beetle density and plant growth stage are considered together, the variation in yield is reduced by 44%.

Figure 4. Contour diagram showing the relationship of yield (total pods per plant) to canola plant growth stage and flea beetle density.

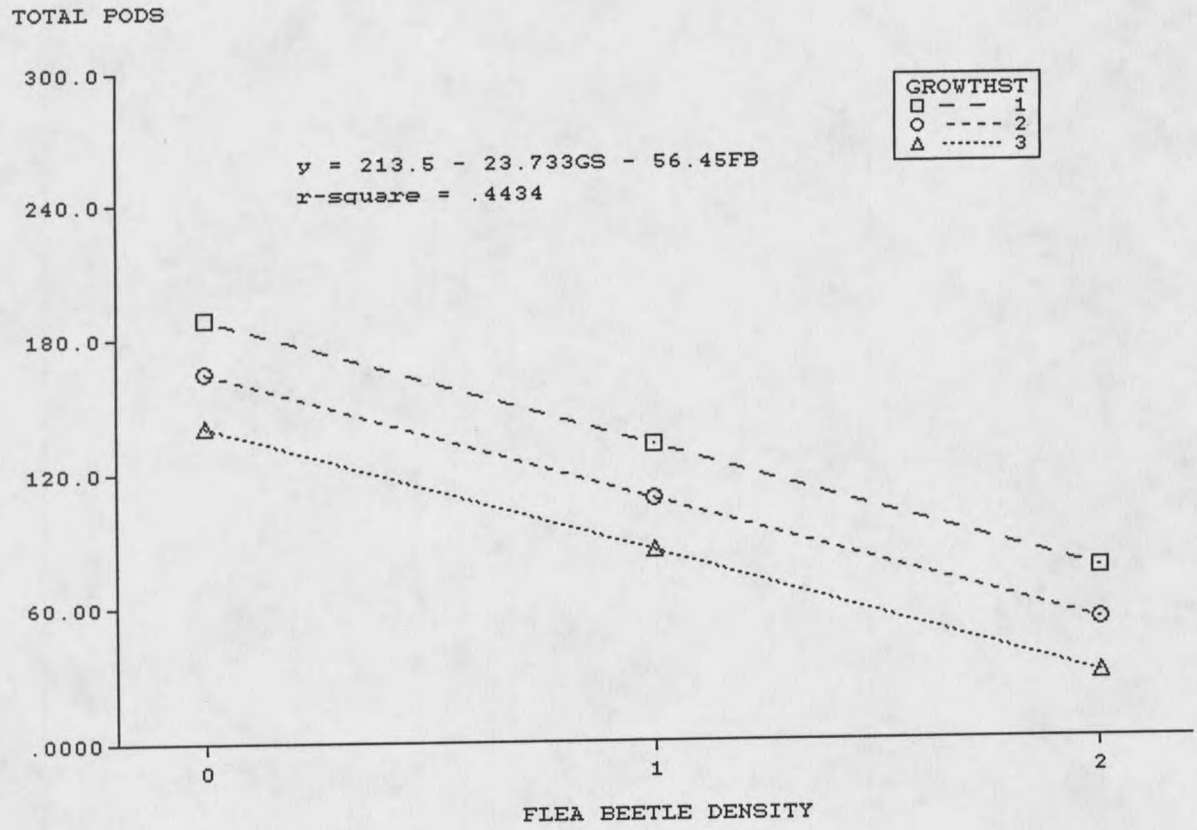
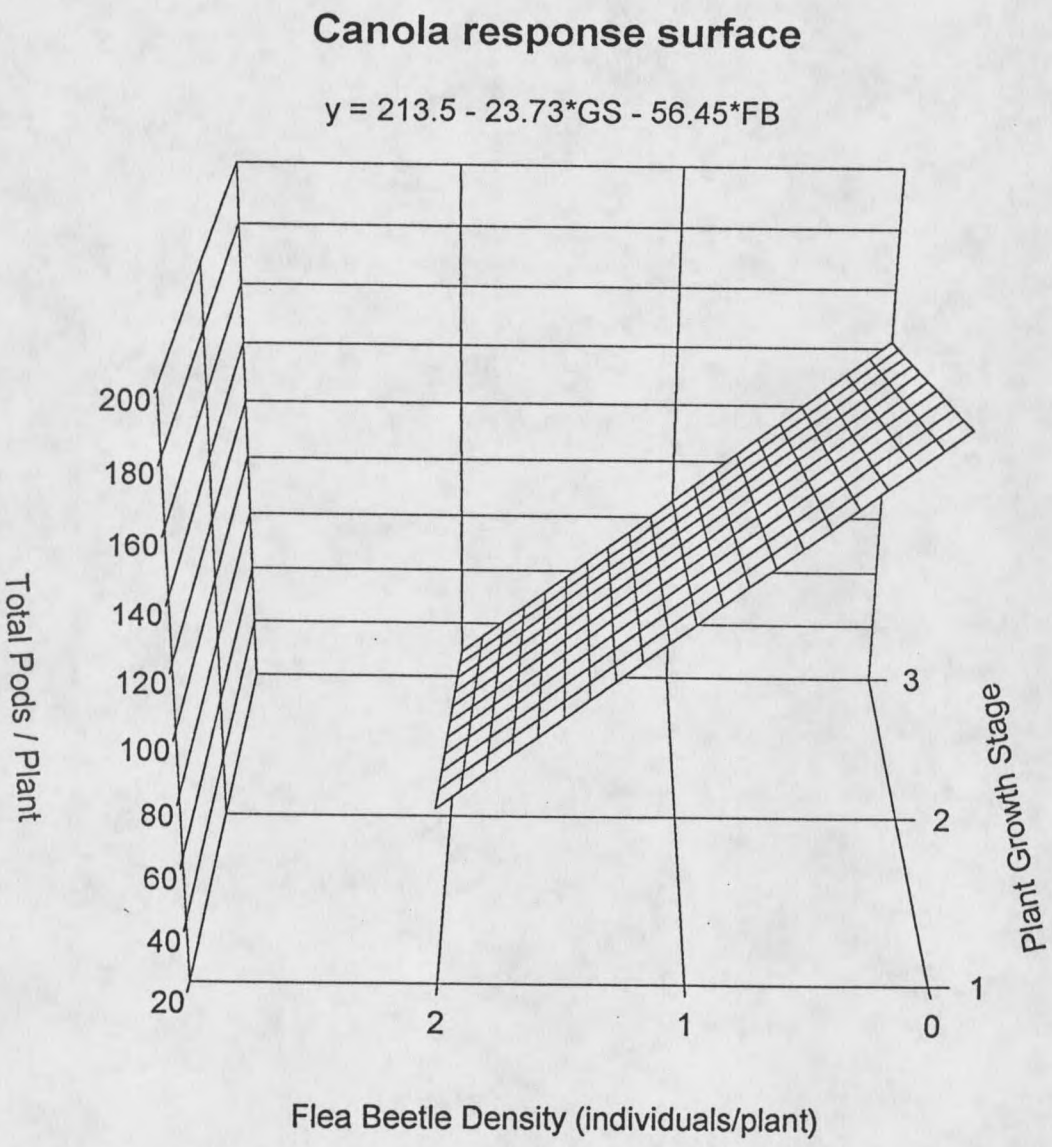


Figure 5. Response surface of total pods per plant at three levels of flea beetle density and three plant growth stages.



CHAPTER 5

DISCUSSION

The main effect of flea beetle density produced the only significant result in the field threshold study. Yield decreased as flea beetle density increased such that one flea beetle per plant was sufficient to cause a significant yield reduction (Figure 1). This finding supports the conclusion of Augustin et al. (1985), who suggest an action threshold of one flea beetle per seedling. However, Weiss et al. (1991), state that the value of this or any action threshold is questionable if materials are not available to provide control once populations reach or exceed the action level.

Flea beetles inflicted the damage that reduced yield at the very early stage (i.e., the cotyledon growth stage). This finding supports the conclusions of Lamb (1984), Bracken and Bucher, (1986) and Augustin et al. (1986) who describe damage at this early plant growth stage as causing delayed plant development, unevenness in height and reduced yield. Damage at the cotyledon growth stage is shown in Figure 1 where the downwards sloping curve represents the damage across all three canola growth stages. If the damage had increased with growth stage, then there would have been a significant growth stage effect and the figure would have shown three separate downward sloping curves. The response curve appears to swing upwards at five flea beetles per plant, but this is most likely an artifact of using the second degree polynomial. More realistically, the rate of decrease tends towards zero, such that

yield is reduced proportionally as infestation increases. While flea beetle densities higher than five per plant were not tested in this experimental design, it is unlikely that increasing numbers to 10, 15 or 20 beetles per plant would show a corresponding increase in yield.

It is doubtful that canola plants would yield as many pods per plant in nature as were recorded in the field experiment. Cages have been used to exclude or include insect pests but plant growth is often affected by the presence of a cage (Matthews 1984). Microclimate effects caused by the cages enabled the plants to grow under greenhouse-like conditions, even with flea beetle herbivory. During the first month of the field experiment, cool temperatures were not uncommon. The protection afforded by the cages to the plants in the early growth stages is the probable cause of a less than realistic number of pods per individual plant.

The main effect of planting date cannot be individually interpreted. The experiment was designed as a split plot design, but because the planting dates were not replicated, this main effect is confounded with block. Therefore, the experiment had to be analyzed as a three factor factorial with the two planting dates treated as blocks. It is questionable however, whether planting date would have yielded a significant main effect even if it had been replicated. Because flea beetles were introduced into the cages, I assume the level of injury inflicted by the respective densities of beetles to be similar regardless of when the crop was planted.

Flea beetles emerge from their overwintering sites at approximately the same time that canola is germinating (Burgess 1977), and will remain in high densities if

there is an adequate food source to sustain them. The advantage of early planting is to avoid attack by emerging flea beetles when canola is at its most vulnerable stage. This was not a factor addressed in the field experimental design, therefore, main effects due to planting date were not important in this study.

Results from the greenhouse threshold study corroborate the field results in the main effect of flea beetle density. One flea beetle feeding per plant was sufficient to cause a yield reduction. However, there was a significant difference in yield for plant growth stage in the greenhouse which was not detected in the field (Figure 4). This is probably because environmental conditions can be controlled to a higher degree in the greenhouse that may have made plant response more sensitive to smaller effects.

Feeding by flea beetles at five per plant was initiated soon after beetles were introduced into the cages. Twenty-four hours after the experiment was initiated, 13 of the 30 plants having a density of five beetles per plant were dead. After six days of feeding, the remainder of those plants died. In contrast, plants exposed to feeding by one beetle often displayed very few feeding pits and the single beetle was observed in places throughout the cage except on the canola seedling. The feeding damage from two flea beetles feeding per plant was intermediate between one and five beetles feeding per plant. Twenty four hours after the experiment was initiated, only one of the 30 plants receiving two beetles per plant had been killed, though after six days, 23 of the 30 plants were dead. While this experiment was not designed to test attractancy, these observations support the findings of Peng and Weiss (1992) who suggest that a flea beetle-produced aggregation pheromone increases the attractiveness

of flea beetles to canola. However, in a caged experiment where five beetles are confined with a single seedling for a set period of time, these results are not unexpected.

In both the field and greenhouse study, while one beetle was sufficient to cause a yield reduction, it rarely did enough damage to kill the plant. With two beetles feeding per plant, the amount of damage increased significantly. Two beetles were often sufficient to kill plants. However, in some instances, two flea beetles did not kill plants, and yield, though greatly reduced, was obtained. The reasons for this are unclear. I found no mention in the literature of possible repellency or interference of members of the same sex, when beetles are confined. Because beetles were unsexed in these studies, this could be a possible reason. Another possible explanation is that once females lay their eggs, feeding is reduced or even stopped. Randomly selected female beetles may have been post-ovipositional, near death and not interested in feeding.

Five flea beetles per plant were sufficient to kill all the plants in the greenhouse experiment. However, some plants survived in the field experiment. A possible reason for the survivorship in the field is due to placement of the cages under field conditions. While every attempt was made to seal and secure the cages to ensure that beetles neither escaped nor entered cages, it was obvious from the amount of missing data that this attempt failed. Therefore, it is possible that beetles may have escaped from some cages that were supposed to be confined with five per cage. Because cages could not be moved, it was almost impossible to detect beetles in cages

other than controls after the plants grew their first set of true leaves.

The mechanical damage studies show that a canola seedling can lose as little as 25% of its cotyledon surface area and show a significant yield decrease. Furthermore, if the percent of cotyledon surface area is not removed until five days after the plant has germinated, the plants will still show a significant yield decrease.

In the second mechanical damage study, the apical meristems were damaged because I observed flea beetles in the field concentrating their feeding on the meristematic region of the emerging seedling. It appeared that plants remained in the cotyledon growth stage under field conditions longer because beetles were actively feeding on the meristems. The results from this second study show that canola plants were stunted when meristems were damaged in combination with cotyledon surface area removal when compared to results from the first mechanical damage study. I believe this is what is occurring under field conditions; flea beetles attack the tender growing tip of the emerging seedling, thereby reducing plant vigor.

Studies have confirmed that glucosinolates may not be solely responsible for the attraction of flea beetles to canola (Lamb 1988, Bodnaryk & Palaniswamy 1990, Peng & Weiss 1992). It may be possible that the meristems are more attractive to flea beetles because they don't contain these secondary plant compounds. The extensive literature documenting glucosinolates in the seed and foliage of canola make no mention of their presence in the meristem.

In spite of this observation, canola has a remarkable ability to compensate after a percentage of its cotyledon surface area has been removed. For example, in

the first mechanical damage study, plant heights at the first true leaf stage were all significantly different from each other. By the rosette growth stage, the plants had begun to compensate. There were no significant differences between control plants and plants with 25% cotyledon removal, between 25% and 50% removal, or between 50% and 75% removal. Significant differences in plant height only occurred when plants with 75% cotyledon removal are compared to either control plants or plants with 25% cotyledon removal (Table 6). At harvest, there were no detectable differences in plant height. There were differences in yield however, suggesting that even though canola plants could outgrow the effects of leaf removal at an early stage, they cannot compensate on yield. However, in the first mechanical damage study, the numbers of pods produced by individual plants were not dramatically different from one another. Further studies need to be completed that document progressive leaf surface area removal to see what effect that will have on yield.

The effect of flea beetle feeding may be direct, through the killing of plants and subsequent stand reduction, indirect, by reducing plant vigor, or it may be a combination of the two, where losses resulting in reduced vigor of surviving plants are compounded by losses due to plant mortality. However, Lamb (1984) concludes that the indirect effect of flea beetle feeding is more important than the direct effect in reducing seed yields. The results of all of the studies described herein support Lamb's conclusions.

In Canada, where most of the canola production occurs, an economic injury level is of little value because an in-furrow treatment of a systemic insecticides is the

standard preventative measure used against flea beetles. They are unavailable to Montana farmers because they are not registered for use in the U.S. Yet farmers lack an effective insecticide. An economic injury level would be valuable for use with foliar sprays, which are the plausible form of control in this country. Current efforts are underway in the U.S. to register effective foliar treatments (Jim Gray, Intermountain Canola, personal communication). If an economic threshold were established for this host/pest complex, it may provide incentive to get farmers into their fields to examine their pest population. This would provide a more accurate basis for the application of a pesticide instead of relying on a reactionary or preventative one (R. Lamb, personal communication).

Any attempt at defining an EIL is useless unless quantification of the pest in question can be accomplished with accuracy. This is especially difficult in the case of the ubiquitous crucifer flea beetle. Furthermore, a definitive scouting and evaluation program needs to be addressed. Finally, farmers need effective, registered insecticides to be used when scouting, quantification and evaluation reveal potential economic losses. The studies described above provide only a small introduction into the lengthy research needed to quantify the effects of flea beetle feeding on canola.

Minor crops have the potential to play a significant role in Montana's agricultural future. As farmers look for options to diversify, new crops such as canola offer many advantages to traditional small grain farming practices. With the advent of effective, registered insecticidal compounds coupled with knowledge and implementation of threshold levels for pests such as the flea beetle, canola has the

potential for a successful future.

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