

**INSECT-SPECIFIC RESPONSES OF *BRASSICA NAPUS* AND *SINAPIS ALBA*
TO HERBIVORY BY SEVERAL SPECIES OF INSECTS**

BY

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FORWARD

This thesis is presented in the manuscript form. Chapter 3 is written in the style of the *Annals of Applied Biology*, and Chapters 4 and 5 in the style of *Ecology*.

ABSTRACT

Gavloski, John Edward. Ph.D., The University of Manitoba, October, 1998. Insect-specific responses of *Brassica napus* and *Sinapis alba* to herbivory by several species of insects. Major Professor: Dr. Robert J. Lamb.

This thesis explores some of the components of the interactions between herbivorous insects and their host plants. Two cruciferous plants, *Brassica napus* L. and *Sinapis alba* L., and several insects which feed on these plants, were used to test the following hypotheses: 1) that the specific impacts vary for different insects feeding on a particular species of plant; 2) that plant compensation for defoliation by insects is specific to a particular herbivore, and not a generalized response to a certain level of injury; and 3) that the specificity of plant compensation for herbivory is related to the pattern of defoliation.

Specific impact was measured as the reduction in plant biomass per unit gain in insect biomass for moth larvae, *Mamestra configurata* (Walker) and *Plutella xylostella* (L.), a beetle larva, *Entomoscelis americana* Brown, beetle adults, *Phyllotreta cruciferae* (Goeze) and *Phyllotreta striolata* (F.), and aphids *Myzus persicae* (Sulzer) and *Lipaphis erysimi* (Kaltenbach). Specific impact was different for different insects, and was highest for *Phyllotreta* spp., intermediate for *Pl. xylostella* and the two aphids, and lowest for *E.*

americana and *M. configurata*. There was more than an order of magnitude difference between specific impacts of some insects.

Laboratory and field experiments were performed to determine the level of compensation of *B. napus* and *S. alba* for equal amounts of injury to seedlings by *M. configurata*, *Pl. xylostella*, and *Ph. cruciferae*. Plants compensated most for injury by *M. configurata* and least for injury by *Ph. cruciferae*. *Sinapis alba* recovered more from the feeding than did *B. napus*. Seedling injury alone caused reductions in the number of seeds and average weight of seeds in both plants. Complete recovery of leaf tissue does not always imply that there will be a complete recovery of plant fitness.

Seedlings of *B. napus* and *S. alba* were artificially injured in ways that simulated feeding by *M. configurata*, *Pl. xylostella*, and *Ph. cruciferae* to determine the effect of pattern of injury on a plant's compensatory ability. Some of this artificial injury resulted in patterns of compensation and levels of recovery similar to those from the insect injury that artificial injury was meant to simulate. Plants generally compensated most for concentrated injury, characteristic of feeding by *M. configurata*, and least for perforations of cotyledons and injury to the apical meristem, characteristic of feeding by *Ph. cruciferae*. Injury to the apical meristem resulted in *S. alba* producing multiple stems, and resulted in damage to true leaves in both plant species.

These results support the following conclusions: 1) that plants respond differently to attack by different insects; 2) that the differences in plant response to injury by different insects is due in part to the ways a plant compensates for herbivory; and 3) that the level of plant compensation for insect injury is determined by the tissue which is

injured, or the pattern of injury to a particular plant tissue.

CHAPTER 1

GENERAL INTRODUCTION

Phytophagous insects are common elements of most terrestrial ecosystems (Mattson and Addy 1975). Several species of phytophagous insects are usually associated with every plant species, and seldom does a plant occur without its ubiquitous consumers (Mattson and Addy 1975). Many foliage feeding insects and their host plants have had a long history of association and coevolution (Knerer and Atwood 1973, van Emden 1973). This implies that insect-plant interactions are not weak, but intricate and intensive. Contrary to this, however, is the hypothesis that the evolution of insect-plant relationships results primarily from independent evolutionary events, namely from heritable functional changes within the insects nervous system that determine plant recognition and ultimately host plant specificity (Jermy 1993). These changes are not evoked by selection, although ecological factors play a secondary role, either by supporting or preventing the establishment of the new genotype with the new food preference.

Types of herbivory and the range of plant responses to herbivory are diverse. Herbivores may affect growth rate directly by reducing the photosynthetic area (leaf removal or leaf mining), by altering the carbohydrate balance (sap suckers or fruit feeders), by interfering with water and nutrient uptake (root feeders, xylem suckers), by weakening the physical structure of the plant (shoot or stem borers) (Crawley 1983), or by injecting host metabolism-modifying chemicals (Trumble *et al.* 1993). Herbivores that remove leaf tissue can be divided into 1) grazers, which take large quantities of leaf comparatively unselectively, 2) leaf miners, which feed on the mesophyll within the leaf without destroying the epidermis, and 3) strip miners, which feed from the leaf surface, rasping through the epidermis into the underlying mesophyll (McNaughton 1983).

Physiological responses to herbivory include decreases in root growth (Evans 1971, Caloin *et al.* 1990), movement of reserves from root to shoot (Bokhari 1977, Caldwell *et al.* 1981, Ta *et al.* 1990), increases in resource acquisition in roots (Chapin and Slack 1979, Chapin 1980a, Ruess 1988) and shoots (Caldwell *et al.* 1981, Welter 1989), activation of new meristems (Caldwell *et al.* 1981, Maschinski and Whitham 1989, Whitham *et al.* 1991), alteration of patterns of leaf senescence (Nooden and Leopold 1988), and increases in photosynthetic rate in remaining leaf tissue (Welter, 1989).

Theoretically, hundreds of thousands of plant species and hundreds of thousands of phytophagous insect species form a potential for millions of insect-host plant relationships (dePonti 1982). The variation in impacts that herbivorous insects have on their host is still not clear, although attempts have been made to study and compare impacts of different insects on a host plant (Moran and Whitham 1990, Meyer 1993). These attempts to compare the impact of different herbivores have been limited because it is usually not clear whether there is a species specific effect by the herbivore, a herbivore specific response by the plant, or more herbivory by one insect than another.

The efficiency of a plant-insect system quantifies the interaction in a way that allows for the comparison of the impact of different herbivores on their host plant. The efficiency of the interaction may be measured as the ratio of the dry biomass gained by the insect and the simultaneous reduction in the biomass of the plant. Such a measurement was used by Lamb and MacKay (1995) to quantify tolerance of three cereals to aphids. This ratio tests whether the impact of herbivores on plants differs for different interactions (herbivore-host plant pairs).

Characteristics of both the plant and the insects may affect the efficiency of the interactions between a host plant and its herbivores. Efficiencies may vary if the level of recovery by the plant after feeding by the different insects varies. The level of recovery likely depends on what compensatory responses occur in the plant due to the feeding. The compensatory responses may in turn depend on the type of feeding by the insect. The tissue injured and the pattern of feeding may both be important in determining the type and magnitude of the recovery by the plant.

To study the impacts of different insect herbivores on a host plant and the factors that affect the impacts requires an insect-plant system that is well known and easily manipulated. Agricultural crops are often useful for studying basic ecological processes because agricultural communities are often simpler than natural communities (Via 1990), and much is often known about crop plants and the way they are damaged by insects. Canola, *Brassica napus* L., and yellow mustard, *Sinapis alba* L., are both cruciferous plants that host a similar insect fauna. In Canada, defoliators of these plants include the crucifer flea beetle, *Phyllotreta cruciferae* (Goeze), the striped flea beetle, *Phyllotreta striolata* (F.), larvae of the bertha armyworm, *Mamestra configurata* (Walk), larvae of the diamondback moth, *Plutella xylostella* (L.), and red turnip beetles, *Entomoscelis americana* (Brown), while sap feeders include the mustard aphid, *Lipaphis erysimi* (Kaltenbach), and the green peach aphid, *Myzus persicae* (Sulzer) (Lamb 1989). An increased knowledge of the impact of these insects on *B. napus* and *S. alba* and the compensatory ability of these plants will aid in the development of control measures for these insects.

This research tested three general hypotheses: 1.) That the specific impacts vary for different insects feeding on a particular species of plant. This hypothesis was tested by determining the loss of tissue of *B. napus* and *S. alba* required to produce insect tissue for five defoliating and two phloem feeding species of insects. 2.) That plant compensation for defoliation by insects is specific to the injury caused by a particular insect, and is not a generalized response to a certain level of injury to the plant. This hypothesis was tested by measuring the responses of *B. napus* and *S. alba* seedlings to feeding injury by crucifer flea beetles, diamondback moth larvae, and bertha armyworm larvae. 3.) That herbivore-specific compensation for tissue removal is related to the pattern of defoliation. *B. napus* and *S. alba* seedlings were artificially injured to mimic the pattern of feeding by flea beetles, bertha armyworm larvae, and diamondback moth larvae. By comparing artificial injury to insect herbivory the importance of pattern of injury to the compensatory responses of the plant was assessed.

CHAPTER 2

LITERATURE REVIEW

Plants have been subject to intense and recurrent natural selection to reduce herbivore impact upon them, and to compensate for attack when herbivory does occur. Conversely, herbivores have been selected for means of penetrating plant defenses (Brues 1924, Gillett 1962, Ehrlich and Raven 1964). It is also likely that herbivores have been under selection for reduction in the deleterious effects of their attacks on essential food sources, because herbivores that are overly destructive will perish with their food sources (McNaughton 1983).

Plants may be defended from herbivores by being hard to find. Plant apparency to enemies may be reduced by neighbouring plants of different species. This has been referred to as associational resistance (Tahvanainen and Root 1972). Odours from tomato, *Lycopersicon lycopersicum* Mill. (=esculentum), and ragweed, *Ambrosia artemisifolia* L., plants interfere with the ability of *Ph. cruciferae* to find crucifer host plants (Tahvanainen and Root 1972).

If a herbivore does find the plant, other mechanisms of defense may come into play. Chemical and morphological features may make the plant undesirable to herbivores. Plant traits that reduce contact between the herbivore and its host are referred to as non-preference (Painter 1951). Kogan and Ortman (1978) proposed the term antixenosis to replace non-preference as a more grammatically acceptable term, although the credibility of this change has been debated (Wiseman 1985). Painter (1968) separated non-preference into two distinct actions of choice; a choice to oviposit or feed when several cultivars are grown, or a choice to oviposit and feed when only one cultivar is grown. Owens (1975) proposed that these two kinds of non-preference be classified as relative or

absolute. Chemical inhibition of feeding by phytophagous insects has been reviewed by Chapman (1974) and Saxena (1987). Many morphological features may make a plant unattractive as a source of food. Thorns may be effective feeding deterrents against large herbivores, prostrate growth forms may make the plant less accessible to large grazers (Stapledon 1928), and trichomes may prevent the survival or feeding of some insects (Gilbert 1971, Levin 1973, Norris and Kogan 1980).

To be a good source of food for a herbivore, the host plant must be nutritionally adequate, nontoxic, and physically suitable for the host once feeding on the host begins. Plant characters that exert an adverse influence on the insect's growth, multiplication, and survival have been classified as antibiotic (Painter 1951). Antibiosis can be due to either biochemical or biophysical factors of the plant (Pathak 1970). Biochemical factors resulting in antibiosis can be classified as physiological inhibitors or nutritional deficiencies (Beck 1965). Substances produced by plants that are harmful to insect growth and multiplication have been reviewed by Levin (1976). Primary plant metabolites, particularly imbalances of sugar and amino acids, have been shown to confer nutritional antibiosis (Tingey 1981). Fecundity of the brown planthopper, *Nilaparvata lugens* Stal, is reduced on rice cultivars deficient in the amino acid asparagine (Pathak 1970). Examples of biophysical factors resulting in antibiosis are reviewed by Beck (1965) and Pathak (1970).

Even if fed upon some plants show an ability to grow and reproduce, or to repair injury, in spite of supporting a population of herbivores equal to that injuring a susceptible host. This is referred to as tolerance (Painter 1951). Tolerance does not affect

the herbivore in any negative way. Tolerant plants thus have value in preventing selection of virulent genotypes of the herbivore and in maintaining predator and parasite populations (Horber 1972). Tolerance to herbivory is a complex trait involving the interaction of both intrinsic and extrinsic factors (Rosenthal and Kotanen 1994). Intrinsic factors are those determined genetically by the plant. Extrinsic factors include a broad range of variables such as the availability of resources in the environment to support regrowth, the type of herbivory experienced and its spatial distribution within the plant. Tolerance in crop plants to insect pests has been reviewed by Velusamy and Heinrichs (1986). The use of tolerance in resistance studies requires a thorough understanding of the ways in which insects injure plants as well as the ways in which plants may repair the injury (Painter 1951).

One or more of these resistance mechanisms may be functioning in a given plant to help reduce insect injury or its impact on the plant. Different conditions associated with the plant, the herbivore, and the environment in which the plant is growing may determine which resistance mechanism is most suitable. Belsky *et al.* (1993) stated that tolerance is favoured over increased defence if 1) a species cannot increase its investment in protective structures or secondary compounds because of ecological, genetic, or physiological constraints, 2) an incremental increase in defence does not reduce damage, 3.) increases in defence are more expensive than replacing the damaged tissue, or 4) if annual plant-losses are minimal.

This review will discuss the factors associated with the plant, the herbivore, and environmental factors that may influence the impact that herbivory may have on a plant.

The plants and insects used in the research of this thesis will also be discussed.

Terminology used to discuss the way plants respond to or are affected by herbivory will also be clarified.

Defining Terminology

The terms injury and damage are often used interchangeably in the literature. The definitions for injury and damage used by Pedigo *et al.* (1986) will be used in this thesis. Injury is thus the effect of insect activities on host physiology that is usually deleterious. Damage is the measurable loss of host utility, most often including yield quantity or quality or aesthetics. A certain level of injury may not produce damage or yield loss.

The terms plant tolerance and compensation are also often used interchangeably, i.e. a plant that compensates effectively for injury is tolerant. Bardner and Fletcher (1974) defined compensation as the process through which the plant or crop mitigates or nullifies the effects of injury. Belsky (1986) divided compensatory growth into three categories based on the level of compensation. Overcompensation occurs when the cumulative total dry weight (including removed tissue) of the grazed or clipped plant is greater than the total dry weight of the uninjured plant. Exact compensation occurs when the cumulative dry weight of the injured plant equals that of an uninjured plant. Undercompensation occurs when the cumulative dry weight of the injured plant is less than that of an uninjured plant.

The cumulative total dry weight of plant tissue, including removed tissue, is not a satisfactory way to measure compensation because it is difficult to quantify and may lead to

misinterpretations about how a plant is responding to injury. It is difficult to quantify because the weight of removed tissue may not be measurable. It may lead to misinterpretations because dry weight changes in one part of the plant or at one point in development may not reflect the overall level of recovery, although such changes may be part of the recovery process of the plant.

Maschinski and Whitham (1989) also divided compensatory ability into three classes. Overcompensation, where herbivory is beneficial, occurs when grazed plants produce significantly greater numbers of fruits and seeds than ungrazed plants. Equal compensation, where herbivory has no lasting effect, occurs when grazed plants produce equal numbers of fruits and seeds as ungrazed plants. Undercompensation, where herbivory is detrimental, occurs when grazed plants produce significantly fewer fruits and seeds than ungrazed plants.

The definitions of compensation used by Belsky (1986) and by Maschinski and Whitham (1989) are clearly different. Both are useful measurements of how a plant responds to herbivory but focus on different plant tissues, and hence the terminology should reflect this. The number of progeny a plant produces and the survival ability of those progeny measures plant fitness. The ability of an injured plant to produce the same number and quality of progeny as would have been produced in the absence of the injury is the ultimate measure of whether or not a plant recovers from injury. The term recovery thus seems a more appropriate description of how plant fitness is affected by herbivory. Compensation is then a measure of how individual aspects of plant growth change in the short term as a result of injury as a plant recovers. After injury a plant may adjust its distribution of resources among different tissues for seed production and/or vegetative reproduction to be maximized. Thus

growth rate of some plant tissues may increase while that of other tissues decreases; and both overcompensation and undercompensation may be vital for the plant to recover from the injury (Iwasa and Roughgarden 1984, Wilson 1988).

The following definitions of compensation and recovery will be used in this thesis. A quantifiable and meaningful assessment of the compensatory response of a plant tissue is the dry weight of the tissue, not including any tissue removed by herbivory, relative to the weight of the tissue had the injury not occurred. Overcompensation is any increase in dry weight as a result of the injury, and undercompensation is any decrease in the total dry weight of the tissue due to injury. Exact compensation would occur when the dry weight of tissue from the injured plant equals the dry weight of tissue from a plant not receiving the injury. Recovery is the production of an equal quantity and quality of progeny as produced by an uninjured plant. The compensatory processes of the plant results in partial or complete recovery.

Plant tolerance was defined by Painter (1951) as the ability of a plant to grow and reproduce itself or to repair injury to a marked degree in spite of supporting a population of insects approximately equal to that damaging a susceptible cultivar. Tolerance is thus a measure of plant response to injury relative to other cultivars of the plant. Although developed as an agricultural term, plant tolerance is also used in ecological literature. Rosenthal and Kotanen (1994) defined tolerance as the capacity of a plant to maintain its fitness through growth and reproduction after sustaining herbivore damage. This definition of tolerance is not based on plant response relative to other varieties of the plant. It is appropriate that plant tolerance has different meanings depending on whether it is used in an

agricultural or an ecological context. When used in an agricultural context, the ultimate measure of tolerance (yield) may refer to the production of seeds, leaves, roots, or other parts of the plant that have some economic value. In an ecological context plant fitness is the measurement that defines plant success, and in this context a tolerant plant is one that recovers more completely from injury than an intolerant plant. Although the plants used in the experiments in this thesis are agricultural crops, this thesis is an ecological study and the ecological meaning of the term tolerance will be used.

Our ability to predict the level of compensation that will occur in any given system is still relatively poor, in part because of a lack of information regarding interactions between ecosystem components (Trumble *et al.* 1993). Agriculture, especially modern agriculture, is an extreme form of ecosystem disruption (dePonti 1982). Thus understanding how plants compensate for herbivory under various conditions can have ecological and agricultural implications.

Environmental Factors Affecting Plant Response to Herbivory

Tolerance is perhaps more subject to variation as a result of environmental conditions than are antixenosis and antibiosis (Painter 1951). Variations in water and nutrients, and the level of competition for these resources, can determine how tolerant a plant will be to feeding injury.

Water Availability

Responses of plants to water stress have been reviewed by Hsiao (1973). Insufficient water can result in a reduced ability to replace tissue removed by herbivores. The compensatory ability of tansy ragwort, *Senecio jacobaea* L., to feeding by cinnabar moth, *Tyria jacobaeae* L., was positively correlated with moisture availability when plants were grown under different irrigation regimes (Cox and McEvoy 1983). Increased levels of irrigation resulted in increases in the amount of growth following defoliation, the number of nodes which produced this growth, and the number of flower heads (capitula) produced following defoliation. Antibiosis may be increased in some plants due to water stress however. Water stress caused a significant increase in the mortality of twospotted spider mites, *Tetranychus urticae* Koch, feeding on bitter cucumber, *Cucumis sativus* L., varieties, but did not affect mortality of the mites on a nonbitter variety (Gould 1978). Bitter cucumber varieties which have been water stressed have higher concentrations of cucurbitacin-C than those which have never been water stressed (Haynes and Jones 1975).

Morphological and physiological changes in the plant due to a water deficit may influence the feeding behaviour of a herbivorous insect. Host plant suitability in relation to water stress has been reviewed by Holtzer *et al.* (1988). White (1984) observed that young Lepidoptera larvae move to younger tissues on water-stressed plants.

Nutrient Availability

Variation in nutrient availability can produce a gradient of plant compensatory responses (Whitham *et al.* 1991). When wheat *Triticum aestivum* L. was infested with Russian wheat aphids (*Diuraphis noxia* Mordvilko), the yield loss caused by aphid infestation was reduced by increased levels of nitrogen in the nutrient solution (Riedell 1990). In general, environments rich in nutrients will be more likely to promote complete compensation or overcompensation of plants, whereas sites low in nutrients will be more likely to result in undercompensation (Belsky 1986, 1987, Maschinski and Whitham 1989).

Arthropods may at times significantly enhance the nutrient status of an environment. Swank *et al.* (1981) observed increased standing crops of nitrifying bacteria and increased $\text{NO}_3\text{-N}$ export associated with defoliation of mixed-hardwood forests by the fall cankerworm, *Alsophila pometaria* (Harris). Owen and Wiegert (1976) and Owen (1978) proposed that aphid honeydew, being rich in sugars, could stimulate nitrogen fixation beneath the host plant, and hence, help in nutrient cycling. This effect has proven difficult to demonstrate experimentally however (Choudhury 1984, Petelle 1980).

Nitrogen levels in foliage affect herbivory. Mattson (1980) concluded that foliar nitrogen concentration was positively correlated with herbivory, and that low nitrogen concentrations in foliage limited insect populations. Low nitrogen availability in foliage, however, may lead to increased ingestion, which is often correlated with low rates of assimilation (Slansky 1982).

Plant Density

High plant density may decrease the ability of plants to recover from defoliation. Lee and Bazzaz (1980) found that when velvetleaf, *Abutilon theophrasti* Medic., was grown at low densities it could withstand 75% defoliation without significant losses in reproduction. Seed production declined by 50%, however, when it was grown at high densities at identical levels of defoliation. This result may reflect differences in nutrient availability resulting from increased inter-plant competition at high densities.

The Influence of Neighbouring Plants

Rhoades (1983) proposed that plants could receive pheromonal signals emitted by nearby attacked trees. To test this hypothesis, Baldwin and Schultz (1983) confined individually potted sugar maple (*Acer saccharum* Marsh) seedlings in two growth chambers. They then tore leaves on some of the plants in one chamber and compared the phenolic chemistry of the injured plants, uninjured plants in the same chamber (communication controls) and uninjured plants in the separate chamber (true controls), for several days following the injury. Levels of total phenolics and tannins were increased in leaves of the injured plants and in the communication controls compared to the true controls. Similar results were obtained with poplar (*Populus x euroamericana*). These results support the hypothesis of pheromonal communication between plants in response to injury, and demonstrate that an antibiosis response can be induced by herbivory. Whether herbivory also

results in induced tolerance responses is not known. In contrast to the above results, artificial injury to birch, *Betula pubescens* Ehrh., saplings did not provide evidence for either induced defences by the plant, or for communication between plants (Fowler and Lawton 1985).

The Resource Availability Hypothesis

To understand why plants differ so much in the type and extent of defenses they possess, it is important to look at the conditions under which the plants evolved and the type of selection pressures that were present. Such information should enable us to understand why certain plants are able to compensate for much of the feeding injury that occurs, while other plants compensate little for feeding injury but employ more chemical-based defenses (i.e. nonpreference and antibiosis).

The amount of resources available to the plant is proposed as the major determinant for both the level and type of plant defense (Coley *et al.* 1985, Chapin 1991). Chapin (1991) hypothesized that plants growing in infertile sites, shaded habitats, or arid areas share a common suite of physiological traits such as slow growth, low potential for resource capture, effective chemical defense, and a well-developed capacity for reserve storage. Coley *et al.* (1985) proposed that when resources are limited, plants with inherently slow growth are favoured over those with high growth rates. Slow growth in turn favours large investments in antiherbivore defenses. Such plants grow slowly even in the most favourable environments and have low capacity to photosynthesize and absorb nutrients (Grime 1977, Chapin 1980 a and b).

This hypothesis suggests that plants growing under conditions with relatively few resource limitations should use nonchemical based defenses more often as a means of responding to herbivore feeding. Thus plants that have evolved in resource limited environments should commonly use antibiosis and nonpreference as defensive mechanisms, while compensatory growth should be used more by plants that have evolved under conditions of little resource limitation.

Plant Characteristics Affecting Compensation for Herbivory

Stage of Plant When Defoliated

The plant is most vulnerable to being killed due to defoliation when its seed reserves are exhausted and it first relies on the products of its own photosynthesis for growth and survival (Crawley 1983). If herbivory occurs during the seedling stage or after tissues have matured, mortality or little compensatory growth will occur (Whitham *et al.* 1991).

Seedlings can be destroyed in large numbers by various enemies. Darwin (1859) dug and cleared a piece of ground three feet long and two feet wide and marked all the seedlings of native weeds as they came up. Out of 357 weeds, at least 295 were destroyed, chiefly by slugs and insects. Steenbergh and Lowe (1969) found that insects killed 3 to 30% of seedlings of the giant saguaro cactus, *Cereus giganteus* Engelm., in their first year of life. Rodents killed another 6 to 26%. With complete defoliation of the tropical palm *Astrocaryum mexicanum* (Liebm.), immature plants suffered 82% greater mortality than

mature ones (Mendoza *et al.* 1987). These studies show the lack of ability of seedling plants to compensate for or protect themselves from defoliators.

A lack of compensatory response and high seedling mortality may have a beneficial role in natural communities by preventing single species stands from occurring. In a study of seedling predation in tropical forests, Connell (1971) observed that each tree species has host-specific enemies which attack it and any of its offsprings which are close to the parent plant. The healthy parent tree supports a large population of these enemies without itself being killed, but the seedlings, whose growth is suppressed by the heavy shade, succumb to the attack of insects and other enemies which come from the parent tree itself or from the soil below it. In the rainforests of Queensland he found that the death rate of small saplings was 38% when the nearest neighbour was of the same species, but only 18% when it was of another species. Large saplings had the same death rate irrespective of the species of their neighbour.

In agriculture the rate of seedling survival is important, and single species stands are often desired. Thus, it is important in agriculture to increase our knowledge of the compensatory ability of seedlings of major crop species.

Compensatory Photosynthesis

Compensatory photosynthesis was defined by Nowak and Caldwell (1984) as an increase in the photosynthetic rate of foliage on partially defoliated plants relative to the rate for foliage of the same age on undefoliated plants. They found that compensatory

photosynthesis occurred in many leaves of *Agropyron desertorum* (Fisch. ex Link) Schult. and *Agropyron spicatum* (Pursh) Scribn. and Sm. during at least part of their development. For both species, compensatory photosynthesis was related primarily to delayed leaf senescence and increased soluble protein concentration, but not to an improvement in the water status of clipped plants.

Another way photosynthesis may be enhanced is through a change in the age composition of foliage. Following severe defoliation of *A. desertorum* and *A. spicatum* the younger regrowing foliage on defoliated plants had higher net photosynthetic rates than the relatively older foliage on undefoliated plants (Caldwell *et al.* 1981). The increase in photosynthesis that accompanies a change in age composition is expected because younger foliage usually exhibits greater photosynthetic capability. Alderfer and Eagles (1976) found that removal of leaves above the first trifoliate leaf of bean plants, *Phaseolus vulgaris* L., induced more rapid leaf expansion, a larger final leaf area, higher dry weight, lower specific leaf area, and higher photosynthetic rates in the first trifoliate leaf compared with undefoliated controls.

Palisade mesophyll tissue removed from mature leaves of lima beans, *Phaseolus lunatus* L., by the leafmining herbivore *Liriomyza trifolii* (Burgess) was replaced with photosynthetically active cells, permitting virtually complete recovery from injury (Martens and Trumble 1987). Decreases in photosynthesis did not exceed 10% for leaves with approximately one-fourth of the leaf area mined. Net photosynthesis rates were significantly increased in opposite, uninjured leaves of the infested plants.

Partial defoliation induces rejuvenation in photosynthetic rates of the remaining

leaves in alfalfa plants (*Medicago sativa* L. cv. Hunter River) (Hodgkinson 1974). Young and middle-aged leaves rejuvenate to rates comparable to those of recently expanded leaves, but old leaves only partially rejuvenate. The time after defoliation to attain peak photosynthetic rates increases as leaves age. It was also observed that partial defoliation leads to an increase in the leaf area and decline in specific leaf area, i.e., thickening of the leaves. These changes may be caused by an elongation of the cells of the palisade mesophyll. Increase in palisade cell size might be caused by an accumulation of starch grains in chloroplasts, by an increase in chloroplast population per cell, or by some other process leading to increased cell volume.

Mauricio *et al.* (1993) suggested that there may be a threshold effect of injury in individual leaves. Leaves with less injury than the threshold may be able to compensate for injury by elevating photosynthetic rates in the remaining tissue. Leaves with more injury than the threshold may have lower photosynthetic rates (per unit area) than leaves with no injury or leaves with less injury than the threshold.

Assimilate Allocation Within the Plant

There is some controversy over the importance of stored energy and nutrient reserves for the ability of plants to compensate for injury. van der Meijden *et al.* (1988) stated that regrowth capacity implies saving and storing of energy and nutrients in organs that are relatively free from attack. Bokhari and Singh (1974), however, suggested that the extent to which carbohydrate reserves are utilized for regrowth may depend upon the photosynthetic

capability remaining after herbage removal, which in turn depends on height of cutting, growth habit of the plant, phenological status, and age of the remaining leaves.

If the leaf tissue remaining after defoliation is adequate for the regrowth of tissue, then root reserves may not be necessary for regrowth. When all leaf tissue above the ligule of leaf 3 in unicum barley plants was removed, the two older leaves supplied the assimilate used in the regrowth of new leaf tissue immediately after defoliation (Ryle and Powell 1975). Before leaf removal, the two older leaves exported most of their assimilates to the roots. There was no evidence that reserves were mobilized to meet the needs of regrowth at leaf meristems. Current photosynthesis supplied sufficient assimilates to account for all observed growth. The reallocation of assimilates from roots to leaf meristems following defoliation severely retarded root growth.

Growth rates in western wheatgrass, *Agropyron smithii* Rydb., were higher in clipped plants than unclipped controls except for the first 10 days following the clipping (Bokhari and Singh 1974). The greatest growth rate occurred under moderate clipping (clipped at 10 cm) and the next greatest under severe clipping (clipped at 5 cm) compared to the control plants. Shoots of clipped plants continued to grow throughout the 80-day experimental period, but those of unclipped plants ceased to show any positive dry matter accumulation after 50 days. The reserves in roots and crowns did not have a major role in the regrowth of shoots following clipping.

Using carbohydrate reserves in plant roots to maintain regular leaf growth may be one way some plants are able to tolerate insect feeding. Dyer and Bokhari (1976) suggested that perhaps the largest single effect exerted by aboveground insect grazers on grassland plants

is an increase in belowground respiration and root exudation. Carbohydrate reserves are the primary source of readily available energy that govern and limit plant growth following defoliation (Archer and Tieszen 1986).

Defoliation of sheathed cotton-grass, *Eriophorum vaginatum* L., and water sedge, *Carex aquatilis* Wahlenb., resulted in a large nutrient investment in leaf regrowth (Chapin 1980a). Roots increased phosphate absorption and respiration rates, which helped to maintain plant nutrient reserves. Root growth rates decreased following defoliation, but root mortality increased only after severe, chronic defoliation.

Root Weight Ratio

It is hypothesized that under a particular set of environmental conditions an allometric relationship exists between the amount of biomass a plant allocates between its roots and shoots, such that the root weight ratio is conserved (Wilson 1988). This has been viewed as an optimum strategy which maximizes relative growth rate (Iwasa and Roughgarden 1984). In response to tissue loss to herbivores, plants generally reallocate resources to new, restitutive or replacement tissues at the expense of other growth and metabolic centres (Trumble *et al.* 1993). Disturbance of the root weight ratio, either by root grazing or by defoliation, alters the growth pattern such that the original ratio is restored rapidly. Failure to restore the root weight ratio may reflect an inability to compensate fully for tissue lost to herbivores (Crawley 1983, 1988).

Relative Growth Rate

Inherent growth rate has been suggested to be the major determinant of a plant's capacity to regrow and compensate for lost photosynthetic tissue (Chapin 1980b, Bryant *et al.* 1983, Coley *et al.* 1985, Gulmon and Mooney 1986). It is suggested that plants with high inherent growth rates have the potential to produce new growth rapidly and compensate for the lost tissues. Plants with low inherent growth rate have limited capacity to refoliate and are less capable of compensating for lost tissue.

Several studies have suggested that compensatory growth may not always be related to inherent growth rates. Sagebrush, *Artemisia tridentata* ssp. *vaseyana* Rydb., has a relatively high growth rate, yet it does not compensate for lost tissue and is relatively intolerant of browsing (Welch and MacArthur 1979, Bilbrough and Richards 1991, 1993). On the other hand, bitterbrush, *Purshia tridentata* Pursh, has a relatively low growth rate, yet it compensates for lost tissues and tolerates even severe herbivory (Bilbrough and Richards 1991, 1993).

Seedling growth rate is not correlated with level of tolerance to flea beetle injury in *Brassica juncea* L., *B. napus* L., *B. rapa* L. (= *B. campestris*), and *S. alba* L. (Brandt and Lamb 1994). *Sinapis alba* has the highest tolerance to flea beetle feeding, and yet when uninjured, *S. alba* consistently has intermediate rates of growth. The high growth rates of uninjured *B. napus* seedlings at the cotyledon and first true leaf stages do not increase tolerance to flea beetles. *B. napus* is the least tolerant of injury by flea beetles.

Whether a plant is able to increase its growth rate as a result of a particular type of

feeding injury may be more important to a plant's compensatory ability than a plant's inherent growth rate. The ability of a plant to increase its growth rate due to feeding may be dependent on the herbivore.

Herbivore Characteristics Affecting Plant Compensation for Herbivory

Effect of Type of Tissue Injured

Within a single population of plants, numerous herbivores may selectively feed upon different plant tissues, creating a gradient of compensatory responses from positive to negative. The thistle *Jurinea mollis* Ascherson responds in different ways to feeding by different insect herbivores (Inouye 1982). Multiple rosettes are produced on individual roots after two unidentified species of lepidopteran larvae eat the central part of the basal rosette, and each of these rosettes eventually produces a flower stalk. Plants with multiple stalks can produce up to three times as many seeds as those without multiple stalks. Injury caused by moth caterpillars, *Cochylis posterana* Hoffmannsegg, and tephritid fly larvae, *Terellia serratulae* L., to the receptacles of flower heads interrupts the development of seeds, affecting the plant's ability to produce viable seeds. This demonstrates how the level of compensation can depend on the plant tissue injured.

Turnip, *B. rapa* L., was affected more from injury to the leaves by the larvae of the beetle *Phaedon cochleariae* (F.) than from injury to the leaves by larvae of the diamondback moth *Plutella xylostella* (Curt.) (Taylor and Bardner 1968). Different methods of feeding by

these insects affects compensatory growth by the plant differently. *Phaedon* larvae sever leaf veins and rasp the leaf surface, thus killing more leaf tissue by desiccation than they eat. They remain on and greatly injure the older leaves, and their feeding reduces yield. Diamondback moth larvae feed on leaves of all ages, which they eat cleanly, leaving the veins and only injuring the area actually eaten. They do less injury than *P. cochleariae* to older leaves, which grow larger and live longer than corresponding leaves on intact plants.

Pattern of Feeding

Removal of younger foliage may be more detrimental to carbon balance and regrowth than removal of older foliage, because younger foliage typically has greater photosynthetic rates and can utilize higher light intensities than does older foliage (Janzen 1979, Dirzo 1984). Younger foliage also has a greater potential future output of photosynthates than does older foliage. When equal amounts of foliage are removed from different locations of *Agropyron desertorum* (Fisch.) Schult., the rate of regrowth and the above-ground biomass production over the growing-season differ (Gold and Caldwell 1989). When lower (older) leaves are removed, regrowth is greater than after removal of the same quantity of foliage higher in the canopy (younger leaves). These differences in regrowth are due to differences in the rate of new tissue production rather than differences in the timing of senescence. Greater yield reduction in sorghum, *Sorghum bicolor* (L.) Moench, results from removing leaf area from upper than from lower portions of the plants (Stickler and Pauli 1961). Thus a higher level of recovery is likely to be expressed when insects feed on the older leaves than

the younger leaves.

Different spatial patterns of defoliation within a plant might influence regrowth not only because foliage of different age is removed, but also due to effects on canopy structure and the resulting microenvironment of the remaining foliage (Gold and Caldwell 1989). Some defoliation patterns may result in removal of meristematic tissue critical for rapid regrowth (Briske 1986).

Several studies have demonstrated that plants recover better from injury dispersed over many leaves than from injury concentrated on just a few leaves. When equivalent amounts of leaf area are removed in different patterns from wild radish, *Raphanus sativus* L., plants where the injury is most dispersed have significantly higher flower number, reproductive biomass, and total biomass than an intermediate injury treatment and significantly more reproductive biomass than the concentrated injury treatment (Mauricio *et al.* 1993). When coachwood, *Ceratopetalum apetalum* D. Don, seedlings are defoliated by removing either 25% of each leaf, 50% of each leaf, one of every four leaves, one of every two leaves, or no leaves, seedlings with all leaves partially clipped recover more successfully than those with some leaves completely removed (Lowman 1982).

The hypothesis that dispersed injury is less detrimental to the plant than concentrated injury is further supported by a study of defoliation pattern on growth of the understorey tropical wet forest shrub *Piper arieianum* C.DC. in Costa Rica (Marquis 1992). When 10% of the plant's leaf area is removed but the loss is spread throughout the canopy, seed production is not significantly reduced compared to uninjured plants. When 10% injury is restricted to a single branch, growth and seed production of both the branch and the entire

plant are significantly reduced. Branches abort in all cases when the number of leaves on an injured branch is reduced to less than three. It is suggested that there may be selection on the plant for mechanisms either to reduce the occurrence of concentrated herbivory, or instead, to avoid the impact of concentrated herbivory when it does occur.

Characteristics of the plant that lead to dispersal of injury may at times be considered a form of tolerance because damage to the plant may be decreased compared to more concentrated injury. Knowing the pattern of injury may be as important as knowing the total injury in determining the effect on the plant and the ability of the plant to tolerate the injury. Because different insects will disperse their feeding differently, the level of tolerance that a plant displays will depend on the herbivore that is doing the feeding and is not solely a response by the plant to a given level of injury.

The response of plants to insect feeding may be influenced by the pattern of feeding within a leaf, as well as the pattern of feeding among the leaves collectively. Photosynthesis in apple, *Malus sylvestris* Mill, leaves is reduced more when 20% of the leaf area is removed as small holes than when 20% of the leaf area is removed as large holes (Hall and Ferree 1976). The average circumference when injury is by smaller holes is greater than when injury is by larger holes. Feeding injury in the form of many small holes can be expected to have a greater impact on leaf net photosynthesis than an equal amount of injury occurring from larger holes. This study showed that dispersed injury can be more detrimental to leaf net photosynthesis than concentrated injury. Whether the growth rate of the injured leaves or newly formed leaves is changed is not known however. It is possible that re-allocation of plant assimilates may compensate for a decrease in photosynthesis.

Effect of Saliva

In addition to mechanical defoliation during grazing, plants may be affected by growth regulators contained in herbivore saliva (Dyer and Bokhari 1976). Several plant growth regulators, such as cytokinins (Englebrecht *et al.* 1969) and auxins (Miles 1968) have been found in relatively high concentrations in the salivary system of some insects. Growth stimulations have been obtained by applying ungulate saliva or one of its constituents, thiamine (vitamin B₁), to manually clipped leaves of sideoats grama, *Bouteloua curtipendula* (Michx.) Torr (Reardon *et al.* 1972, 1974). Thiamine acts as a root growth hormone (McNaughton 1983). In contrast to these results, saliva from the lubber grasshopper (*Brachystola magna* Girard) was found to reduce biomass and tiller production in blue grama, *Bouteloua gracilis* [H.B.K.] Griffiths (Detling and Dyer 1981).

The Herbivorous Insects and their Host Plants Used in the Studies

A complete understanding of how an insect herbivore affects the growth of a plant requires an understanding of how factors relating to the insect, the plant, and the environment all interact. Agricultural systems provide good models for studying insect-plant interactions because many of the environmental factors are controlled and many crop plants and their herbivores have been studied in detail. The crucifer plants *B. napus* and *S. alba* and several insect herbivores of these crops will be used to test the hypotheses of this thesis.

Insects

Flea Beetles. The striped flea beetle, *Phyllotreta striolata* (F.) and the crucifer flea beetle, *Ph. cruciferae* (Goeze) are the most important pests of canola or oilseed rape, *B. rapa* and *B. napus*, in Canada (Lamb 1989). They are univoltine in the prairie provinces of Canada. Adults overwinter mainly in litter in hedgerows, windbreaks, and wooded areas (Burgess 1981). In early spring they feed on cruciferous weeds and later may move into fields of newly-emerged canola. Eggs are laid in soil adjacent to food plants where the larvae feed on roots (Westdal and Romanow 1972). Larvae pupate in the soil and adults emerge from late July to October. After emergence, adults feed for several weeks before moving to hibernation sites.

Phyllotreta cruciferae and *Ph. striolata* are oligophagous herbivores that feed primarily on plants in the family Brassicaceae (=Cruciferae). Adult beetles feed on the cotyledons and slender stems of seedling cruciferous plants, and continue to attack the leaves as the plant develops (Feeny *et al.* 1970). The characteristic type of flea beetle injury to plants consists of small holes or pits in the epidermis of leaves. Although the feeding injury does not penetrate the leaf completely, the tissues below the injury eventually dry up and break or fall out giving a shot-hole appearance (Westdal and Romanow 1972).

Flea beetles feed more on the upper surfaces, bases, and edges of cotyledons and first true leaves in *B. napus* and *S. alba* (Brandt and Lamb 1993). *Phyllotreta cruciferae* has a greater preference for the upper surface of *S. alba* cotyledons than for the upper surface of *B. napus*. First true leaves of *B. napus* have greater than 90% of the feeding injury along the

edge, compared with <70% for *S. alba*. The size of feeding pits does not differ on the upper and lower surfaces of the cotyledons and first true leaves for *B. napus*, but *S. alba* has smaller feeding pits on the lower surface. *Sinapis alba* has smaller feeding pits on the upper surface of its cotyledons than *B. napus*, and *S. alba* first true leaves have feeding pits about one-half the size of the feeding pits of *B. napus*. These findings differ from those of Anderson *et al.* (1992) which show most injury occurs on the lower surfaces of *B. napus* and *S. alba*. The reason for the discrepancy between the two studies is not known.

Small shallow feeding pits may indicate that feeding is initiated, but rapidly terminated in response to plant antixenosis (Anderson *et al.* 1992). One of the defences may be high concentrations of the glucosinolate sinalbin in the cotyledons of *S. alba* (Bodnaryk 1991). However, because the feeding pit size remains small in true leaves of *S. alba*, where sinalbin levels are too low to act as a deterrent, the presence of another factor is suggested (Brandt and Lamb 1993).

Diamondback Moth. The diamondback moth, *Plutella xylostella* (L.) feeds only on plants in the mustard family (Brassicaceae) (Talekar and Shelton 1993). The newly hatched larva crawls to the lower surface of the leaf and bores through the epidermis (Harcourt 1957). During the first instar it mines the leaf tissues. Typically the mines are shallow; the larva usually feeds in the spongy mesophyll and avoids the comparatively tough palisade layer. The mines show up as numerous white markings on the leaf. At the end of the first instar, the larva emerges from the mine, spins a few protective threads and moults beneath, selecting a sheltered site such as a depression on the leaf or near an edge that is slightly curled. Older larvae feed from the lower leaf surface and usually consume all tissue except the wax layer

on the upper surface, thus creating a window in the leaf (Talekar and Shelton 1993).

Loss of leaf area due to diamondback moth feeding probably causes little yield reduction in any but the most extreme cases (Philip and Mengersen 1989). Late in the season when the leaves become senescent, larvae feed on pods and this can result in significant yield reductions.

Bertha Armyworm. Bertha armyworm, *Mamestra configurata* Walker, is a serious pest of canola in the prairie provinces of Canada (Turnock and Philip 1977). It causes widespread damage across the Parkland Region during some years and then becomes uncommon or rare for a number of years until the next outbreak (Lamb *et al.* 1985). The causes of outbreaks are not completely understood. There is a significant negative relationship between mortality and snow depth up to about 20 cm measured at the end of January .

Larvae of *M. configurata* are general feeders on broad-leaved plants, including flax, rape, sweetclover, alfalfa, cabbage, corn, peas, beans, turnips, beets, tobacco, and garden flowers (Beirne 1971). On canola, the larvae of bertha armyworms feed initially on the leaves. The last two larval instars feed when the plants have finished flowering and the pods are maturing (plant stages 5.1-5.3, Harper and Berkenkamp 1975), and some of these larvae feed on the pods (Bracken 1984). Most of the yield loss from larvae of bertha armyworms is attributed to direct feeding on pods because in canola plants, from stage 5.2 onwards, the pod surface is of increasing importance, relative to leaves, in supplying photosynthates to the developing seeds (Major *et al.* 1978, Clarke 1978). Eighty percent of the total plant tissue eaten by larvae occurs during the sixth and final instar (Bailey 1976). These large larvae can

consume leaf or pod tissue rapidly.

The order of feeding preference on parts of *B. napus* by sixth instar larvae of *M. configurata* is leaves, bracts, immature pods, and mature pods (Bracken 1984). Larvae fed exclusively leaves from the middle of the fourth instar onwards pupated sooner, formed heavier pupae, and gained more dry weight than larvae fed on mature or immature pods. Mortality of larvae fed mature pods was 30% compared with no mortality for larvae fed leaves or immature pods.

Red Turnip Beetle. The red turnip beetle, *Entomoscelis americana* Brown, is an oligophagous insect which feeds on plants in the family Brassicaceae (Stewart 1973). Red turnip beetles are native to the north temperate region of North America (Gerber 1984). The eggs overwinter and hatch soon after snow melt in the spring (Gerber and Lamb 1982). The larvae feed on the cotyledons and the first true leaves of seedling volunteer canola and commercial mustards (*B. juncea* and *S. alba*) and on cruciferous weeds (Gerber and Obadofin 1981). Larvae and pupae develop in the spring and adults usually emerge by the end of June (Gerber 1982). Larvae pupate in the top 3 cm of the soil. The adults aestivate in July in the southern part of their range, but probably do not aestivate in the north (Gerber 1982). After aestivation the adults recommence feeding on crucifers. From early August to late October, they oviposit near the beetle's host plants under cover on the soil surface, in shallow crevices in the soil, or in loose soil to a depth of 6 mm (Gerber 1978, 1981).

Green Peach Aphid and Turnip Aphid. Aphids ingest plant sap from the phloem. During the course of probing, aphids inject pectinase from their saliva into the intercellular spaces in the plant (Dreyer and Campbell 1987). The injected pectinase catalyses the

digestion or depolymerization of the middle lamellar pectin. The activity of this injected pectinase system mediates the rate of insect probing through the intercellular pectin.

Lipaphis erysimi (Kaltenbach), commonly known as the turnip aphid or the mustard aphid, feeds on many genera and species of Brassicaceae, including *Brassica* and *Sinapis* (Blackman and Eastop 1984). *Lipaphis erysimi* is restricted to plants containing glucosinolates, and possesses enzymes (glucosinolases) capable of hydrolysing glucosinolates to glucose, sulphate, and isothiocyanates or rearrangement products (MacGibbon and Beuzenberg 1978). *Brassica juncea* is more resistant to *L. erysimi* than are cultivars of *B. rapa* and *B. napus* (Bakhetia and Sandhu 1973, Bakhetia *et al.* 1984, Ronquist and Åhman 1990). This resistance has been attributed to the content and composition of glucosinolates (Malik *et al.* 1983, Narang and Atwal 1985). They are believed to interfere with the aphid's probing and feeding behaviour (Dilawari and Atwal 1987). Pods are the most suitable plant part for the development of *L. erysimi*, followed by stalks and tender leaves, with hard leaves being the most unsuitable (Tripathi *et al.* 1986).

The green peach aphid, *Myzus persicae* (Sulzer), causes direct injury to leaves and stems of canola, *B. napus* (Buntin and Raymer 1994). *M. persicae* is able to transmit over 100 virus diseases of plants; these plants represent about 30 different families, and include many major crops such as beans, sugar beet, sugar cane, brassicas, potatoes, tobacco, and citrus (Kennedy *et al.* 1962). Green peach aphids prefer older leaves of crucifers and are usually evenly distributed within a field (Trumble 1982, McLeod and Lowery 1989). The ecology of *M. persicae* was reviewed by van Emden *et al.* (1969).

Defensive Ecology of *Brassica napus* and *Sinapis alba*

The defensive ecology of crucifers seems to typify that of many ephemeral herbaceous plants, which rely to a great extent on being hard to find (unapparent) in natural habitats. Such plants often contain low concentrations of effective toxins (Feeny 1977). They probably benefit from a diversity of chemical defences in any one species and from association with other plants of different chemistry (Rhoades and Cates 1976, Feeny 1976). Most members of the Brassicaceae invade open ground and are quickly displaced by grasses or woody plants during normal ecological succession (Howe and Westley 1988).

Brassica napus is the result of a natural interspecific cross between *B. rapa* and *B. oleracea*. It is generally agreed that *B. napus* originated in southern Europe (Downey 1983). Varieties of *B. napus* and *B. rapa* with low erucic acid content in the oil (less than 5% erucic acid) and low glucosinolate content in the meal (less than 30 $\mu\text{mol/g}$) are referred to as canola (Sernyk and Stefansson 1983). The canola cultivars were developed because erucic acid was a suspected health hazard for humans and glucosinolates caused the meal to be unpalatable or toxic to farm animals (Lamb 1988).

Sinapis alba grows wild in the Mediterranean region, preferring wet and fertile conditions (Tsunoda 1980). It has been developed into a cultivated crop plant.

Oilseed *Brassica* plants produce glucosinolates (formerly referred to as mustard oil glycosides), which are characteristic of the Brassicaceae and a few other plant families (Underhill 1980, Lamb 1989). Glucosinolates are antifeedants for many polyphagous herbivores, but most insect pests of *Brassica* crops use glucosinolates or their breakdown

products as attractants or feeding stimulants (Feeny 1977, Lamb 1989). The glucosinolates comprise about 80 naturally occurring thioglucosides with side chains of varying structure (Larsen 1981, Rodman 1981). These compounds are stored in glucosidic form in plant tissues, separately from the thioglucosidases (= myrosinases) that co-occur in all plants that contain glucosinolates (Rodman 1981). Following injury to tissue, glucosinolates undergo hydrolysis catalysed by myrosinase to produce a complex array of products which include volatile isothiocyanates and several compounds with goitrogenic activity (Mithen 1992). Many of these products have been implicated in the interaction between *Brassica* and their pests and pathogens, and some may have a role in defence mechanisms. The role of glucosinolates and their hydrolysis products in the interaction between insect pests and *Brassica* has been reviewed by Chew (1988).

Glucosinolate levels in cruciferous seeds have been manipulated by plant breeders. Mustard quality has been enhanced by increasing the level of glucosinolates, whereas breeding from selected lines of oilseed rape has reduced the glucosinolate levels in canola seed (Fenwick *et al.* 1989).

Glucosinolates occur in all tissues of oilseed rape plants (Mithen 1992). Low glucosinolate oilseed rape cultivars have similar concentrations of leaf glucosinolates as high glucosinolate oilseed rape cultivars, and low glucosinolate cultivars are no more susceptible to pests and pathogens than high glucosinolate cultivars (Lamb 1988, Rawlinson *et al.* 1989, Williams *et al.* 1991, Inglis *et al.* 1992). The reduction in glucosinolates in low glucosinolate cultivars is specific to the seed (Milford *et al.* 1989, Inglis *et al.* 1992).

The major glucosinolate in the cotyledons of *B. napus* 'Westar' is glucobrassicin (3-

indolylmethyl glucosinolate), but lesser amounts of an unidentified aliphatic glucosinolate also occur. Glucobrassicin occurs in the lowest concentrations and amounts at seedling emergence in *B. napus* 'Westar' (Bodnaryk and Palaniswamy 1990).

The cotyledons of *S. alba* contain high concentrations of sinalbin, which does not occur in *B. napus*, and are fed on by flea beetles at one-half the rate of *B. napus* cotyledons (Bodnaryk 1991). Concentrations of sinalbin found in the cotyledons of *S. alba* may be responsible for the antixenosis exhibited by this species (Bodnaryk and Lamb 1991).

High concentrations of sinalbin in young cotyledons (up to 20 mM) and young leaves (up to 10 mM) of *S. alba* deter feeding of both *Ph. cruciferae* and *M. configurata*. Tiny young leaves of *S. alba* may also be protected by their high concentration of sinalbin. These deterrent levels, however, are transitory, and the concentration of sinalbin in the cotyledons and leaves rapidly declines in the developing plant, largely due to dilution by the increasing tissue biomass. The lower concentrations of sinalbin found in the older *S. alba* plant (2-3 mM) offer little or no protection against *Ph. cruciferae* and may actually stimulate the feeding of this crucifer specialist. The concentrations of sinalbin in the leaves of older plants are still effective in reducing the level of feeding by larvae of the more generalist feeder *M. configurata* (Bodnaryk 1991).

In crucifers, a general response to wounding is an increase in the concentration of glucosinolates (Koritsas *et al.* 1989, 1991, Birch *et al.* 1990, Bodnaryk 1992), the compounds that stimulate feeding in some crucifer feeding flea beetles (Nielsen 1977, 1988, Larsen *et al.* 1985, Birch *et al.* 1990). Mechanical wounding increases the glucobrassicin concentration in *B. napus* 'Westar', and 4-hydroxyglucobrassicin and glucobrassicin concentrations in *B.*

rapa 'Tobin', but the sinalbin concentration of *S. alba* remains unchanged (Bodnaryk 1992). However induced resistance is more readily and consistently detected in *S. alba* than in *B. napus* and *B. rapa* (Palaniswamy and Lamb 1993). It is therefore likely that factors other than glucosinolates play a role in the induced resistance to flea beetles in *S. alba*.

S. alba has a dense mat of pubescence on the undersurface of its cotyledons, stems, and petioles, but *B. napus* does not. Although pubescence has been connected with the decreased susceptibility of plants to some insect pests (Hoxie *et al.* 1975, Lyman and Cardona 1982), it does not seem to be an important resistance factor for seedlings of *S. alba* (Brandt and Lamb 1993). The stiff hairs on the pods of *S. alba* appear to protect the pods from feeding by flea beetles, however (Lamb 1980).

Brassica villosa Biv. and *B. villosa* Biv. subsp. *drepanensis*, which have trichome densities of >2172 per cm^2 , are highly resistant to flea beetle feeding (Palaniswamy and Bodnaryk 1994). A high density of trichomes on uninjured *B. villosa* leaves acts as a physical barrier to flea beetle feeding by preventing the flea beetles from firmly settling on the leaf surface to initiate feeding. Flea beetles readily initiate feeding on areas of *B. villosa* leaves where trichomes are inadvertently injured, suggesting the plant does not contain significant concentrations of feeding deterrents. Trichomes are responsible for the resistance in *B. villosa* plants to flea beetle feeding.

The two Brassicaceae species, yellow mustard, *S. alba* 'Ochre', and canola, *B. napus* 'Westar' have different levels of tolerance to flea beetle feeding (Bodnaryk and Lamb 1991, Brandt and Lamb 1994). Seedlings of *S. alba* are tolerant to low levels of flea beetle feeding injury to their cotyledons and continue to grow at the same rate as uninjured seedlings,

whereas the growth of *B. napus* declines continuously with the level of feeding injury.

Summary

Although there has been an abundance of research on plant compensation for herbivory, much of the research focuses on specific plant-herbivore interactions. Few studies have compared plant compensation for feeding by different insect herbivores. This may be because of problems comparing damage from different herbivorous insects. A detailed understanding of how plant-herbivore systems function must address not only the general ways that a plant responds to herbivory, but also the specific responses a plant may have to individual insects.

With much research already having been performed on secondary plant compounds in crucifers and how these compounds interact with insect herbivores, this insect-plant system provides a good model to test hypotheses regarding insect-plant interactions. The insect fauna associated with *Brassica* and *Sinapis* species has a diverse range of feeding patterns. Each of these insects is regarded as a pest of cruciferous crops, although some are considered more destructive than others. What makes one insect more destructive than another is not always clear, and methodology for quantifying the impacts that different insects have on plants has only recently begun to emerge. The goal of the following research is to provide a basis so that the impacts of different insects can be compared, and to determine some of the plant and insect factors that determine the level of impact an insect herbivore has on a plant.

CHAPTER 3

A COMPARISON OF THE SPECIFIC IMPACTS OF A DIVERSE GROUP OF INSECT HERBIVORES ON CRUCIFER SEEDLINGS

Abstract

This research tests the hypothesis that the specific impacts vary for different insects feeding on a particular species of plant. The specific impact is estimated by a biomass conversion ratio (reduction in plant biomass / gain in insect biomass). A series of three experiments was performed with two defoliating herbivores, *Mamestra configurata* (Walker) and *Entomoscelis americana* (Brown), to estimate their specific impacts on seedlings of *Brassica napus* L. and *Sinapis alba* L., and the effect of insect density on the specific impacts. Then, experiments were performed that allow comparisons of specific impacts among herbivores, first between two closely related flea beetles that defoliate seedlings, and then a diverse group of defoliators and sap feeders. The specific impact of *M. configurata* was dependent on insect density in *S. alba*, but not in *B. napus*. The specific impact of *E. americana* did not differ between plant species, but in an experiment on *B. napus* there was a density dependent effect. There were no significant differences in specific impact between species of flea beetles. *Plutella xylostella* (L.) had a higher specific impact than *M. configurata* or *E. americana*. Specific impacts varied from 3 to 18 for larval defoliators, 11 - 16 for aphids, and 80 - 150 for flea beetles. This research supports the hypothesis that the specific impact of an insect feeding on a plant varies for different insect herbivores.

Introduction

Insect herbivores are diverse and attack plants in a variety of ways. Few studies, however, have compared the impacts of different types of insects feeding on the same host plant (Inouye 1982, Moran and Whitham 1990, Strauss 1991, Meyer 1993, Lamb and MacKay 1995). A herbivore may have a high impact on a plant either because it has a severe, species-specific effect on plant growth (a high specific impact) or because its population increases rapidly (a high numeric impact) (MacKay and Lamb 1996).

Although attempts have been made to compare the impact that herbivores have on their host plants, the results of such studies have often been complicated by different interpretations of what constitutes impact. Lamb's-quarters, *Chenopodium album* L., was not significantly affected by the root feeding aphid *Pemphigus betae* Doane, but overall host mass and seed set was reduced by the leaf gall forming aphid *Hayhurstia altriplicis* L. (Moran and Whitham 1990). The relative growth rate of goldenrod, *Solidago altissima* L., was reduced by both a xylem sap-feeding spittlebug, *Philaenus spumarius* L., and a leaf-chewing beetle, *Trihabda* sp., but not by a phloem-sap feeding aphid, *Uroleucon caligatum* Richards (Meyer 1993). The impact of the spittlebug was more severe than that of the beetle. These attempts to compare the impact of different herbivores are limited by the fact that it is not clear whether there was a species specific effect of the herbivore, a herbivore specific response by the plant, or more herbivory by one insect than another. Determining which insect species are most damaging to a plant requires a quantifiable measurement of the specific impact that each species of herbivore has on its host plant. MacKay and Lamb

(1996) used a biomass conversion ratio, the biomass lost by the plant due to feeding divided by the biomass gained by the insect, to measure the specific impacts (per unit of insect biomass) of five species of aphids feeding on barley, *Hordeum vulgare* L. The specific impacts were similar, with a 3.4 mg reduction in plant growth for each milligram gained in aphid biomass. Whether a specific impact of 3.4 is unique to this group of aphids, or is common for many insect-plant systems needs to be determined.

Agricultural crops are often useful for studying basic ecological processes because agricultural communities may be simpler than natural ones (Via 1990), and much is often known about crop plants and the way they are damaged by insects. Crop plants are usually more uniform and more easily manipulated than uncultivated ones and are therefore amenable to more precise experimentation.

Canola, *Brassica napus* L., and yellow mustard, *Sinapis alba* L., are attacked by a variety of insects (Lamb 1989) and *S. alba* is more tolerant of some insect injury than *B. napus* (Bodnaryk and Lamb 1991, Brandt and Lamb 1994). The insects feed in different ways on these plants. The green peach aphid, *Myzus persicae* (Sulzer), and the mustard aphid, *Lipaphis erysimi* (Kaltenbach), ingest plant sap from the phloem of leaves and stems of *B. napus* (Buntin and Raymer 1994). Larvae of the bertha armyworm, *Mamestra configurata* (Walker), diamondback moth, *Plutella xylostella* (L.), and the red turnip beetle, *Entomoscelis americana* Brown, and adults of the flea beetles *Phyllotreta cruciferae* (Goeze) and *Phyllotrata striolata* (F.) are all defoliators of *B. napus* and *S. alba*, but the patterns of defoliation differ.

Feeding by *Ph. cruciferae* and *Ph. striolata* results in small holes or pits in the

epidermis of leaves. Although the feeding injury does not penetrate the leaf completely, the tissues below the injury eventually dry up and break or fall out giving a shot-holed appearance (Westdal and Romanow 1972). First instar larvae of *Pl. xylostella* mine the leaf tissue (Harcourt 1957). Older larvae feed from the lower leaf surface and usually consume all tissue except the wax layer on the upper surface, thus creating a window in the leaf (Talekar and Shelton 1993). Larvae of *E. americana* feed on the cotyledons and first true leaves of their cruciferous hosts, consuming large contiguous areas of tissue, especially when they are in later instars (Gerber and Obadofin 1981). Larvae of *M. configurata* feed initially on the leaves, but later instars may also attack maturing pods (Bracken and Bucher 1977). They also consume large contiguous areas of tissue.

This research tests the hypothesis that the specific impact of an insect feeding on a plant varies for different insect herbivores. Specific impacts were estimated by a biomass conversion ratio for five defoliating and two phloem feeding species of insects on *B. napus*, and two defoliators on *S. alba*.

Materials and Methods

A series of three experiments was performed on two defoliating herbivores to estimate their specific impacts on seedlings of *B. napus* and *S. alba*, and the effect of insect density on specific impact. Then, experiments were done that allow comparisons of specific impacts among herbivores, first between two closely related flea beetles that defoliate seedlings, and then a diverse group of defoliators and sap feeders.

Bertha Armyworm on *B. napus* and *S. alba*

Three seeds of *B. napus* 'Excel' were planted 2 cm deep in each of 60 plastic pots, 11 cm diam. by 10 cm high, containing a clay loam soil with added sand and peat. Two days later, three seeds of *S. alba* 'Ochre' (which germinates and grows more rapidly) were planted in an additional 60 pots. Pots were placed in a chamber with a photoperiod of 18 h light and 6 h dark, and a day:night temperature of 24:18°C. All pots received 150 ml of water when soil moisture became low. Shortly after germination, one healthy plant per pot was selected and the other plants discarded. After 14 days, 100 ml of a 6.67g/l solution of Plant-prod® 20-20-20 fertilizer was added to each pot.

When *B. napus* plants were in the 3-4 leaf stage (18 days after planting), and *S. alba* plants were in the 5-6 leaf stage (16 days after planting), the 40 most uniform plants of each species were selected for use in the experiment and other plants were discarded. Plants were paired by size, within species, resulting in 20 pairs for each species. Either one or two larvae

were assigned randomly to one member of each pair. The experiment was set up as a randomized complete block design with 10 replications per treatment.

Each plant was covered with a plexiglass cage, 10 cm diam. by 26 cm high, ventilated with a fine mesh cover both at the top, and through two 5 cm diam. holes in the sides. Probes from a YSI tele-thermometer were placed to determine temperature inside and outside one of the cages. Temperature in the growth cabinet was regulated to provide a daytime temperature inside the cage of 24°C. Fifteen larvae of similar size to those placed on the plants were chosen from the insect colony, killed in 95% ethanol, dried, and weighed. A mean larval weight at the time of infestation was calculated.

Insects fed on the plants for one week. Insects from each cage were killed in 95% ethanol in separate small, preweighed aluminum foil dishes. Plants were separated into leaves and support tissue. Insects, leaves, and support tissue were dried to constant weight at 80°C and the weights recorded.

Red Turnip Beetle on *B. napus* and *S. alba*

The above experiment was repeated using 3rd instar larvae of the red turnip beetle. *B. napus* was infested 14 days after planting, and *S. alba* was infested 12 days after planting.

Another experiment was conducted with red turnip beetles on *B. napus*, using one, two, or four 2nd-instar-larvae per plant. Plants were grown as described previously. Thirteen days after planting, leaf length was measured and summed for each plant. Plants with a similar leaf length (0.1 cm or less difference between plants) were matched by computerized

sorting of leaf lengths. Forty-five pairs of plants were chosen giving 15 replicate pairs for each larval density. Fourteen days after planting, plants were infested randomly among pairs, using a camel hair brush to place larvae on one plant in each pair, and all plants were caged.

For both experiments, plants were arranged in a randomized complete block design. Fifteen larvae of the same stage as those placed on the plants were taken from the insect colony, killed in 95% ethanol, dried, and weighed. A mean larval weight at the time of infestation was calculated.

Insects fed on the plant for seven days. Harvest procedures were the same as reported previously.

Two Species of Flea Beetles on *B. napus*

The specific impacts of *Ph. striolata* and *Ph. cruciferae* on *B. napus* seedlings were estimated in separate experiments. For *Ph. striolata*, 100 plants were grown as described above. Six days after planting the widths of the two cotyledons were measured for each plant. Plants were sorted into 20 groups of three, based on similarity of cotyledon width, and caged. Adult beetles were collected from the field using traps baited with allyl isothiocyanate (Burgess and Wiens 1980). Seven days after planting, five or 10 female *Ph. striolata* were added to one of the caged plants, selected randomly from each of the 20 groups, and one plant was left uninfested as a control. Seventeen beetles were also taken from the flea beetle collection, killed in 95% ethanol, dried, and weighed to determine the mean weight at the time of infestation. Insects were left to feed on the plants for five days and then the caged

pots were frozen so that the small active beetles could be recovered. Harvest procedures were as reported above.

Because it was difficult to recover all *Ph. striolata* from the soil surface after the soil was disturbed by removing the cage, a different caging system was used for *Ph. cruciferae*. Plants were grown in styrofoam cups and caged using ventilated plastic bags that fitted tightly around the outside of each cup's rim. Plants were paired by size as described above and five flea beetles of unknown sex were placed on one of the two plants in a pair, chosen randomly. Twenty-one replicates were used.

Three Defoliators and Two Sap-Feeders on *B. napus*

Brassica napus 'Excel' was grown as described above for bertha armyworm. Thirteen days after planting the total leaf length for each of 166 plants was measured, and pairs of plants were matched as described above. Fifty pairs of plants were arranged in the growth chamber in a randomized complete block design with 10 replicates for each of the five insects. Plants were infested 14 days after planting. Insects and their densities were; eight 2nd-instar diamondback moth larvae, one 3rd-instar bertha armyworm larva, two 2nd-instar red turnip beetle larvae, five 7-day-old mustard aphids (young adults), and five 6-day-old green peach aphids (young adults). Insects were placed on one plant of each pair and caged as described for bertha armyworm. Fifteen insects of each species, similar in age and size to those placed on the plants were taken from the insect colonies, killed in 95% ethanol, dried, and weighed to determine the mean weight at the time of infestation. Only nine red turnip

beetle larvae were available for weighing. Insects fed on the plant for seven days and harvest procedures were as reported for bertha armyworm.

Statistical Analyses

Reduction in shoot biomass due to insect feeding was calculated by subtracting the total aboveground plant biomass of the insect injured plant from that of the paired control plant at the time insects were removed. Biomass gained by the insects was calculated by subtracting the initial insect weight from the final insect weight. Differences in biomass for plants or insects were analysed using totals, and on a per insect basis, using analysis of variance (ANOVA) or unpaired *t*-tests (SAS Inst. 1989). Where the *F*-value of an ANOVA was significant, Tukey's Studentized Range Test was used to discriminate means. Data for reduction in dry biomass of shoots per insect was log transformed prior to analysis for the experiments where red turnip beetles fed on *B. napus* and *S. alba*, and where five different insects fed on *B. napus*. Data for gain in dry biomass of insects per insect was also log transformed prior to analysis in the experiment where five insects fed on *B. napus*. Log transformations were performed to reduce the variability in error among samples. Transformed data were reexamined to confirm that variances were stabilized before proceeding with the analysis.

Specific impacts (MacKay and Lamb 1996) were estimated as biomass conversion ratios for each insect-plant interaction and each insect density:

$$\sum(\text{shoot biomass of control plant} - \text{shoot biomass of injured plant}) / \sum(\text{final weight of insects} -$$

initial weight of insects), i.e. mg reduction in shoot biomass per mg gain in insect biomass. Standard errors for the specific impacts, which are ratios, were calculated according to Cochran (1977). Differences in specific impacts between treatments were analysed using unpaired *t*-tests (SAS Institute 1989). A significance level of $P < 0.05$ was used.

Results

Bertha Armyworm Larvae on *B. napus* and *S. alba*

Two bertha armyworm larvae feeding on a plant caused a greater reduction in shoot biomass ($F=38.77$; $df=1,26$; $P=0.0001$) and a greater gain in total insect biomass ($F=7.27$; $df=1,26$; $P=0.0121$) than one larva feeding on a plant. When two larvae were added, approximately half of the foliage was consumed (Table 1), and in two *B. napus* plants and one *S. alba* plant all the leaf tissue was eaten. The reduction in shoot biomass per insect was not significantly different between the two levels of insects ($F=3.49$; $df=1,26$; $P=0.0729$), although there was a trend towards an increased reduction in shoot biomass per insect when a second larva was feeding on the plant (Table 1). The gain in biomass per insect, was also not significantly different when two larvae were feeding on a plant than when one larva was feeding on the plant ($F=4.13$; $df=1,26$; $P=0.0523$). There was no significant interaction between type of plant and level of infestation for either reduction in dry biomass of the shoot/insect ($F=0.11$; $df=1,26$; $P=0.7469$), or gain in dry biomass of insects/insect ($F=1.33$; $df=1,26$; $P=0.2601$).

The specific impact of bertha armyworm larvae was dependent on insect density in *S. alba*, but not in *B. napus*. On *S. alba*, two larvae per plant resulted in a higher specific impact ($t=2.18$; $df=18$; $P=0.0426$) than one larva per plant (Table 1). Density of larvae had no effect on specific impacts in *B. napus* ($t=1.87$; $df=18$; $P=0.0771$), although the trend was in the same direction as for *S. alba*. There were no differences in specific impact between *B.*

Table 1. Mean specific impact, reduction in shoot biomass per insect, and gain in insect biomass per insect, for larvae of the bertha armyworm, *Mamestra configurata*, feeding for seven days on canola, *Brassica napus*, and yellow mustard, *Sinapis alba*

Plant	Insects /plant ¹	n	Specific impact ² $\pm$ SE	Reduction in dry biomass of the shoot / insect, $\text{mg} \pm \text{SE}^3$	% reduction in dry biomass of the shoot	Gain in dry biomass of insects / insect, $\text{mg} \pm \text{SE}$	Mean initial dry biomass (mg $\pm$ SE) of larvae ⁴
Canola	1	10	3.9 ± 1.0	193 ± 36	19 ± 3	50 ± 8	4.7 ± 0.4
Canola	2	10	6.2 ± 0.7	272 ± 40	51 ± 8	44 ± 6	9.4 ± 0.7
Mustard	1	9	3.8 ± 0.8	220 ± 46	21 ± 4	57 ± 10	4.7 ± 0.4
Mustard	2	10	7.5 ± 1.5	270 ± 30	49 ± 8	36 ± 4	9.4 ± 0.7

¹Canola was infested 18 days after planting, and mustard was infested 16 days after planting.

²Specific impact = reduction in shoot biomass / gain in larval biomass.

³Mean shoot biomass of a control plant at the end of the experiment was $1,072 \pm 48$ mg for *Sinapis alba* (n=19) and $1,043 \pm 36$ mg for *Brassica napus* (n=20).

⁴Mean initial dry biomass of insects = mean biomass at the time of infestation of 15 larvae similar to those placed on the plants multiplied by the number of insects placed on the plant.

napus and *S. alba* with either one ($t=-0.04$; $df=18$; $P=0.9677$) or two ($t=0.79$; $df=18$; $P=0.4403$) larvae per plant.

Red Turnip Beetle Larvae on *B. napus* and *S. alba*

Infesting *S. alba* and *B. napus* with either one or two 3rd-instar red turnip beetle larvae resulted in no difference in reduction of shoot biomass per insect between levels of infestation ($F=0.30$; $df=1,19$; $P=0.5898$) and species of plant ($F=0.74$; $df=1,19$; $P=0.4006$) (Table 2). The gain in insect biomass production per insect did not differ when one or two larvae were placed on the plant ($F=0.32$; $df=1,19$; $P=0.5769$). The different species of plants did not result in differences in insect biomass production ($F=0.10$; $df=1,19$; $P=0.7608$). The specific impact of red turnip beetle larvae did not differ between plant species at either the high ($t=-0.05$; $df=14$; $P=0.9645$) or low ($t=-0.44$; $df=14$; $P=0.6660$) level of infestation, or between levels of infestation in *B. napus* ($t=-0.46$; $df=13$; $P=0.6535$) or *S. alba* ($t=0.13$; $df=15$; $P=0.8980$).

In a second experiment, two or four red turnip beetle larvae feeding on canola caused less of a reduction in shoot biomass per larva than an individual larva ($F=10.34$; $df=2,27$; $P=0.0005$). This is the opposite of the trend that was noted for larvae of the bertha armyworm. Four red turnip beetle larvae per plant caused less of a gain in insect biomass per larvae than one or two larvae ($F=22.48$; $df=2,27$; $P=0.0001$). In cages with four larvae, the gain in dry biomass of an individual larva was half that of a larva in cages that received two larvae per cage. Two red turnip beetle larvae per plant resulted in a lower specific impact

Table 2. Mean specific impact, reduction in shoot biomass per insect, and gain in insect biomass per insect for larvae of the red turnip beetle, *Entomoscelis americana*, feeding for seven days on canola, *Brassica napus*, and yellow mustard, *Sinapis alba*

Plant	Larvae/ plant ^{1,2}	n	Specific impact ³ $\pm$ SE	Reduction in dry biomass of the shoot / insect, mg $\pm$ SE ^{4,5,6}	% reduction in dry biomass of the shoot	Gain in dry biomass of insects / insect, mg $\pm$ SE ⁶	Mean initial dry biomass (mg $\pm$ SE) of insects ⁷
Experiment 1							
Canola	1	8	18.0 $\pm$ 4.9	145 $\pm$ 33	39 $\pm$ 10	8.1 $\pm$ 1.4	2.7 $\pm$ 0.3
Canola	2	7	15.3 $\pm$ 3.1	125 $\pm$ 19	71 $\pm$ 12	8.2 $\pm$ 0.9	5.4 $\pm$ 0.5
Mustard	1	8	14.0 $\pm$ 7.6	121 $\pm$ 62	34 $\pm$ 15	8.7 $\pm$ 0.8	2.7 $\pm$ 0.3
Mustard	2	9	15.1 $\pm$ 3.8	109 $\pm$ 28	63 $\pm$ 15	7.2 $\pm$ 1.2	5.4 $\pm$ 0.5
Experiment 2							
Canola	1	14	13.6 $\pm$ 1.8	167 $\pm$ 26a	44 $\pm$ 5	12.3 $\pm$ 0.9a	0.9 $\pm$ 0.04
Canola	2	15	10.3 $\pm$ 1.3	99 $\pm$ 12b	52 $\pm$ 6	9.6 $\pm$ 1.0a	1.9 $\pm$ 0.09
Canola	4	15	15.6 $\pm$ 1.2	75 $\pm$ 07b	83 $\pm$ 6	4.8 $\pm$ 0.5b	3.7 $\pm$ 0.17

¹Canola was infested 14 days after planting in both experiments 1 and 2, mustard was infested 12 days after planting.

²Plants were infested with 3rd instar larvae in experiment 1, and 2nd instar larvae in experiment 2.

³Specific impact = reduction in shoot biomass / gain in larval biomass.

⁴Mean shoot biomass of control plants at the end of experiment 1 was 374 $\pm$ 19 mg for *B. napus* (n=15) and 345 $\pm$ 45 mg for *S. alba* (n=17). Mean shoot biomass of control plants at the end of experiment 2 was 372 $\pm$ 20 mg (n=44).

⁵For experiment 1, log transformed values of the numbers presented were analyzed.

⁶Means within a column followed by the same letter are not significantly different ($P > 0.05$; Tukey's studentized range (HSD) test [SAS Institute 1989]).

⁷Mean initial dry biomass of insects = mean dry biomass at the time of infestation of 15 larvae similar to those placed on the plants multiplied by the number of larvae placed on the plant.

than four red turnip beetle larvae per plant ($t=2.90$; $df=28$; $P=0.0072$) (Table 2). By the end of the seven-day feeding period, 10 of the 15 plants infested with four larvae were completely defoliated, with only support tissue remaining. Feeding and growth of larvae may have been restricted on plants that were completely defoliated.

Green leaf tissue was found on the soil in some cages, due to the larvae feeding in a pattern that resulted in uneaten plant tissue being destroyed. Some of the larvae were in the soil when insects were recovered after the seven-day feeding period, probably indicating that the larvae had stopped feeding before the end of the experiment and were preparing to pupate.

Two Species of Flea Beetles on *B. napus*

Ten *Ph. striolata* reduced shoot biomass more than five *Ph. striolata* ($t=-2.20$; $df=31$; $P=0.0357$), although there was no difference in reduction in shoot biomass per insect for the two densities ($t=0.65$; $df=23$; $P=0.5215$) (Table 3). Reduction in shoot biomass did not differ between insect species ($t=-2.00$; $df=20.5$; $P=0.0588$), although reduction in dry biomass of shoots was higher for *Ph. striolata* than for *Ph. cruciferae* and may have been significant with a larger sample size. *Ph. striolata* gained more biomass per insect than *Ph. cruciferae* ($t=-4.12$; $df=36$; $P=0.0002$). There was no difference in biomass per insect between the two densities of *Ph. striolata* ($t=1.57$; $df=31$; $P=0.1268$).

There were no significant differences in specific impact between species of flea beetles ($t=0.94$; $df=36$; $P=0.3526$) or densities of *Ph. striolata* ($t=-0.49$; $df=31$; $P=0.6277$)

Table 3. Mean specific impact, reduction in shoot biomass per insect, and gain in insect biomass per insect, for the adult flea beetles *Phyllotreta striolata* and *Ph. cruciferae* feeding for five days on canola, *Brassica napus*

Flea Beetle	Beetles / plant ¹	n	Specific impact ² $\pm$ SE	Reduction in dry biomass of the shoot / insect, mg $\pm$ SE ³	% reduction in dry biomass of the shoot	Gain in dry biomass of insects / insect, mg $\pm$ SE	Mean initial dry biomass (mg $\pm$ SE) of insects ⁴
<i>Ph. striolata</i>	10	16	96 $\pm$ 20	4.6 $\pm$ 0.6	54 $\pm$ 4	0.048 $\pm$ 0.009	3.1 $\pm$ 0.1
<i>Ph. striolata</i>	5	17	81 $\pm$ 22	5.5 $\pm$ 1.2	34 $\pm$ 6	0.067 $\pm$ 0.008	1.6 $\pm$ 0.1
<i>Ph. cruciferae</i>	5	21	148 $\pm$ 67	2.9 $\pm$ 0.5	31 $\pm$ 5	0.020 $\pm$ 0.008	1.8 $\pm$ 0.1

¹Plants were infested 7 days after planting.

²Specific impact = reduction in shoot biomass / gain in insect biomass.

³Mean shoot biomass of control plants at the end of the experiments was 85.2 $\pm$ 8.2 mg for 10 *Ph. striolata* per plant (n=16), 80.9 $\pm$ 8.5 mg for 5 *Ph. striolata* per plant (n=17), and 46.1 $\pm$ 1.3 mg for 5 *Ph. cruciferae* per plant (n=21).

⁴Mean initial biomass of insects = mean biomass of a random sample of insects at the time of infestation multiplied by the number of insects placed on the plant.

(Table 3). The specific impacts due to flea beetle feeding were higher than for the other insects used in the study, but standard errors were high as well.

Three Defoliators and Two Sap-Feeders on *B. napus*

Reductions in the dry biomass of shoots per insect were not significantly different for the different insect species ($F=1.20$; $df=4,36$; $P=0.3267$) (Table 4). High standard errors associated with the means may have contributed to the differences not being significant, since large differences exist between the means. A bertha armyworm larva caused a reduction in shoot biomass at least 14 times that of either an individual green peach aphid or mustard aphid, and more than ten times greater than that of a diamondback moth larva. The gain in biomass of individual insects of each species differed ($F=309.78$; $df=4,36$; $P=0.0001$). A bertha armyworm larva gained more than 20 times more biomass than a diamondback moth larva or the aphid species. Gain in biomass by a red turnip beetle larva was intermediate between that of a bertha armyworm and that of an individual of either of the aphids.

Diamondback moth larvae had a higher specific impact than bertha armyworms ($t=-2.97$; $df=18$; $P=0.0082$) or red turnip beetle larvae ($t=-3.26$; $df=18$; $P=0.0044$) (Table 4). Although the total reduction in shoot weight was similar for the two Lepidoptera, a single bertha armyworm larva produced almost three times as much insect biomass as eight diamondback moth larvae.

There were no consistent differences in specific impacts between defoliators and

Table 4. Mean specific impact, reduction in shoot biomass per insect, and gain in insect biomass per insect for red turnip beetle larvae, *Entomoscelis americana*, diamondback moth larvae, *Plutella xylostella*, bertha armyworm larvae, *Mamestra configurata*, the green peach aphid, *Myzus persicae*, and the mustard aphid, *Lipaphis erysimi*, feeding for seven days on canola, *Brassica napus*. n = 10 in all treatments

Insect ¹	Insects /plant ²	Specific impact ³ ± SE	Reduction in dry biomass of the shoot / insect, mg ± SE ^{4,5}	% reduction in dry biomass of the shoot	Gain in dry biomass of insects / insect, mg ± SE ⁵	Mean initial dry biomass (mg ± SE) of insects ⁶
Red turnip beetle	2	3.5 ± 1.5	49 ± 19a	11 ± 5	13.57 ± 0.71b	1.7 ± 0.1
Diamond-back moth	8	11.7 ± 2.0	22 ± 4a	21 ± 3	1.93 ± 0.03c	0.7 ± 0.1
Bertha armyworm	1	5.0 ± 1.1	224 ± 62a	28 ± 8	44.96 ± 6.54a	4.5 ± 0.2
Green peach aphid	5	15.9 ± 6.1	16 ± 6a	9 ± 3	1.01 ± 0.09d	0.7 ± 0.1
Mustard aphid	5	11.6 ± 8.3	12 ± 8a	7 ± 4	0.99 ± 0.10d	0.7 ± 0.1

¹Plants infested 14 days after planting.

²The number of insects placed on a plant at the beginning of the feeding period. The aphid species were reproducing during the feeding period.

³Specific impact = reduction in shoot biomass / gain in insect biomass.

⁴Mean shoot biomass of control plants at the end of the experiment was 821 ± 15 mg (n=50).

⁵Means within a column followed by the same letter are not significantly different ($P > 0.05$; Tukey's Studentized Range (HSD) Test [SAS Institute 1989]). Log transformed values of the numbers presented were analyzed.

⁶Mean initial biomass of insects = mean biomass of a random sample of insects at the time of infestation multiplied by the number of insects placed on the plant.

phloem feeders (Table 4). The two species of aphids, phloem feeders, both had high specific impacts, but also had higher standard errors associated with the specific impacts than did the defoliators. Two of the defoliators, red turnip beetle larvae and bertha armyworm larvae, had much lower specific impacts than the phloem feeders, but diamondback moth larvae had a specific impact similar to that of the aphids.

There were no differences in specific impact between crucifer specialists and generalists (Table 4). Bertha armyworm larvae, which are generalist feeders, had a similar specific impact to that of the red turnip beetle, a crucifer specialist, but a lower specific impact than another crucifer specialist, the diamondback moth. The generalist green peach aphid had a specific impact similar to that of the mustard aphid, a crucifer specialist.

Discussion

This research supports the hypothesis that the specific impact of an insect feeding on a plant varies for different insect herbivores. Specific impacts varied from 3 to 18 for larval defoliators, 11 - 16 for aphids, and 81 - 148 for adult flea beetles.

Different specific impacts of defoliators on a plant may be due to the plant compensating differently for injury by different insects, and differences in the efficiency with which plant tissue removed or injured by the consumer is converted into insect biomass. The level of plant compensation for insect injury may be determined by what part of the plant is consumed (Inouye 1982), the pattern of tissue removal (Mauricio *et al.* 1993), and circumference of leaf area lost (Hall and Ferree 1976) by leaf-chewing insects. Compounds in insect saliva may also affect the compensatory ability of the plant (Detling and Dyer 1981). Some insects may feed in a manner that results in the destruction of tissues not eaten while other insects may only destroy the tissue that is consumed (Lowman 1982), which may result in different efficiencies of converting plant tissue consumed or injured into insect biomass.

The reason that higher densities of bertha armyworm larvae resulted in an increased specific impact on *S. alba* are not clear. Both a weakened compensatory response by *S. alba* and a reduced insect growth rate due to competition among larvae may have contributed to the higher specific impact. The density dependent response of bertha armyworm larvae on specific impact in *S. alba* is not a general response to insect feeding, however. A density dependent effect on specific impact was not found for red turnip beetle larvae feeding on *S.*

alba or *B. napus*, or for five species of aphids feeding on barley, *Hordeum vulgare* L. (MacKay and Lamb 1996).

Data from the literature can be used to calculate a specific impact for bertha armyworms feeding on more mature *B. napus* plants, which is their natural host, than were used in these experiments. Yield loss of canola per larva was found to be 0.402g/larva (Bracken and Bucher 1977). Mean dry weight of larvae produced on *B. napus* was 0.119g (Bailey 1976). Thus, reduction in yield per g of larvae produced = $0.402/0.119 = 3.38$. This estimate of specific impact is similar to those for bertha armyworm larvae obtained in these short-term experiments on young plants.

Specific impacts for bertha armyworms feeding on *B. napus* were similar in the different experiments presented here. Specific impacts were not consistent between experiments where red turnip beetle larvae were feeding on *B. napus*, however. Specific impact was higher in the experiments where only red turnip beetle larvae were used (Table 2) than in the experiment where five insect species were used (Table 4). Dry biomass of the shoot per insect was reduced less due to feeding by larvae of red turnip beetles in the latter experiment. The reason for this difference in specific impacts is not known, however plants in the latter experiment were larger than plants in the former experiments. The lower specific impact for red turnip beetles in table 4 than table 2 may be because the larger plants were able to compensate better for the feeding.

Flea beetles had much higher specific impacts than those of the other insects. Adult flea beetles were used in this experiment. All other insects used in this study were immatures, except for the aphids. Aphids were born during the feeding period however, and immatures

would have accounted for most of the insect biomass gain and some of the reduction in plant biomass. Insect biomass on the plants increased by several times for all insects except for flea beetles, where production of new insect biomass was only 5-22% of the initial biomass. The lower production of biomass by flea beetles was expected because adults were used. Flea beetles are more mobile than the other insects used in the study, and may have spent a greater proportion of their energy moving around and less on growth than the other insects. Seven day old plants were infested in the experiments with flea beetles, whereas in the other experiments plants were 14 days old or older. This difference in plant age may be another factor contributing to higher specific impacts for flea beetles than for the other insects.

The specific impacts for the aphids *M. persicae* and *L. erysimi* are high in comparison with those found for other aphid-plant systems. The specific impacts of the aphids *Metopolophium dirhodum* (Walker) and *Rhopalosiphum padi* (L.) feeding on wheat and barley were close to 3.0, regardless of aphid species, plant cultivar, or aphid density (Lamb and MacKay 1995). This suggests a very efficient insect-plant interaction compared with most of the herbivores studied on *B. napus*. One possible reason for the differences in the specific impacts for these aphids (Lamb and MacKay 1995) and the present study may be due to differences in the way grasses and cruciferous plants respond to herbivory (Trumble *et al.* 1993).

There is much uncertainty about the impact of sap feeding insects on plants relative to the impact of leaf-chewing insects. Sap feeding insects are regarded by some as less damaging than leaf chewers (Coulson and Witter 1984, Fitter and Hay 1987) because presumably they can feed on the products of photosynthesis without destroying the

photosynthetic machinery (Llewellyn 1972). The data presented here does not support this hypothesis, however.

The volume of resource removed, and not just the type, must be considered to determine how an insect will affect plant growth. Aphids are specifically adapted to tap directly the metabolites in the phloem. The process is potentially wasteful, because to assimilate the amino acids they need, aphids use only a small part of the carbohydrate they ingest, the rest being excreted as honeydew (Osborne 1973, Dreyer and Campbell 1987). Mittler (1958) suggested that in one day a single willow aphid could account for the photosynthetic products of 5-20 cm² of leaf. Thus it is difficult to predict whether leaf feeders or sap feeders should lead to a greater reduction in plant weight per gram of insect produced.

The relative growth rate of goldenrod (*Solidago altissima* L.) was reduced more by a leaf-chewing beetle (*Trirhabda* sp.) than by a phloem sap-feeding aphid (*Uroleucon caligatum* Richards) (Meyer 1993). The present studies, however, show that there are large differences in the specific impacts that leaf chewing insects have on plants. Thus trying to determine whether leaf or phloem feeding insects are more destructive to a plant may be complicated by the fact that with both of these types of feeding there are factors other than type of food consumed that affect how a plant responds to the injury.

Too much or too little insect injury to the plant may reduce the precision with which specific impacts can be calculated. Some experiments had high standard errors associated with the means for the specific impacts. High standard errors often occurred where there was a low level of damage to the plant. In any of the treatments where the percent reduction in dry biomass of the plant was less than 15%, the standard error was at least 35% of the mean

specific impact. When percent reduction in dry biomass of plants was above 40%, the standard error was always less than 26% of the mean specific impact. To minimize the variation associated with the specific impacts, injury levels must be great enough to overcome the effect of natural variation between plants, and yet not high enough to result in the death of the plant. A percent injury level of 20-90% should be aimed for to reduce the chances of the standard errors associated with the specific impacts being high.

This research shows that the specific impacts of different insects on a plant varies. The different specific impacts that defoliating insects have on *B. napus* may be due to *B. napus* plants compensating better for injury by certain insects, differences in the efficiency with which plant tissue removed or injured by the consumer is converted into insect biomass, or a combination of the above two reasons. Further experiments are required to determine why these insects have different specific impacts on plant growth.

CHAPTER 4**COMPENSATION BY TWO CRUCIFEROUS PLANTS FOR HERBIVORY BY
THREE INSECT DEFOLIATORS**

Abstract

This research tested the hypothesis that plant compensation for defoliation by insects is specific to the injury caused by particular insects, and is not a generalized response to a certain level of injury to the plant. The hypothesis was tested by measuring the responses of *Brassica napus* L. and *Sinapis alba* L. to injury to seedlings by *Phyllotreta cruciferae* (Goeze), larvae of *Plutella xylostella* L., or larvae of *Mamestra configurata* (Walker). Six controlled environment studies tested the responses of *S. alba* 'Ochre' and *B. napus* 'Excel' to the three insects. Plants in the cotyledon stage received either 0, 25 or 75% feeding injury. The dry weight of leaves, support tissue, and roots were measured for six plants from each treatment for four weeks after the injury. In a field study, *S. alba* and *B. napus* received 0, 25, or 75% injury by the three species of insects to plants in the cotyledon stage. Leaf length was measured at 9, 16, 25, and 31 days after the injury, and growth stage of the plants was measured at 9, 16, 25, 31, 45, 52, 58, 65, and 73 days after the injury. After plants were harvested, the number of seeds, average weight of seeds, and rate of germination were measured for each treatment. In both *B. napus* and *S. alba* the growth of leaves, support tissue, and roots were all dependent on the type of insect injury to seedlings. Although injured to the same levels by all insects, plants compensated most for injury by *M. configurata*, and least for injury by *Ph. cruciferae* in both experiments. *B. napus* recovered better from the injury than *S. alba*. Both plant species compensated better for 25% injury to the cotyledons than for 75% injury to the cotyledons. Laboratory experiments in controlled environments showed similar patterns of recovery for leaf growth as the field experiment,

but recovery was more rapid in the controlled environments. *Sinapis alba* with 75% injury by *Ph. cruciferae* produced fewer seeds than uninjured plants, even though leaf length had recovered by 31 days after the injury. Complete recovery of leaf tissue does not always imply that there will be a complete recovery of plant fitness. This research supports the hypothesis that plant compensation for defoliation by insects depends on the insect causing the injury, and is not a generalized response to injury.

Introduction

The specific impact that insect herbivores have on a plant varies for different insects (Chapter 3). One possible reason for the difference in specific impacts among insects is that plant compensation is specific to the injury caused by particular herbivores.

Plant compensation is the process through which an injured plant mitigates or nullifies the effect of injury (Bardner and Fletcher 1974). Different methods have been suggested for measuring compensation. Belsky (1986) divided compensatory growth into three categories based on the cumulative total dry weight (including removed tissue) of the injured plant in comparison to the total dry weight of uninjured plants. Maschinski and Whitham (1989) divided compensatory ability into three classes based on the fruit and seed production of injured plants compared to uninjured plants. In this thesis, plant compensation will be viewed as the changes in individual aspects of plant growth as a result of injury, and measured as the dry weight of the tissue relative to the weight of the tissue had the injury not occurred. Recovery will refer to the production of an equal quantity and quality of seeds as an uninjured plant.

Belsky *et al.* (1993) hypothesized that regrowth in plants is a generalized response by plants to all types of injury - fire, trampling, desiccation, disease, wind, frost, among many others - and not an adaptation to specific types of herbivory. Regrowth is a major part of a plant's compensatory ability, and this hypothesis raises the question, how general a response is plant compensation?

The level of injury and the stage of the plant that is injured may both be important

factors in determining to what level a plant compensates for injury by insect feeding. A plant is most vulnerable to defoliation when its seed reserves have run out and it first relies on the products of its own photosynthesis for growth and survival (Crawley 1983). If herbivory occurs during the seedling stage, or after tissues have matured, mortality or little compensatory growth may occur (Whitham *et al.* 1991).

Pattern of tissue removal may affect plant growth (Hall and Ferree 1976, Lowman 1982, Marquis 1992, Mauricio *et al.* 1993). It is still not certain, however, whether compensation is specific to injury by particular herbivores, or is just a reaction by the plant to losing a certain amount of plant tissue. A thorough knowledge of plant compensation for herbivory would be an asset to developing sound integrated pest management programs for agricultural pests (Trumble *et al.* 1993).

Because agricultural communities are often simpler than natural ones, they can provide good models for the study of basic evolutionary processes (Via 1990). Much is often known about crop plants and the way that they are damaged by insects, and crop plants can be manipulated in the field in ways that noncultivated plants may not tolerate.

Much research has been performed on the impact of insect herbivory on the Brassicaceae (=Cruciferae) species *Brassica napus* L. and *Sinapis alba* L. For example, yellow mustard, *S. alba*, and canola (oilseed rape), *B. napus*, have different levels of tolerance to feeding by the crucifer flea beetle, *Phyllotreta cruciferae* (Goeze) (Bodnaryk and Lamb 1991, Brandt and Lamb 1994). Seedlings of *S. alba* are tolerant of low levels of injury to their cotyledons and recover rapidly, whereas the growth of *B. napus* declines continuously with the level of feeding injury. This difference occurs although flea beetles

feed in similar ways on the two plants (Brandt and Lamb 1993).

Cruciferous plants are attacked by a variety of defoliating insects which feed in different manners. The crucifer flea beetle, *Ph. cruciferae*, and the striped flea beetle, *Ph. striolata* (F.), are oligophagous herbivores primarily of crucifers. Adult beetles feed on the cotyledons and slender stems of seedlings, and also attack true leaves (Feeny et al. 1970, Brandt and Lamb 1993). The characteristic injury consists of small holes or pits in the epidermis of leaves. Although the feeding injury does not penetrate the leaf completely, the tissues below and surrounding the injury eventually dry up and break or fall out giving a shot-hole appearance (Westdal and Romanow 1972).

The diamondback moth, *Plutella xylostella* (L.), feeds only on plants in the mustard family Brassicaceae (Talekar and Shelton 1993). First instar larvae burrow into the leaf from the lower surface and feed on the mesophyll, making a shallow whitish mine (Beirne 1971). Older larvae feed from the lower leaf surface and usually consume all tissue except the wax layer on the upper surface, thus creating a window in the leaf (Talekar and Shelton 1993).

Larvae of the bertha armyworm, *Mamestra configurata* (Walker), are general feeders on broad-leaved plants, including canola, flax, sweetclover, alfalfa, cabbage, corn, peas, beans, turnips, beets, tobacco, and garden flowers (Beirne 1971). On canola the larvae feed initially on the leaves. During the last two instars larvae attack the maturing pods as well. Little measurable economic loss occurs until pods are injured (Bracken and Bucher 1977). Eighty percent of the total plant tissue eaten by larvae occurs during the sixth and last instar (Bailey 1976). When bertha armyworm larvae are placed on seedlings of *B. napus* or *S. alba* they feed in a manner that results in little injury to tissue not consumed (personal

observations).

The purpose of this research is to test the hypothesis that plant compensation for defoliation by insects is specific to the injury caused by particular insects, and is not a generalized response to a certain level of injury to the plant. This hypothesis will be tested by measuring the responses of *B. napus* and *S. alba* to injury to seedlings by crucifer flea beetles, diamondback moth larvae, or bertha armyworm larvae.

Materials and Methods

Controlled Environment Study

The experiments were performed six times, sequentially, so the responses of *S. alba* 'Ochre' and *B. napus* 'Excel' to the three insects could be tested. The protocol presented below uses *B. napus* and diamondback moth larvae as examples.

Two seeds of *B. napus* were planted in each of 160 plastic pots, 11 cm diam. by 10 cm high, containing a clay loam soil with added sand and peat. Pots were randomly placed in a growth chamber with an 18h light:6h dark photoperiod, and a day:night temperature of 24:18°C. Plants were watered as needed with 150 ml of water per pot, and fertilized 14, 21, and 28 days after planting with an aqueous solution of 0.67 g/pot of Plant-Prod® 20-20-20 (N-P-K) fertilizer. Shortly after germination, one plant per pot was chosen for use in the experiment and the other plant pulled out.

When plants were in the cotyledon stage (seven or eight days after planting), 140 plants having approximately equal cotyledon area were selected for further use and the other plants discarded. Fifty plants were infested with two second instar diamondback moth larvae, and 50 plants were infested with four second instar diamondback moth larvae to achieve a low (25%) and a high (75%) level of injury to the plants. Larvae were transferred to plants using a camel hair brush. Larvae were caged on individual plants using inverted 3.5 cm diam. by 6.2 cm diam. vials, with a 1.5 cm diam. mesh-covered hole in the bottom of the vial providing ventilation. Forty plants were left uninfested as controls, and had cages placed over

them.

Larvae were removed from plants when approximately 25% or 75% of the area of the cotyledons was injured. Thirty-four plants at both levels of feeding injury were selected for further use in the experiment and the rest of the injured plants discarded.

Ten plants were randomly chosen from each treatment and the fresh weight of the cotyledons and support tissue measured. For both injury levels, estimates of the percent feeding injury were determined by comparing the mean fresh weight of cotyledons from injured plants with the mean fresh weight of cotyledons from control plants.

Six plants from each treatment were harvested each week for four weeks after the insect injury. Leaves were removed from plants and fresh weight measured. The remaining above ground plant tissue (support tissue) was removed and weighed. Roots were recovered by washing soil through a screen. Leaves, support tissue, and roots were placed in an oven at 60°C until dry, and weights taken.

The experiments with the other two insects and with *S. alba* were similar. Two and four second instar diamondback moth larvae were also used to achieve the appropriate injury levels when the experiment was performed using *S. alba*. One or two third instar bertha armyworm larvae and five or 10 adult crucifer flea beetles were used to achieve the desired injury levels on *B. napus* and *S. alba*, respectively. Bertha armyworm larvae were transferred to plants using either tweezers or fingers. Flea beetles were transferred onto the caged plants using an aspirator. Plants were injured in the cotyledon stage because injury is easier to rate at this stage than in older plants, a long period is available for measurements of compensation, and this plant stage is frequently injured by insects, although bertha

armyworm larvae do not usually attack such young plants.

The experimental design was a two-factor (type of insect injury and injury level) completely randomized design with six replications. Data for each plant species and for each harvest were analyzed separately to reduce variability in error. Because of significant interactions between insects and injury level, data for these two factors were analyzed separately. Data for leaf weight, weight of support tissue, and root weight were transformed to natural logarithms before analysis to reduce the variation in error among samples. Transformed data were reexamined to confirm that variances were stabilized before proceeding with the analysis. For each type of insect injury, Dunnett's two-tailed *t*-test was used to test for differences between means of treated plants and means of control plants. Because there was a separate set of control plants for each combination of plant and insect species, data were adjusted by subtracting the mean of the transformed data for control plants from the transformed value of the injured plants for the corresponding type of injury. To test for differences between different types of insect injury, data were analyzed using analysis of variance for a completely randomized design (SAS Inst. 1989). Tukey's Studentized Range Test was used to discriminate means. Pearson correlation coefficients were used to determine the correlation between leaf and root weight. Separate correlation coefficients were calculated for each plant species and harvest time.

Field Study

This study was performed in Winnipeg, Manitoba during the summer of 1994.

Brassica napus 'Excel' and *S. alba* 'Ochre' seeds were planted 2 cm deep in a clay loam soil using a motorized four-row seeder. Six replications, each consisting of four 5-m rows of each plant species were planted. Thirty cm spacings were left between rows within a replication.

For each plant species a split-plot design was used with six replications. Main-plots were the type of insect injury (bertha armyworm larvae, diamondback moth larvae, or flea beetles). Each main-plot consisted of four 1-m-long rows, and each of the three main-plots occurred once in each replication. Subplots were level of injury (0, 25, or 75% of cotyledon tissue injured). Each level of injury occurred once in every main-plot. A 1-m guard row was left at both ends of each replication.

Plants were injured when they were in the cotyledon stage. *Sinapis alba* and *B. napus* were infested 10 days and 11 days after planting, respectively, when the two species were at the same stage and similar in size. Insects were caged on groups of four or five plants by placing a 10 cm diam. by 26 cm high plexiglass cage over the plants. The top of each cage was a fine mesh and two 5 cm diam. holes covered with mesh in the sides of each cage allowed for ventilation. To achieve 75% injury either five or six 3rd-instar bertha armyworm larvae, 15 to 20 2nd-instar diamondback moth larvae, or 50 adult flea beetles were placed inside a cage. To achieve 25% injury either three bertha armyworm larvae, eight to 10 diamondback moth larvae, or 25 flea beetles were placed in a cage. Only groups of uniform plants in the middle two rows of a main-plot were used, and cages were spaced by at least 15 cm within a row. Cages were also placed over uninjured control plants until the appropriate feeding levels were reached on injured plants.

Insects were removed when either 25, or 75% of the surface area of the cotyledons

of at least two plants in each cage were injured. All cages were removed when insects were removed. In each caged section of row, two plants representative of a level of injury were selected for further use in the experiment, and a third plant with the same level of injury was labelled as a backup plant in case of subsequent loss of one of the experimental plants.

Plants within 15 cm on both sides of a group of treated plants were artificially injured at the same level as the treated plants so that plants injured by insects were competing with plants having a similar level of injury. Artificial injury was done by removing with scissors either 25 or 75% of tissue from the edge of both lobes of each cotyledon to simulate diamondback moth feeding, or removing with scissors either one or three lobes of the cotyledons to simulate bertha armyworm feeding. Flea beetle injury was simulated by removing 25 or 75% of the cotyledons by making 0.5 mm diam. holes with the tube of a mechanical pencil which had the lead removed, and by combining this perforation injury with meristem injury. Meristem injury was done by cutting the developing leaves in the apical meristem with a pair of dissecting scissors.

All plants that were injured by insects were rated for feeding pattern and injury to the apical meristem. Feeding pattern was rated as either concentrated injury, dispersed injury, or shot-hole injury. Injury to plants was rated as concentrated if feeding was very localized on the cotyledons. Injury was rated as dispersed if it was spread out over many areas of the cotyledon. Injury was rated as shot-hole if it occurred as small holes and necrotic areas spread over the surface of the cotyledons. Injury to apical meristems was rated on a scale of 0-3 (0=no injury, 1=1-50% injury, 2=51-99% injury, 3=meristem completely destroyed).

The length (mm) of each leaf blade was measured with a ruler at 9, 16, 25, and 31

days after injury to the plants. Growth stage of the plant was measured at 9, 16, 25, 31, 45, 52, 58, 65, and 73 days after injury. Growth stage was based on a modification of the key to the growth stages by Harper and Berkenkamp (1975). To avoid opening seed pods to assess growth stage the following classifications were used for the ripening stage (stage 5): 5.1 - all petals off the plant, upper pods small (< half the size of the lower pods and no sign of seed development); 5.2 - upper pods approximately half the size of lower pods, upper pods starting to fill; 5.3 - upper pods filled.

Beginning in August, pods were opened periodically to assess seed development on plants that had been injured artificially. Plants were harvested when seeds in the lower pods turned brown. *Sinapis alba* and *B. napus* were harvested 91 and 98 days after planting, respectively. Plants were cut at ground level and placed in cloth bags. Pods were removed from plants and weighed. Seeds were removed from the pods and the number of seeds from each plant counted using an electronic seed counter (Halross Instruments Corporation Limited, Winnipeg, Manitoba). The fresh weight of seeds from each plant was then measured. Plants were separated into leaves and support tissue, dried at 60°C, and weighed. Thirty seeds from each plant were selected randomly, weighed and placed on moist filter paper in a dark growth chamber at 20°C. The number of seeds that germinated after approximately seven days was recorded. Seeds not used in the germination test were dried at 60°C, and weighed.

Data for each species of plant and each period of measurement were analysed separately to reduce the variation in error for the treatments. For an individual plant, data for leaf length was added, and the total leaf length for each plant was used in the analysis. Data

for leaf length, and number of seeds were transformed to natural logarithms before analysis to reduce variation in error among samples. Transformed data were reexamined to confirm that variances were stabilized before proceeding with the analysis. Since the rating scale for growth stage was not continuous, data for growth stage were ranked prior to analysis so there were equal differences between consecutive values. Dunnett's two-tailed t -test was used to test for differences between means of treated plants and control plants. For each plant measurement, the mean of the transformed data for control plants was subtracted from the transformed value of the injured plants of the corresponding type of injury. To test for differences among main-plots, data were analyzed using analysis of variance for a split-plot design (SAS Inst. 1989). Tukey's Studentized Range Test was used to discriminate means. Pearson correlation coefficients were used to test for correlations between leaf length and the number of seeds, and between leaf length and average weight of seeds. Separate correlation coefficients were calculated for each species of plant and level of injury.

Results

Description of Insect Injury

Ratings of the level of injury in controlled environment experiments were on average within 10% of the desired injury level for all insects (Table 5). Thus, the ratings assigned to injured plants are assumed to be a good approximation of the actual level of injury. In the field experiment, methods of rating injury were similar to those used in the controlled environment experiments. Thus, injury ratings from the field experiment should have a similar degree of accuracy to those from the controlled environment experiments. Extraneous insect injury to the plants in the field experiment was negligible.

The three insects fed on crucifer seedlings in different ways. Flea beetles produced their characteristic shot-hole injury. Injury by bertha armyworm larvae was generally more concentrated than injury by diamondback moth larvae, although this trend was stronger in *B. napus* than in *S. alba* (Table 6). Feeding by diamondback moth larvae on the cotyledons did not result in the windowing that is often seen when the larvae feed on older leaves. Tissue was eaten away cleanly, with many feeding sites on the cotyledons. For bertha armyworms the higher injury level resulted in more concentrated injury, whereas for diamondback moth the higher injury level resulted in more dispersed injury (Table 6). Bertha armyworm injury appears more dispersed on *S. alba* than on *B. napus*. The amount of injury to the apical meristem was dependent on the insect (Fig. 1), with the largest amount of injury to the apical meristem done by flea beetles, and the least by bertha armyworm larvae.

TABLE 5. Actual levels of injury for 3 insects feeding on *Brassica napus* and *Sinapis alba* in controlled environment conditions.

Plant	Insect	Injury by weight (%) for target injury level of:	
		25%	75%
<i>B. napus</i>	Flea beetle	27.9 ¹	67.3
<i>B. napus</i>	Diamondback moth	32.6	78.2
<i>B. napus</i>	Bertha armyworm	32.9	72.4
<i>S. alba</i>	Flea beetle	— ²	— ²
<i>S. alba</i>	Diamondback moth	23.7	76.3
<i>S. alba</i>	Bertha armyworm	20.1	75.5

¹Estimates of the % of cotyledon tissue injured based on a comparison of the average fresh weight of cotyledons from 10 injured and 10 uninjured plants.

²Data unavailable.

TABLE 6. Pattern of feeding for three species of insects on cotyledons of *Brassica napus* and *Sinapis alba* grown in the field.

Insect	Injury level(%)	n ¹	% of plants where injury was		
			concentrated ²	dispersed ³	very-dispersed ⁴
<i>B. napus</i>					
Flea beetle ⁵	25	11	0	0	100
Flea beetle ⁵	75	6	0	0	100
Diamondback moth	25	22	27.3	72.7	0
Diamondback moth	75	23	4.3	95.7	0
Bertha armyworm	25	11	54.5	45.5	0
Bertha armyworm	75	11	72.7	27.3	0
<i>S. alba</i>					
Flea beetle ⁵	25	22	0	0	100
Flea beetle ⁵	75	3	0	0	100
Diamondback moth	25	13	30.8	69.2	0
Diamondback moth	75	10	10.0	90.0	0
Bertha armyworm	25	20	25.0	75.0	0
Bertha armyworm	75	15	46.7	53.3	0

¹Sample size²Three or fewer feeding sites.³Four to nine feeding sites.⁴Ten or more feeding sites.⁵Injury was in the form of numerous small feeding pits in cotyledons (shot-hole injury).

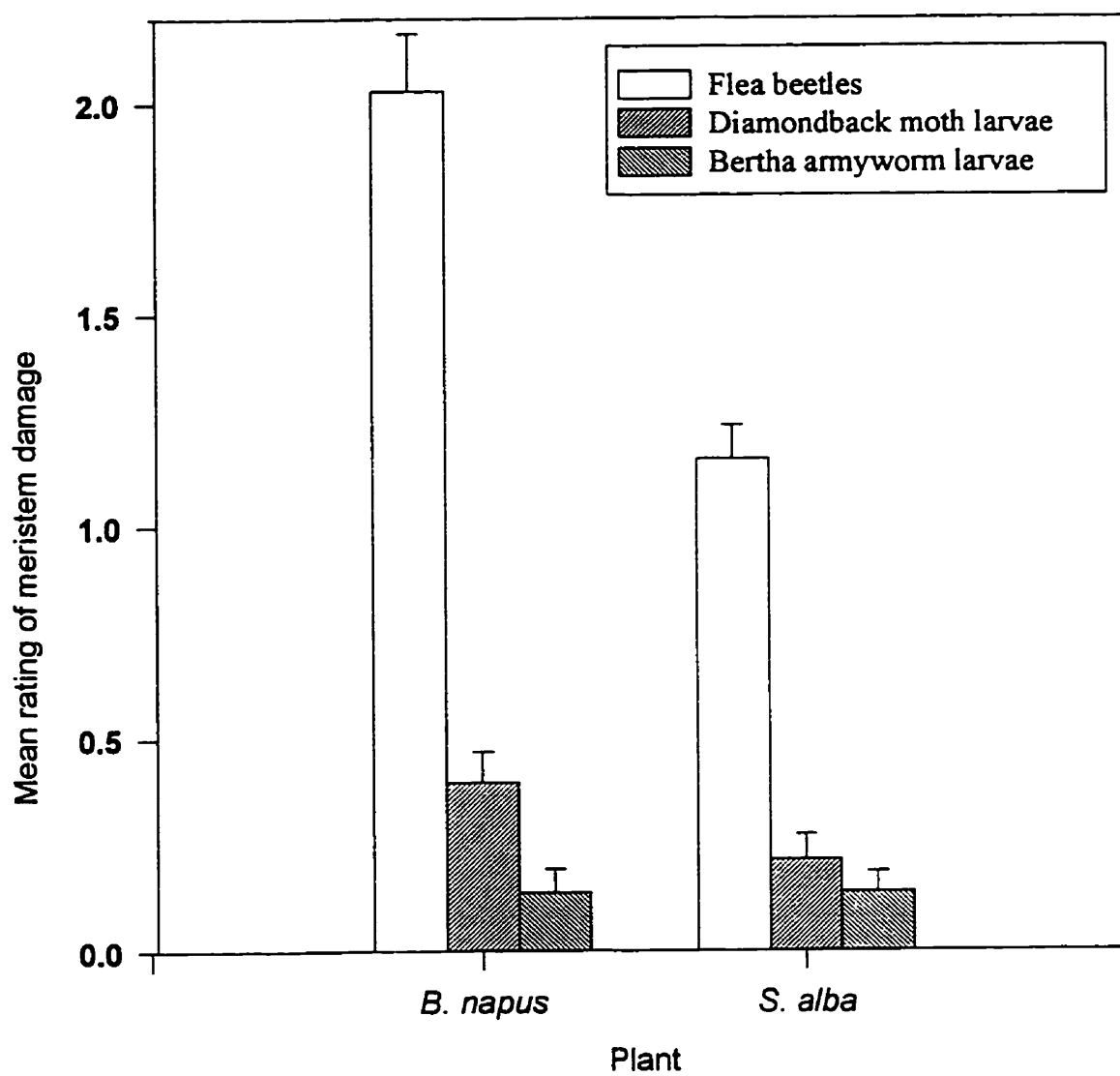


Fig. 1. Rating of injury to the apical meristem in field grown *Brassica napus* and *Sinapis alba* injured by three species of insects.

Plant Response to Herbivory in a Controlled Environment

Figures 2 to 4 demonstrate how growth of leaves, support tissue, and roots were affected by the different insects. The y-axes of these graphs show the reduction in growth of a particular type of plant tissue due to feeding. Since data, which had been transformed to natural logarithms and adjusted for control plants, had negative values for plants where growth was reduced due to the insect feeding, the sign of the value (whether positive or negative) was reversed so the majority of bars faced up in the figures. Positive values thus reflect undercompensation, the level of reduction in plant tissue due to feeding, zero represents exact compensation, and negative values reflect overcompensation, the level of increase in production of plant tissue due to feeding.

Leaf Biomass. Twenty-five percent injury to *B. napus* cotyledons by three species of insects resulted in different effects on leaf biomass only at 14 days after the injury ($F=13.90$; $df=2,15$; $P=0.0004$) (Fig. 2). Flea beetles caused significantly greater reductions in biomass of leaves than bertha armyworm larvae or diamondback moth larvae, but all plants had fully compensated by 21 days. Seventy-five percent injury to *B. napus* cotyledons resulted in differences in leaf biomass at 14 days after the injury ($F=24.35$; $df=2,14$; $P=0.0001$) (Fig. 2). The dry biomass of leaves was least for plants injured by flea beetles, and highest for plants injured by bertha armyworm larvae.

Compensation for insect feeding was quicker in *S. alba* than in *B. napus*. There was complete recovery of leaf weight from 25% injury to *S. alba* by 14 days after the injury. Seventy-five percent injury to *S. alba* caused differential leaf growth at seven ($F=6.22$;

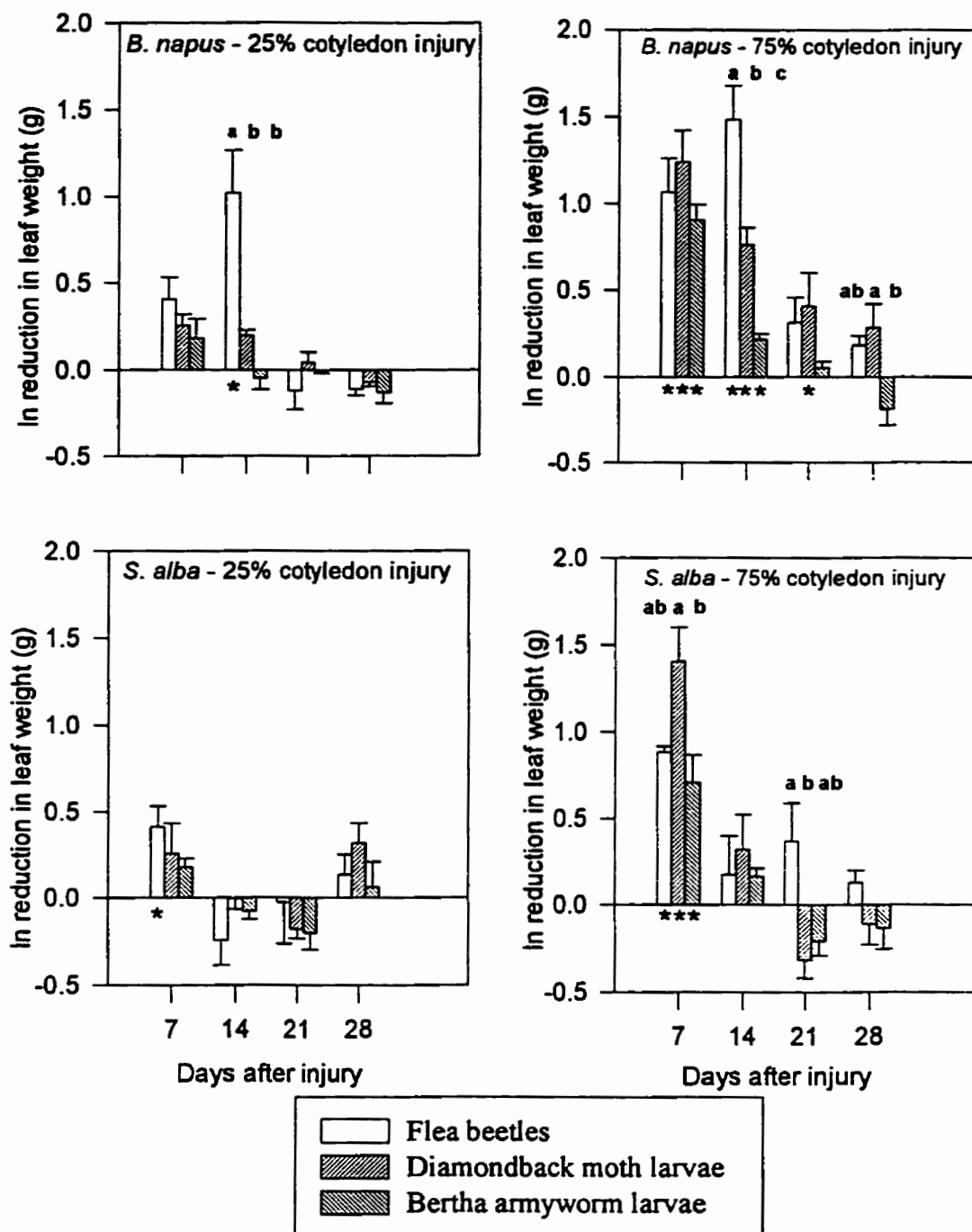


Fig. 2. Reduction in dry biomass of leaves in *Brassica napus* and *Sinapis alba* plants due to injury to seedlings by three species of insects in a controlled environment. Means within a group of bars followed by different letters are significantly different ($P < 0.05$; Tukey's Studentized Range Test). Means within a group of bars with no letters are not significantly different ($P \geq 0.05$; Tukey's Studentized Range Test). *=Significantly different from control plants ($P < 0.05$; Dunnett's two-tailed t -test).

df=2,15; $P=0.0108$) and 21 ($F=5.54$; df=2,14; $P=0.0169$) days after the injury (Fig. 2). Seven days after 75% injury, leaf weight was less in *S. alba* plants injured by diamondback moth larvae than in plants injured by bertha armyworm larvae. Twenty-one days after 75% injury, leaf weight was reduced more from feeding by flea beetles than from feeding by diamondback moth larvae, however.

Support Tissue. Fourteen days after the injury, *B. napus* plants receiving 25% injury to the cotyledons by flea beetles had significantly greater reductions in support tissue than plants injured by bertha armyworm larvae or diamondback moth larvae ($F=8.15$; df=2,15; $P=0.0040$) (Fig. 3). Seventy-five percent injury to *B. napus* caused differential growth of support tissue at 14 ($F=17.26$; df=2,14; $P=0.0002$) and 28 ($F=4.29$; df=2,15; $P=0.0336$) days after the injury. Fourteen days after the injury, weight of support tissue was reduced more for plants injured by flea beetles or diamondback moth larvae than for plants injured by bertha armyworm larvae. Twenty-eight days after the injury, weight of support tissue was reduced more for plants injured by diamondback moth larvae than for plants injured by flea beetles.

For *S. alba* with 25% injury to cotyledons, growth of support tissue did not differ among insects. At 75% injury, compensatory growth of support tissue was greater for plants injured by bertha armyworm larvae than for plants injured by flea beetles 28 days after the injury ($F=4.41$; df=2,15; $P=0.0312$).

Root Growth. Leaves and roots responded similarly to cotyledon feeding in both *B. napus* and *S. alba* (Figs. 2 and 4), so that a reduction in leaf tissue was accompanied by a similar reduction in root tissue. The Pearson correlation coefficients and their associated P -values comparing log reduction in leaf weight and log reduction in root weight were: *B.*

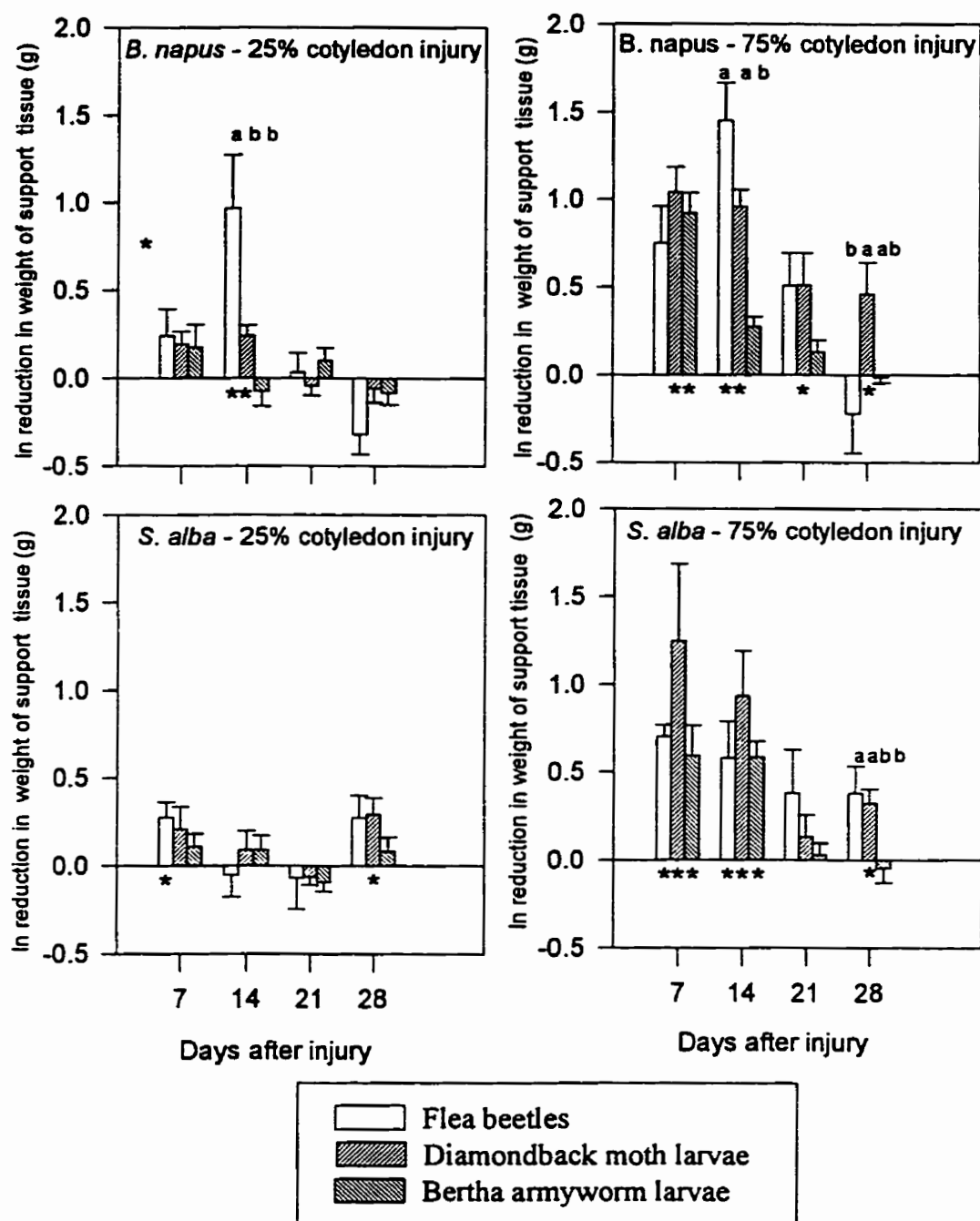


Fig. 3. Reduction in dry biomass of support tissue in *Brassica napus* and *Sinapis alba* due to injury to seedlings by three species of insects in a controlled environment. Means within a group of bars followed by different letters are significantly different ($P < 0.05$; Tukey's Studentized Range Test). Means within a group of bars with no letters are not significantly different ($P \geq 0.05$; Tukey's Studentized Range Test). * = Significantly different from control plants ($P < 0.05$; Dunnett's two-tailed t -test).

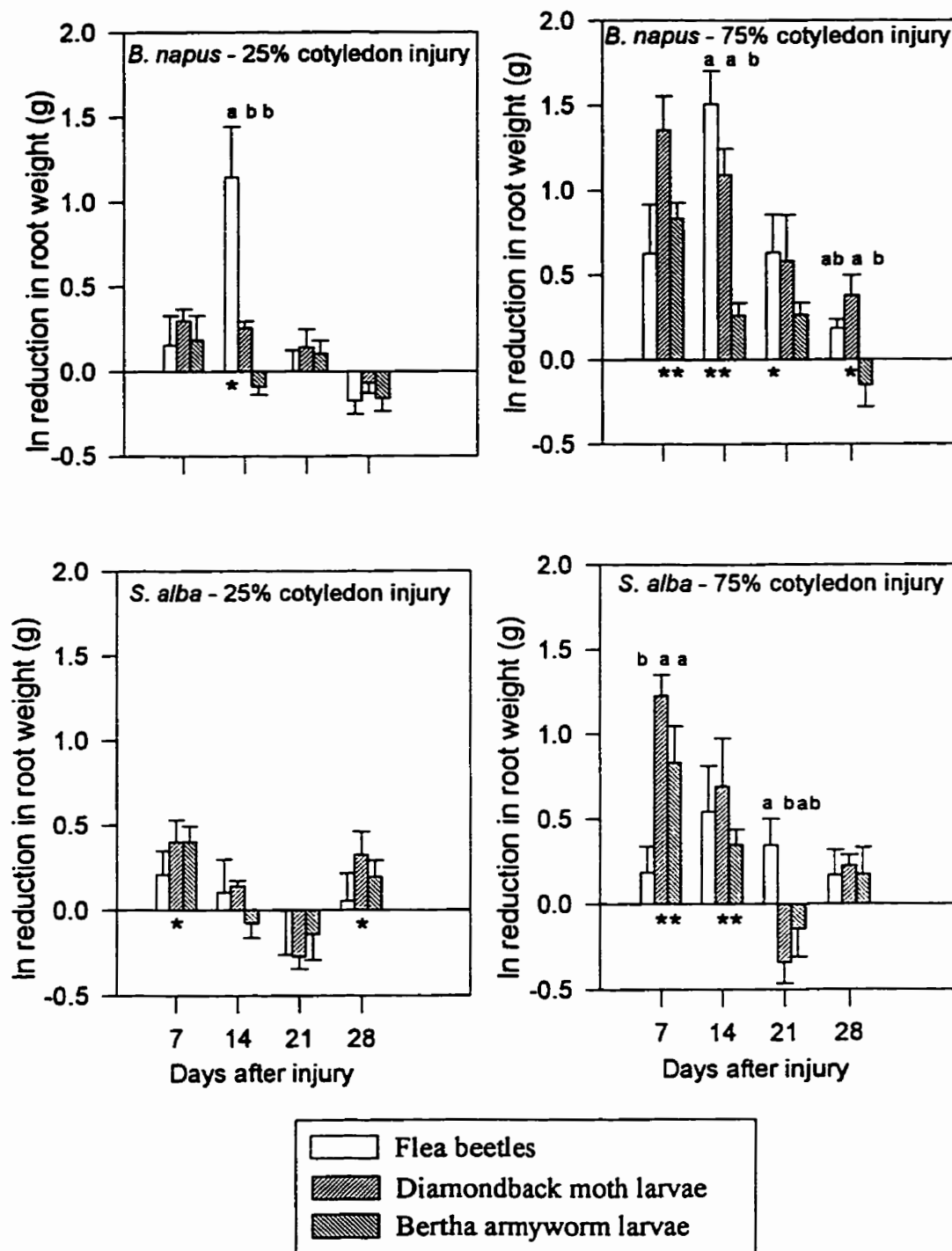


Fig. 4. Reduction in dry biomass of roots in *Brassica napus* and *Sinapis alba* plants due to injury to seedlings by three species of insects in a controlled environment. Means within a group of bars followed by different letters are significantly different ($P < 0.05$; Tukey's Studentized Range Test). Means within a group of bars with no letters are not significantly different ($P \geq 0.05$; Tukey's Studentized Range Test). * = Significantly different from control plants ($P < 0.05$; Dunnett's two-tailed t -test).

napus at seven ($r=0.708$; $n=36$; $P=0.0001$), 14 ($r=0.938$; $n=35$; $P=0.0001$), 21 ($r=0.851$; $n=36$; $P=0.0001$), and 28 ($r=0.783$; $n=35$; $P=0.0001$) days after the injury, and *S. alba* at seven ($r=0.703$; $n=36$; $P=0.0001$), 14 ($r=0.888$; $n=36$; $P=0.0001$), 21 ($r=0.870$; $n=35$; $P=0.0001$), and 28 ($r=0.475$; $n=35$; $P=0.0039$) days after the injury. Twenty-five percent injury to *B. napus* caused differential growth of roots only at 14 days after the injury ($F=13.10$; $df=2,15$; $P=0.0005$) (Fig. 4). *B. napus* plants receiving injury by flea beetles had significantly greater reductions in root tissue than plants injured by bertha armyworm larvae or diamondback moth larvae. Seventy-five percent injury to *B. napus* caused differences in growth of roots at 14 ($F=18.88$; $df=2,14$; $P=0.0001$) and 28 ($F=6.22$; $df=2,15$; $P=0.0108$) days after the injury (Fig. 4). Fourteen days after 75% injury to *B. napus*, reduction in weight of roots was less for plants injured by bertha armyworm larvae than for plants injured by flea beetles or diamondback moth larvae. At 28 days after 75% injury, plants injured by bertha armyworm larvae had a lower reduction in root weight than plants injured by diamondback moth larvae.

For *S. alba* with 25% injury to cotyledons, growth of roots did not differ among insects. Plants injured by diamondback moth larvae had reduced root growth at 7 and 28 days after injury, however. Different types of seventy-five percent injury to *S. alba* caused differences in growth of roots at seven ($F=9.73$; $df=2,15$; $P=0.0020$) and 21 ($F=6.11$; $df=2,14$; $P=0.0124$) days after the injury (Fig. 4). Seven days after the injury the reduction in root weight was less for plants injured by flea beetles than for plants injured by diamondback moth larvae or bertha armyworm larvae. Feeding by diamondback moth larvae resulted in a smaller reduction in root growth than feeding by flea beetles 21 days after the

injury, however. Plants injured by diamondback moth larvae or bertha armyworm larvae had reduced root growth at 7 and 14 days after injury.

Plant Response to Herbivory in the Field

This experiment used leaf length, plant developmental stage, and seed production to measure plant response to defoliation. Appendix A presents data for control plants at the times when measurements were taken. Leaf length was no longer increasing between 37 and 43 days after planting in *S. alba*, but was still increasing in *B. napus*.

Leaf Length. Feeding by flea beetles resulted in greater reductions in leaf growth in *B. napus* than feeding by bertha armyworm larvae or diamondback moth larvae (Fig. 5). Feeding by flea beetles resulted in a larger reduction in leaf length than feeding by either bertha armyworm larvae or diamondback moth larvae at nine ($F=24.48$; $df=2,10$; $P=0.0001$) and 16 ($F=11.91$; $df=2,10$; $P=0.0023$) days after cotyledon injury. Plants injured by flea beetles continued to have a larger reduction in leaf length than plants with injury by bertha armyworm larvae at 25 ($F=10.48$; $df=2,10$; $P=0.0035$) and 31 ($F=7.27$; $df=2,10$; $P=0.0112$) days after the injury. *Brassica napus* plants that received 25% injury by flea beetles had reduced leaf lengths at all four periods of measurement, and plants receiving 75% injury by either flea beetles or diamondback moth larvae had reduced leaf length at all four periods of measurement.

Leaf length was not as severely affected by cotyledon feeding in *S. alba* as it was in *B. napus* (Fig.5). In *S. alba* there were no significant differences in reduction in leaf length

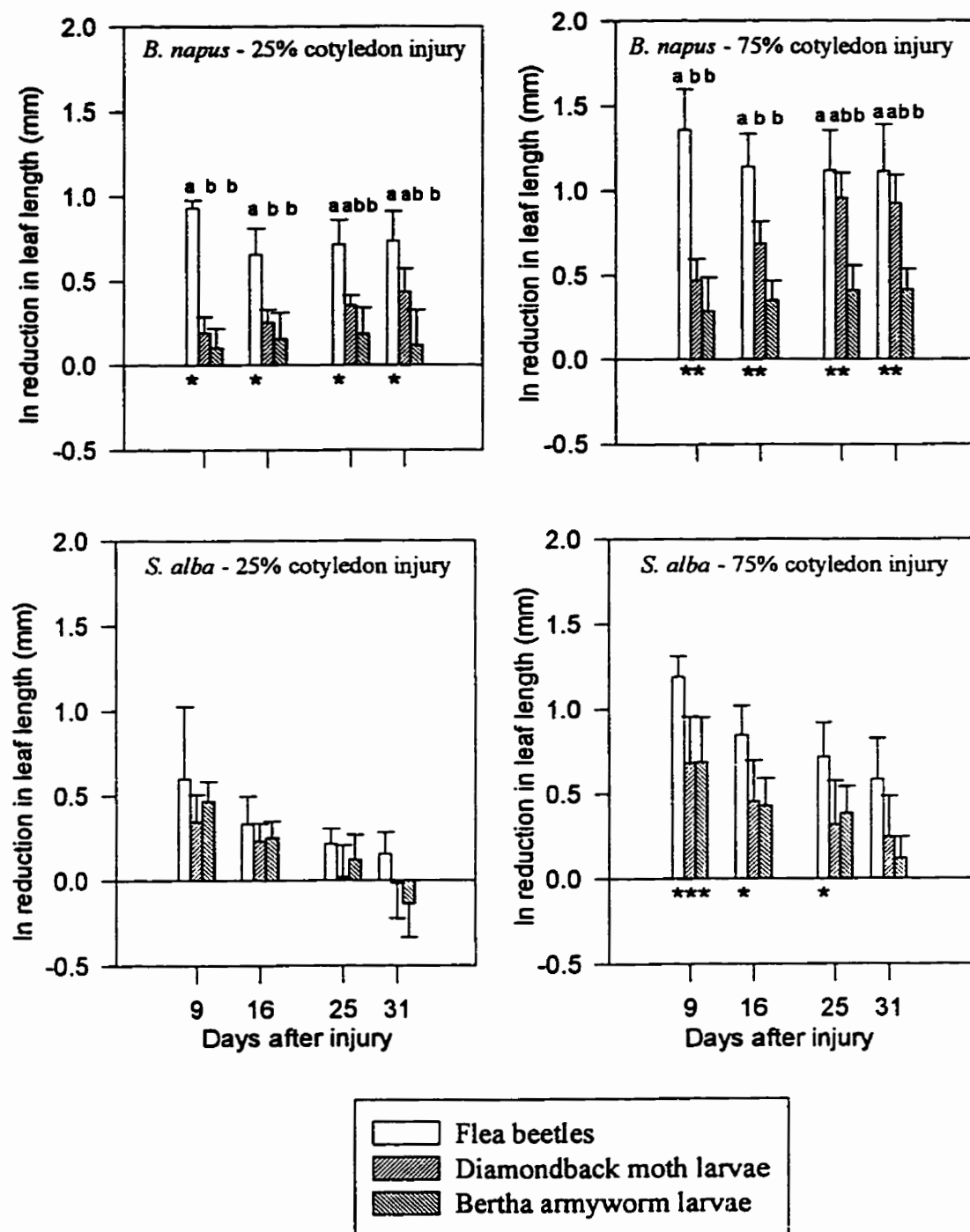


Fig. 5. Reduction in leaf length in *Brassica napus* and *Sinapis alba* plants due to injury to seedlings by three species of insects in the field. Means within a group of bars followed by different letters are significantly different ($P < 0.05$; Tukey's Studentized Range Test). Means within a group of bars with no letters are not significantly different ($P \geq 0.05$; Tukey's Studentized Range Test). * = Significantly different from control plants ($P < 0.05$; Dunnett's two-tailed t -test).

between the different types of insect feeding. For each insect, 75% injury to cotyledons caused a reduction in leaf length at 9 days after injury. Only the 75% injury by flea beetles caused a reduction in leaf length at 16 and 25 days after injury, however.

Although both plant species received the same level of injury, recovery appeared slower and less complete in *B. napus* than in *S. alba*. In *S. alba*, with each period of measurement the reduction in leaf length became less for each insect at both levels of injury (Fig. 5). In *B. napus* the level of reduction in leaf length dropped only very slightly for flea beetles, increased nearly linearly for diamondback moths, and changed very little for bertha armyworms. *Sinapis alba* appeared able to increase its relative growth rate as a result of insect feeding whereas *B. napus* appeared not to increase its relative growth rate to compensate for insect injury.

Reduction in leaf growth due to feeding on seedlings lasted longer in *B. napus* and *S. alba* grown in the field than under controlled environment conditions (Figs. 2 and 5). In controlled environments, leaves of plants from each injury treatment weighed as much as leaves of control plants by 28 days after the injury. For plants grown in the field, plants from some injury treatments still had less total leaf length than control plants 31 days after the injury. In both experiments, plants compensated most for injury by bertha armyworms, and least for injury by flea beetles, however.

Growth Stage. In *B. napus*, plant development was affected less by injury from bertha armyworm larvae than by injury from flea beetles or diamondback moth larvae (Fig. 6). Plants injured by bertha armyworm larvae were at a more advanced growth stage than plants injured by flea beetles at 25 days after injury, and at a more advanced growth stage than

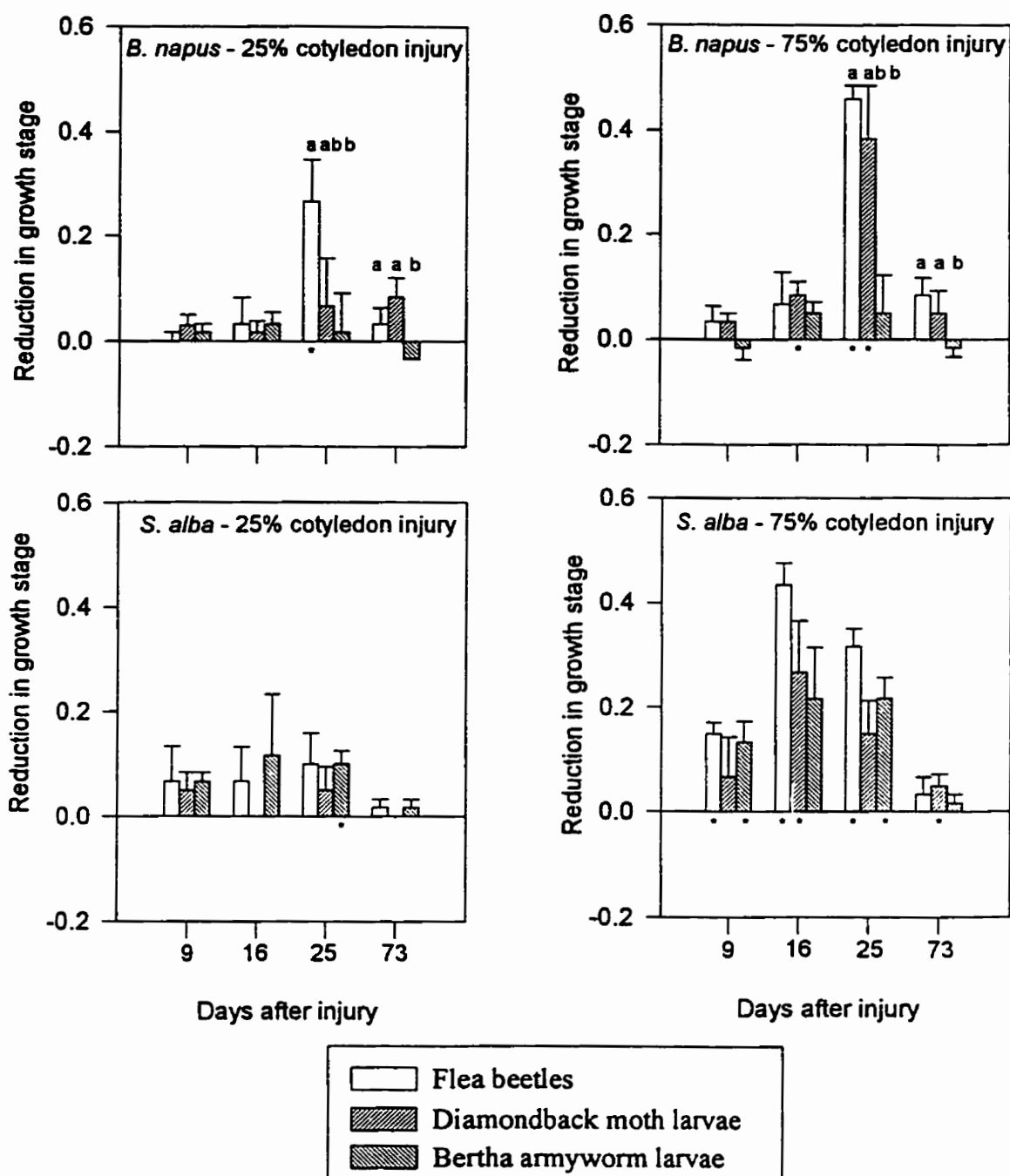


Fig. 6. Reduction in growth stage of *Brassica napus* and *Sinapis alba* plants due to injury to seedlings by three species of insects in the field. Growth stage based on a modification of the system by Harper and Berkenkamp (1975). Means within a group of bars followed by different letters are significantly different ($P < 0.05$; Tukey's Studentized Range Test). Means within a group of bars with no letters are not significantly different ($P \geq 0.05$; Tukey's Studentized Range Test). * = Significantly different from control plants ($P < 0.05$; Dunnett's two-tailed t -test).

plants injured by either flea beetles or diamondback moth larvae 73 days after the injury.

Twenty-five percent injury to *S. alba* cotyledons had little effect on growth stage. Seventy-five percent injury to *S. alba* resulted in a reduction in growth stage for feeding by flea beetles at 9, 16, and 25 days after the injury, for feeding by diamondback moth larvae at 16 and 73 days after the injury, and for feeding by bertha armyworm larvae at 9 and 25 days after the injury.

Seed Size, Number of Seeds, and Germination. Injury to *B. napus* plants at the cotyledon stage alone was sufficient to cause a reduction in average weight of seeds and number of seeds. This reduction was not evident at 25% injury to cotyledons, but 75% injury by flea beetles to *B. napus* caused a reduction in both the average weight of seeds and the number of seeds (Fig. 7). Seventy-five percent injury by diamondback moth larvae also caused a reduction in the number of seeds produced by *B. napus*, although average weight of seeds was not affected.

Twenty-five percent injury to cotyledons also had no effect on average weight of seeds or number of seeds for *S. alba*. Seventy-five percent injury by flea beetles to *S. alba* caused a reduction in the number of seeds. The average weight of *S. alba* seeds was not affected by injury from any of the insects. For neither *B. napus* nor *S. alba* did the number or the average weight of seeds differ due to injury from the different insects, although reductions were always highest for 75% injury by flea beetles.

Germination of seeds did not differ between the different insect treatments in either *B. napus* ($F=2.13$; $df=2,15$; $P=0.1701$) or *S. alba* ($F=0.09$; $df=2,15$; $P=0.9140$). None of the insect injury treatments caused a change in the germination of seeds in either plant species.

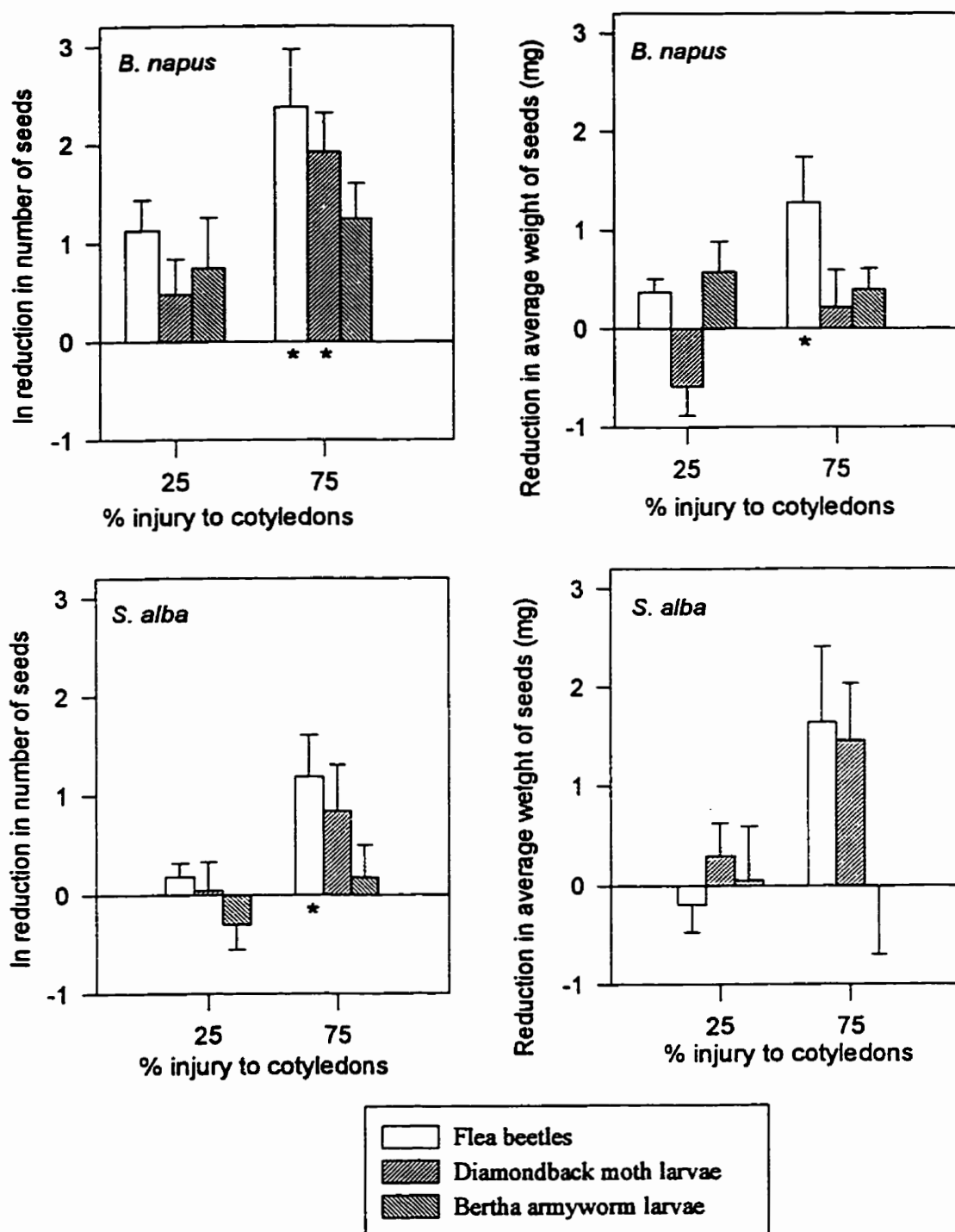


Fig. 7. Reduction in the number of seeds per plant and the average weight of seeds in *Brassica napus* and *Sinapis alba* plants due to injury to seedlings by three species of insects in the field. *=Significantly different from control plants ($P < 0.05$; Dunnett's two-tailed t -test).

Average germination of control plants was 99.3 ± 0.6 for *S. alba* and 83.5 ± 4.6 for *B. napus* (Appendix A).

There was a similar pattern in the reduction in leaf length and the reduction in the number of seeds per plant (Figs. 5 and 7). The Pearson correlation coefficients and their associated *P*-values comparing log reduction in leaf length and log reduction in number of seeds were: *B. napus* at 25% injury ($r=0.791$; $n=18$; $P=0.0001$), *B. napus* at 75% injury ($r=0.938$; $n=18$; $P=0.0001$), *S. alba* at 25% injury ($r=0.887$; $n=18$; $P=0.0001$), and *S. alba* at 75% injury ($r=0.843$; $n=18$; $P=0.0001$). Thus, reductions in leaf length may have resulted in the reductions in the number of seeds. Reductions in leaf length due to insect feeding were not always correlated with a reduction in average weight of the seeds, however (Figs. 5 and 7). The Pearson correlation coefficients and their associated *P*-values comparing log reduction in leaf length and reduction in average weight of seed were: *B. napus* at 25% injury ($r=0.341$; $n=18$; $P=0.1659$), *B. napus* at 75% injury ($r=0.732$; $n=18$; $P=0.0006$), *S. alba* at 25% injury ($r=0.195$; $n=18$; $P=0.4387$), and *S. alba* at 75% injury ($r=0.441$; $n=18$; $P=0.0667$).

Discussion

Compensation by *B. napus* and *S. alba* for injury by the three insects used in this study was dependent on the insect and not just the level of injury. Although injured to the same levels by all insects, plants compensated most for injury by bertha armyworms, and least for injury by flea beetles. The different levels of compensation for feeding by different insects were more evident in *B. napus* than *S. alba*. The latter species recovered better from the injury than the former. Both plant species compensated better for 25% injury to the cotyledons than for 75% injury to the cotyledons.

Laboratory experiments in controlled environments showed similar patterns of compensation for leaf growth as the field experiment, but compensation was more rapid in the controlled environments. Conditions such as temperature, soil moisture, and competition from neighboring plants may have been more favorable for compensatory growth in the controlled environments than in the field. The environment affected the amount of compensation (Trumble *et al.* 1993), but not the relative compensation for the different herbivores.

Reductions in the number and size of seeds indicates that some plants that received 75% insect injury to the cotyledons never did completely recover from the injury, even though all injury was restricted to a short interval in the seedling stage. This inability to completely recover from injury to seedlings was greatest for plants injured by flea beetles.

For *S. alba* with a level of injury of 75%, leaf length had recovered by 31 days after the injury, and might have been judged to have fully compensated for the injury. Yet these

plants produced fewer seeds than uninjured plants when they had been injured by flea beetles. This observation forces an examination of what is implied by the term compensation. If the plant regained some or all of the type of tissue that was destroyed, then it is clear that the plant compensated for the injury. Although compensation occurred, the reduced seed production indicates that recovery was not complete. Full compensation by one plant tissue does not necessarily mean the plant has recovered.

Change in growth rate of root tissue appears to be one of the means by which *B. napus* and *S. alba* respond to defoliation at the seedling stage. Root growth declines with defoliation. Undercompensation of some plant tissues may be necessary for a plant to recover from the injury.

The fact that leaves and roots responded similarly to cotyledon feeding in both *B. napus* and *S. alba* supports the hypothesis that under a particular set of environmental conditions an allometric relationship exists between the amount of biomass a plant allocates between its roots and shoots, such that the shoot : root ratio is conserved (Wilson 1988). This relationship has been viewed as a strategy which maximizes relative growth rate (Iwasa and Roughgarden 1984). In response to tissue loss to herbivores, plants generally reallocate resources to new, restitutive or replacement tissues at the expense of other growth and metabolic centres (Trumble *et al.* 1993). Disturbance of the shoot : root ratio, either by root grazing or by defoliation, alters the growth pattern such that the original ratio is restored rapidly. Failure to restore the shoot : root ratio may reflect an inability to compensate fully for tissue lost to herbivores (Crawley 1983, 1988).

Aboveground herbivory in *B. napus* and *S. alba* not only affects root growth, but the

response is dependent on the species of herbivore and not just the level of injury. Thus, in both *B. napus* and *S. alba* the growth of leaves, support tissue, and roots are all dependent on the type of insect injury to seedlings.

The differences in compensation between *S. alba* and *B. napus* may be due to physiological differences in how these plants compensate for injury by insects, or due to differences in the way the insects fed on these two plants. A herbivore may cause different patterns of feeding on different species or cultivars of plants, and this must be considered, along with morphological and physiological differences in the plants, as a possible reason for differences in levels of compensation. Feeding behaviour will be determined by plant chemistry and how it changes during feeding (Edwards and Wratten 1983), and by morphological features such as trichomes (Palaniswamy and Bodnaryk 1994).

The cotyledons of *S. alba* contain high concentrations of sinalbin, which does not occur in *B. napus*, and are fed on by flea beetles at one-half the rate of *B. napus* (Bodnaryk 1991). Concentrations of sinalbin found in the cotyledons of *S. alba* may be responsible for the antixenosis exhibited by this species (Bodnaryk and Lamb 1991). High concentrations of sinalbin in young cotyledons (up to 20 mM) and young leaves (up to 10 mM) of *S. alba* deter feeding of both *Ph. cruciferae* and *M. configurata* (Bodnaryk 1991). Sinalbin in the cotyledons of *S. alba* may have resulted in more dispersed injury by bertha armyworm than in *B. napus*. Young leaves of *S. alba* may also be protected by their high concentration of sinalbin. Feeding pits by *Ph. cruciferae* on the first true leaves of *S. alba* are about one-half the size of the feeding pits on *B. napus* (Brandt and Lamb 1993). More than 90% of the feeding injury by *Ph. cruciferae* on the first true leaves of *B. napus* is along the edge,

compared with <70% for *S. alba*.

S. alba is injured less by *Ph. cruciferae* on the lower relative to the upper surface of the cotyledon than *B. napus* (Brandt and Lamb 1993). *Sinapis alba* has a dense mat of pubescence on its stems, petioles, and the undersurface of its cotyledons, but *B. napus* does not. These hairs may provide some protection to the lower surfaces of the cotyledons.

The differences in compensation between *S. alba* and *B. napus* were not always consistent with that of previous studies. Bodnaryk and Lamb (1991) found that for *B. napus*, the relationship between the percentage of the cotyledons that had been eaten and the dry weight of seedlings six days after injury was linear. For *S. alba*, low levels of cotyledon injury had little effect on the dry weight of seedlings six days after injury, giving a curvilinear relationship between cotyledon damage and seedling weight. In the present field experiment, feeding by flea beetles resulted in a reduction in leaf length nine days after the injury in both plant species and at both levels of injury, with the exception of *S. alba* at 25% cotyledon injury. In the laboratory experiments, however, feeding by flea beetles resulted in a reduction in dry weight of leaves seven days after the injury in both plant species and at both levels of injury in all but *B. napus* at 25% cotyledon injury. It is not until 14 days after the injury that the compensation in dry weight of leaves for flea beetle feeding is greater for *S. alba* than for *B. napus*. Although *S. alba* appears to ultimately compensate better than *B. napus* for flea beetle feeding in both the present study and the study by Bodnaryk and Lamb (1991), the latter study implies a much higher level of compensation by *S. alba* relative to *B. napus* than do the present studies. The level of compensation in *S. alba*, relative to *B. napus*, is important because *S. alba* has been considered a source for breeding flea beetle tolerant canola

varieties.

Bertha armyworm caused more concentrated patterns of feeding injury than the crucifer flea beetle and the diamondback moth. Fewer bertha armyworm larvae were required than diamondback moth larvae and flea beetles to achieve the same level of injury. Conditions of crowding may thus have been partly responsible for the increased dispersal of injury resulting from diamondback moth and flea beetle feeding. It is possible that high densities of flea beetles on a plant may be a contributing factor to the stem and petiole injury often associated with feeding by these insects on crucifer seedlings. Adult flea beetles are also much more active than the larvae of bertha armyworm and diamondback moth, and consume much less in a bout of feeding, which may also result in their very dispersed feeding pattern.

The difference in compensation for the three types of injury may be due to the type of tissue injured, for example, the degree of meristem feeding; the pattern of feeding, which affects differences such as the amount of injured edge for the remaining tissue; the stimulation of plant growth due to compounds in the insect's saliva; the introduction of a toxin into the plant; or a combination of two or more of the above reasons. Further research is necessary to determine which of these factors have the greatest effect on the compensatory response of *B. napus* and *S. alba* to these three insects.

This research supports the hypothesis that plant compensation for defoliation by insects depends on the insect causing the injury, and is not a generalized response to injury. Thus, *B. napus* and *S. alba* are responding to some factor specific to the insect herbivore. The plants may be responding to the pattern of injury, which varies among herbivores, or a

chemical associated with the feeding or excretion processes of herbivores.

CHAPTER 5**INJURY-SPECIFIC COMPENSATION BY TWO CRUCIFEROUS PLANTS FOR
SIMULATED INSECT HERBIVORY TO SEEDLINGS**

Abstract

The purpose of these experiments was to test the hypothesis that plant compensation for tissue removal is related to the pattern of defoliation. Experiments in controlled environments were conducted on *Sinapis alba* L. 'Ochre' and *Brassica napus* L. 'Excel'. Eight day old plants of *S. alba* and nine-day old plants of *B. napus* received either concentrated injury to cotyledons, dispersed injury to cotyledons, perforation injury to cotyledons, meristem injury, or no injury. Six plants from each treatment were harvested weekly for four weeks after the injury, and plant biomass was measured. *Brassica napus* plants with meristem injury had a reduction in weight of leaves, support tissue and roots 12 days after the injury. *Sinapis alba* plants receiving perforation injury had less than half the weight of leaves and roots as control plants seven days after the injury. In a field experiment, 14 day old plants of *B. napus* 'Excel' and *S. alba* 'Ochre' received perforation injury (to simulate injury by flea beetles, *Phyllotreta cruciferae* (Goeze)), dispersed injury (to simulate injury by diamondback moth, *Plutella xylostella* L., larvae), or concentrated injury (to simulate injury by bertha armyworm, *Mamestra configurata* (Walker), larvae). There were five levels for each pattern of injury, with perforation and dispersed injury having levels that included meristem injury, and concentrated injury having a level of complete removal of both cotyledons. Total leaf length and growth stage were measured weekly. Plants were harvested when seeds in the lower pods had turned brown, and seeds were counted and weighed. Thirty seeds from each plant were selected randomly for a germination test. Total leaf length was reduced most by perforation injury and least by concentrated injury. Injury to the apical

meristem resulted in *S. alba* producing multiple stems, and resulted in damage to true leaves in both plant species. Meristem injury resulted in a reduction in the number of seeds produced in both plant species, and complete removal of cotyledons resulted in a reduction in the number of seeds produced by *B. napus*. This research supports the hypothesis that the pattern of injury to a leaf will affect the ability of the plant to compensate for the injury. The field study described in Chapter 4 was performed concurrent to the field study presented in this paper. Patterns of compensation were similar in the artificial injury treatments of this experiment to the insect injury treatments in Chapter 4. This supports the hypothesis that the important difference between how these insects affect plant growth is the pattern of their injury, and not other insect-specific factors associated with their feeding or excretion.

Introduction

Feeding by different species of insects has different specific impacts on growth of the cruciferous plants canola, *Brassica napus* L., and yellow mustard, *Sinapis alba* L. (Chapter 3). Even insects feeding on the same plant tissue may have different effects on the plant.

One of the reasons that defoliating insects have different specific impacts on plant growth in *B. napus* and *S. alba* is that plant compensation for insect injury is also insect specific (Chapter 4). Seedling injury by flea beetles, *Phyllotreta cruciferae* (Goeze), was compensated for more poorly than injury to the same plant stage by either diamondback moth, *Plutella xylostella* L., larvae or bertha armyworm, *Mamestra configurata* (Walker), larvae. Insect specific factors, and not just the level of injury, affect how plants respond to feeding injury. Pattern of feeding injury may be one of the factors resulting in different levels of compensation by the plants. If so it could then be concluded that different patterns of feeding are one of the factors resulting in the different specific impacts that insects have on plants.

Artificial means of injury designed to simulate herbivory may be a useful technique in determining the importance of pattern of injury on plant compensation. Artificial injury can enable hypotheses on the effects of pattern of herbivory to be tested without the confounding effects of insect-related factors such as compounds in the insect's saliva.

The type of plant tissue injured may have a large effect on how a plant compensates. Recovery from injury requires that shoot meristems are present and that these meristems are

able to initiate growth following the removal of tissue (Archer and Tieszen 1980, Richards and Caldwell 1985; Roundy and Ruyle 1989). In defoliated plants, adequate carbohydrate and nutrient resources must be allocated to those meristems for regrowth (Chapin et al. 1980, 1986). These resources may come from storage, or be acquired through mechanisms such as compensatory photosynthesis (Nowak and Caldwell 1984, von Caemmerer and Farquhar 1984) or compensatory nutrient uptake (Chapin and Slack 1979, McNaughton and Chapin 1985).

The level of dispersal of injury may also affect a plant's ability to compensate for the injury. Injury dispersed over many leaves is less detrimental to the plant than if the same amount of injury is restricted to just a few leaves (Lowman 1982, Mauricio *et al.* 1993). The pattern of feeding within a leaf may also affect how the plant responds to the feeding (Hall and Ferree 1976).

The striped flea beetle, *Phyllotreta striolata* (F.), the crucifer flea beetle, *Ph. cruciferae*, the diamondback moth, *Pl. xylostellata*, and the bertha armyworm, *M. configurata*, are important pests of oilseed *Brassica* crops in Canada (Lamb 1989). These insects are defoliators of *B. napus* and *S. alba*, although the pattern of defoliation varies. Feeding by flea beetles results in small holes or pits in the leaves (Westdal and Romanow 1972), and is more dispersed than injury by diamondback moth larvae or bertha armyworm larvae (Chapter 4). The patterns of injury by these insects can be simulated, but whether the effects on the plant of artificial injury and insect injury are similar is not known. If pattern of feeding is the main factor affecting how the plant is responding to the injury, then the plant should respond similarly to insect and artificial injury if the pattern is closely mimicked.

The purpose of these experiments was to test the hypothesis that plant compensation for tissue removal is related to the pattern of defoliation. *Brassica napus* and *S. alba* seedlings were injured artificially to mimic the patterns of feeding described in Chapter 4 for adult flea beetles, bertha armyworm larvae, and diamondback moth larvae. The field study described in Chapter 4 was performed concurrent to the field study presented in this paper. By comparing artificial injury to insect herbivory, the importance of pattern of injury to the compensatory responses of the plant can be determined.

Materials and Methods

Controlled Environment Study

Experiments were conducted on *S. alba* 'Ochre' and *B. napus* 'Excel' at different times. Two seeds were planted 2 cm deep in each of 160 plastic pots, 11 cm diam. by 10 cm high, containing soil and peat moss. Pots were randomly placed in a growth chamber with an 18 h light:6 h dark photoperiod, and a day:night temperature of 24:18°C. Plants were watered as needed with 150 ml per pot. Plants were fertilized 14, 21, and 28 days after planting with an aqueous solution of 0.67 g/pot of Plant-Prod® 20-20-20 (N-P-K) fertilizer. Shortly after germination, one plant per pot was chosen for use in the experiment and the other plant pulled out.

Treatments. When plants were in the cotyledon stage (eight days after planting for *S. alba* and nine days after planting for *B. napus*), 126 plants having approximately equal cotyledon area were selected for further use and the other plants discarded. Treatments consisted of:

- 1) Concentrated injury to cotyledons: one cotyledon and one lobe of the remaining cotyledon (75% of cotyledon tissue) was removed using scissors.
- 2) Dispersed injury: the outer 75% of each lobe of both cotyledons was removed using scissors.
- 3) Perforation injury: approximately 75% of the cotyledon area was injured by making 1 mm diameter holes in both cotyledons using the tube of a mechanical pencil (Pentel® - 0.5mm

lead) from which the lead was removed. This hole size closely matches the size of feeding pits made by *Ph. cruciferae* (Brandt and Lamb 1993).

4) Meristem injury: developing true leaf buds were removed using micro-dissecting scissors.

5) No injury (control plants).

All treatments were applied to 24 plants of each species.

Plant Measurements. Six plants from each treatment were harvested weekly for the first four weeks after the injury. Leaves were removed from plants and their fresh weight measured. The remaining above ground plant tissue (support tissue) was removed and weighed. Roots were recovered by washing soil through a screen. Leaves, support tissue, and roots were dried at 60°C and weighed.

Data Analysis. A completely randomized design with 6 replications was used. Data for each plant species and each harvest were analyzed separately to reduce variability. Data for weight of leaves, support tissue, and roots were transformed to natural logarithms before analysis to reduce the variation in error among samples. Transformed data were reexamined to confirm that variances were stabilized before proceeding with the analysis. Data were analyzed using analysis of variance for a completely randomized design and Tukey's Studentized Range Test to discriminate means (SAS Inst. 1989).

Field Study

This research was performed in Winnipeg, Manitoba during the summer of 1994 on land routinely used for agricultural plots. *Brassica napus* 'Excel' and *S. alba* 'Ochre' seeds

were planted 2 cm deep in a clay loam soil using a motorized four-row seeder. Six replications, each consisting of four 5-m rows of each plant species were planted with 30-cm spacings between rows.

Experimental Design and Treatments. For each species of plant, a nested design was used with 6 replications. Each replication was divided into three plots. Each plot within a replication contained plants that received a different pattern of injury (perforation injury, dispersed injury, or concentrated injury). For each species of plant, a plot designated for a particular pattern of injury consisted of four 1-m rows. For each pattern of injury, there were five levels of injury, including uninjured control plants. Different levels of injury were in subplots. Each subplot was nested within a particular pattern of injury, and consisting of plants in a 15 cm section of row injured to the same level. A 1-m guard row was left at both ends of each replication. Plants were injured when they were in the cotyledon stage, 14 days after planting.

Perforation injury was designed to simulate injury by flea beetles. Twenty-five or 50% of the cotyledons was removed by making 1 mm diam. holes with the tube of a mechanical pencil which had the lead removed. Two further levels of injury were created by combining the perforation injury with injury to the apical meristem. The developing true leaves in the apical meristem were excised with a pair of micro-dissecting scissors.

Dispersed injury was designed to simulate injury by diamondback moth larvae. Scissors were used to excise 25, 50, or 75% of tissue from the edge of both lobes of each cotyledon. Injury to the apical meristem as described above with no cotyledon injury was done as a fourth level of dispersed injury, because diamondback moth larvae sometimes

attack the meristem.

Concentrated injury was designed to simulate injury by bertha armyworm larvae. Scissors were used to remove one lobe of one of the cotyledons (25% injury), one cotyledon (50% injury), one cotyledon and one lobe of the remaining cotyledon (75% injury), or both cotyledons (100% injury).

One of the plants in the middle 5 cm of a subplot was randomly selected for subsequent measurements. This was done to reduce the effect of plant competition on plant response to the injury treatments. In each plot, a control plant was selected randomly in the middle 5 cm of a 15 cm section of uninjured plants. Only groups of uniform plants in the middle two rows of a plot received injury treatments, and treatments were spaced so there was at least 15 cm between each group of treated plants within a row.

Populations of insect herbivores of *B. napus* and *S. alba* were low or absent throughout the experiment, and thus uncontrolled injury to the plants was considered insignificant. Netting was placed over plants when pods developed, however, to prevent injury by birds.

Plant Measurements. The length (mm) of each leaf blade was measured with a ruler at 8, 14, 24, and 36 days after cessation of injury. Growth stage of the plant was measured at 8, 14, 24, 36, 42, 49, 55, 62, and 70 days after the injury. Growth stage was based on a modification of the key of Harper and Berkenkamp (1975), which relies on destructive sampling of pods. To avoid opening seed pods, the following classifications were used for the ripening stage (stage 5): 5.1 - all petals off the plant, upper pods small (< half the size of the lower pods and no sign of seed development); 5.2 - upper pods approximately half the

size of lower pods, upper pods starting to fill; 5.3 - upper pods filled.

Beginning in August, pods were opened periodically to assess seed development on plants that had been injured but were not being measured. Plants were harvested when seeds in the lower pods turned brown. *Sinapis alba* was harvested 91 days and *B. napus* 98 days after planting. Plants were cut at ground level and placed in cloth bags. Pods were removed from plants and weighed. Seeds were removed from the pods and the number of seeds from each plant counted using an electronic seed counter (Halross Instruments Corporation Limited, Winnipeg, MB). Fresh weight of seeds from each plant was measured. Plants were separated into leaves and support tissue, dried to constant weight at 60°C, and weighed.

Thirty seeds from each plant were selected randomly for a germination test. These seeds were weighed and placed on moist filter paper in a dark growth chamber at 20°C. The number of seeds germinated after seven days was recorded. The remaining seeds not used in the germination test were dried to constant weight at 60°C and weighed.

Data Analysis. Data for each species of plant and each period of measurement were analysed separately to reduce the variation in error for the treatments. For an individual plant, data for leaf length was added, and the total leaf length for each plant was used in the analysis. Data for leaf length and number of seeds were transformed to natural logarithms before analysis to reduce the variation in error among samples. Transformed data were reexamined to confirm that variances were stabilized before proceeding with the analysis. Relative growth rate was calculated as: $(\ln S_2 - \ln S_1) / (7 * (t_2 - t_1))$, where S_2 is the leaf length at time 2, S_1 is the leaf length at time 1, t_2 is time 2 (days), and t_1 is time 1 (days). Data for growth stage were ranked prior to analysis so there were equal differences between

consecutive values. For each type of measurement, Dunnett's two-tailed *t*-test was used to test for differences between means of treated plants and control plants within each pattern of injury. For each plant measurement, the mean of the transformed data for control plants was subtracted from the transformed value of the injured plants of the corresponding type of injury. To test for differences between different patterns of injury, data was analyzed using analysis of variance, with level of damage nested within pattern of damage (SAS Inst. 1989). Contrasts were used to determine differences among treatments. Two-tailed Fisher's exact tests were performed to determine if treatments with meristem injury resulted in multiple stems or injury to leaves.

The effects of artificial injury were compared to the effects of insect injury described in Chapter 4. The comparisons were between the real insect injury and the artificial injury designed to simulate that injury. Data were transformed and adjusted as previously described. *t*-tests (SAS Inst. 1989) were used to determine differences in leaf length, seed weight, and the number of seeds between artificially injured and insect injured plants within each group. For leaf length, *t*-tests were performed at each of 4 different periods after the injury. Since this resulted in 64 *t*-tests being performed for leaf length, a test-wise α of 0.005 was used to determine significance to reduce the risk of a type 1 error.

Results

Controlled Environment Study

Brassica napus. The weight of leaf tissue was dependent on the type of injury at 6 ($F=4.22$; $df=4,25$; $P=0.0095$) and 12 ($F=3.38$; $df=4,24$; $P=0.0250$) days after the injury (Table 7). The weight of support tissue was also dependent on type of injury at 6 ($F=3.18$; $df=4,25$; $P=0.0303$), and 12 ($F=3.64$; $df=4,24$; $P=0.0187$) days after the injury. At six days after the injury *B. napus* plants with dispersed injury were the most severely affected; they had less leaf weight and support tissue than uninjured plants (Table 7).

The weight of roots was dependent on type of injury at 12 ($F=8.94$; $df=4,24$; $P=0.0001$) days after the injury. By 12 days after the injury, plants with dispersed injury equalled control plants in weight of leaves and support tissue, but the weight of roots was reduced. Perforation injury was the only treatment not resulting in a reduction in weight of roots 12 days after the injury.

Plants with meristem injury were the most severely affected by 12 days after the injury, with reductions in weight of leaf tissue, support tissue, and roots. Root weight in plants with meristem injury was less than half the root weight of uninjured plants at 12 days after the injury.

Sinapis alba. The weight of leaf tissue ($F=4.58$; $df=4,25$; $P=0.0065$) and roots ($F=6.68$; $df=4,25$; $P=0.0008$) were dependent on the type of injury 7 days after the injury (Table 8). Plants receiving perforation injury had less than half the weight of leaves and roots

TABLE 7. Mean dry weight ($\pm$ SE) of leaves, support tissue, and roots for *Brassica napus* with different types of artificial injury in a controlled environment.

Treatments	Mean weight (g) and SE of:		
	Leaves ¹	Support tissue ¹	Roots ¹
6 days after injury			
Control	0.139 $\pm$ 0.011a	0.025 $\pm$ 0.003a	0.027 $\pm$ 0.003a
Concentrated	0.087 $\pm$ 0.005ab	0.016 $\pm$ 0.001ab	0.016 $\pm$ 0.003a
Dispersed	0.079 $\pm$ 0.015b	0.015 $\pm$ 0.003b	0.020 $\pm$ 0.005a
Perforation	0.114 $\pm$ 0.013ab	0.019 $\pm$ 0.002ab	0.024 $\pm$ 0.004a
Meristem	0.094 $\pm$ 0.012ab	0.022 $\pm$ 0.002ab	0.022 $\pm$ 0.002a
12 days after injury			
Control	1.072 $\pm$ 0.051a	0.352 $\pm$ 0.035a	0.286 $\pm$ 0.022a
Concentrated	0.785 $\pm$ 0.073ab	0.257 $\pm$ 0.018ab	0.167 $\pm$ 0.021bc
Dispersed	0.830 $\pm$ 0.045ab	0.256 $\pm$ 0.014ab	0.168 $\pm$ 0.020bc
Perforation	0.992 $\pm$ 0.064ab	0.318 $\pm$ 0.023ab	0.206 $\pm$ 0.018ab
Meristem	0.732 $\pm$ 0.113b	0.228 $\pm$ 0.030b	0.120 $\pm$ 0.020c
20 days after injury			
Control	3.125 $\pm$ 0.243a	2.372 $\pm$ 0.092a	1.117 $\pm$ 0.074a
Concentrated	3.259 $\pm$ 0.136a	1.971 $\pm$ 0.201a	1.070 $\pm$ 0.102a
Dispersed	3.355 $\pm$ 0.242a	1.929 $\pm$ 0.124a	1.045 $\pm$ 0.064a
Perforation	2.882 $\pm$ 0.078a	1.806 $\pm$ 0.171a	0.983 $\pm$ 0.058a
Meristem	2.708 $\pm$ 0.133a	1.706 $\pm$ 0.209a	0.807 $\pm$ 0.089a
27 days after injury			
Control	4.507 $\pm$ 0.675a	5.508 $\pm$ 0.547ab	2.556 $\pm$ 0.279a
Concentrated	5.281 $\pm$ 0.469a	4.322 $\pm$ 0.214b	2.334 $\pm$ 0.161a
Dispersed	4.752 $\pm$ 0.226a	5.381 $\pm$ 0.430ab	2.231 $\pm$ 0.244a
Perforation	4.234 $\pm$ 0.268a	6.973 $\pm$ 0.554a	2.578 $\pm$ 0.359a
Meristem	4.682 $\pm$ 0.219a	5.905 $\pm$ 0.357ab	2.628 $\pm$ 0.179a

¹For each period of measurements, means within a column followed by the same letter are not significantly different ($P > 0.05$; Tukey's Studentized Range Test). Analysis performed on natural log transformed values of the data presented.

TABLE 8. Mean dry weight ($\pm$ SE) of leaves, support tissue, and roots for *Sinapis alba* with different types of artificial injury in a controlled environment.

Treatments	Mean weight (g) and SE of:		
	Leaves ¹	Support tissue ¹	Roots ¹
7 days after injury			
Control	0.106 $\pm$ 0.016a	0.020 $\pm$ 0.004a	0.033 $\pm$ 0.005a
Concentrated	0.099 $\pm$ 0.018ab	0.019 $\pm$ 0.004a	0.033 $\pm$ 0.007a
Dispersed	0.053 $\pm$ 0.007b	0.011 $\pm$ 0.001a	0.018 $\pm$ 0.002ab
Perforation	0.051 $\pm$ 0.010b	0.010 $\pm$ 0.003a	0.014 $\pm$ 0.002b
Meristem	0.086 $\pm$ 0.010ab	0.019 $\pm$ 0.003a	0.030 $\pm$ 0.003a
14 days after injury			
Control	0.375 $\pm$ 0.057a	0.161 $\pm$ 0.022a	0.077 $\pm$ 0.012a
Concentrated	0.411 $\pm$ 0.029a	0.175 $\pm$ 0.016a	0.079 $\pm$ 0.005a
Dispersed	0.346 $\pm$ 0.077a	0.125 $\pm$ 0.034a	0.071 $\pm$ 0.016a
Perforation	0.481 $\pm$ 0.075a	0.177 $\pm$ 0.024a	0.084 $\pm$ 0.013a
Meristem	0.474 $\pm$ 0.077a	0.239 $\pm$ 0.036a	0.101 $\pm$ 0.017a
21 days after injury			
Control	0.775 $\pm$ 0.111a	1.273 $\pm$ 0.097a	0.263 $\pm$ 0.029a
Concentrated	0.967 $\pm$ 0.065a	1.373 $\pm$ 0.123a	0.312 $\pm$ 0.025a
Dispersed	0.887 $\pm$ 0.101a	1.118 $\pm$ 0.165a	0.271 $\pm$ 0.023a
Perforation	1.060 $\pm$ 0.067a	1.375 $\pm$ 0.087a	0.325 $\pm$ 0.012a
Meristem	0.774 $\pm$ 0.044a	1.541 $\pm$ 0.075a	0.303 $\pm$ 0.014a
28 days after injury			
Control	0.874 $\pm$ 0.125a	3.106 $\pm$ 0.243a	0.454 $\pm$ 0.054a
Concentrated	0.976 $\pm$ 0.148a	2.877 $\pm$ 0.167a	0.391 $\pm$ 0.023a
Dispersed	1.013 $\pm$ 0.077a	2.766 $\pm$ 0.215a	0.441 $\pm$ 0.045a
Perforation	1.230 $\pm$ 0.068a	2.653 $\pm$ 0.249a	0.470 $\pm$ 0.035a
Meristem	1.100 $\pm$ 0.156a	2.963 $\pm$ 0.238a	0.453 $\pm$ 0.044a

¹For each period of measurements, means within a column followed by the same letter are not significantly different ($P > 0.05$; Tukey's Studentized Range Test). Analysis performed on natural log transformed values of the data presented.

as control plants 7 days after the injury. Plants with dispersed injury also had reduced leaf weight 7 days after the injury. Plants receiving concentrated and meristem injury had equal leaf, support tissue, and root weights as control plants at all harvests. Injury to meristems had little effect on growth of any of the plant tissues in *S. alba*, although in plants with meristem injury the first true leaves were often deformed.

Compensation from injury to cotyledons and the apical meristem was complete in leaves, support tissue, and roots by 14 days after the injury in *S. alba*, whereas in *B. napus* complete compensation took 20 days (Tables 7 and 8).

Field Study

This experiment used leaf length, plant developmental stage, average weight of seeds, number of seeds, and germination as measures of plant performance after the injury. The y-axes in Figures 8 to 14 show the reduction in growth of a particular type of plant tissue due to the injury. Since data had negative values for plants where growth was reduced due to the injury, the sign of the value (whether positive or negative) was reversed so the majority of bars faced up in the figures. Positive values thus reflect undercompensation, the reduction in plant tissue due to the injury in relation to an uninjured control plant; 0 represents exact compensation; and negative values reflect overcompensation, the increase in production of plant tissue due to the injury in relation to an uninjured control plant.

Leaf Length. Total leaf length was reduced most by perforation injury and least by concentrated injury (Figs. 8 and 9). Within pattern of injury, leaf length tended to be most

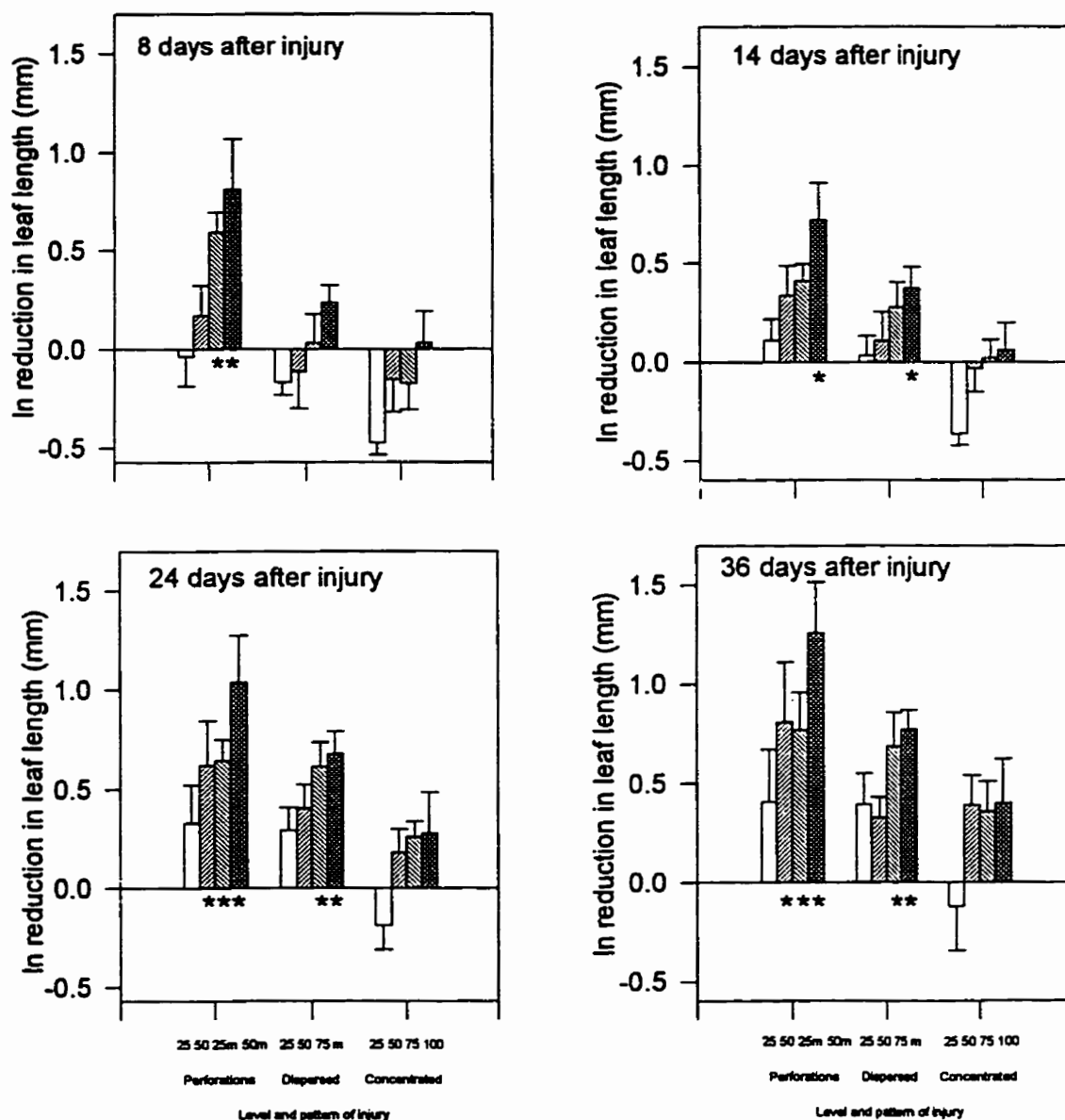


Fig. 8. Reduction in leaf length in *Brassica napus* plants due to injury to seedlings by different types of artificial injury. *=Significantly different from control plants ($P < 0.05$; Dunnett's two-tailed t -test). Numbers for treatments indicate the % of the area of the cotyledons injured. m=injury to the apical meristem.

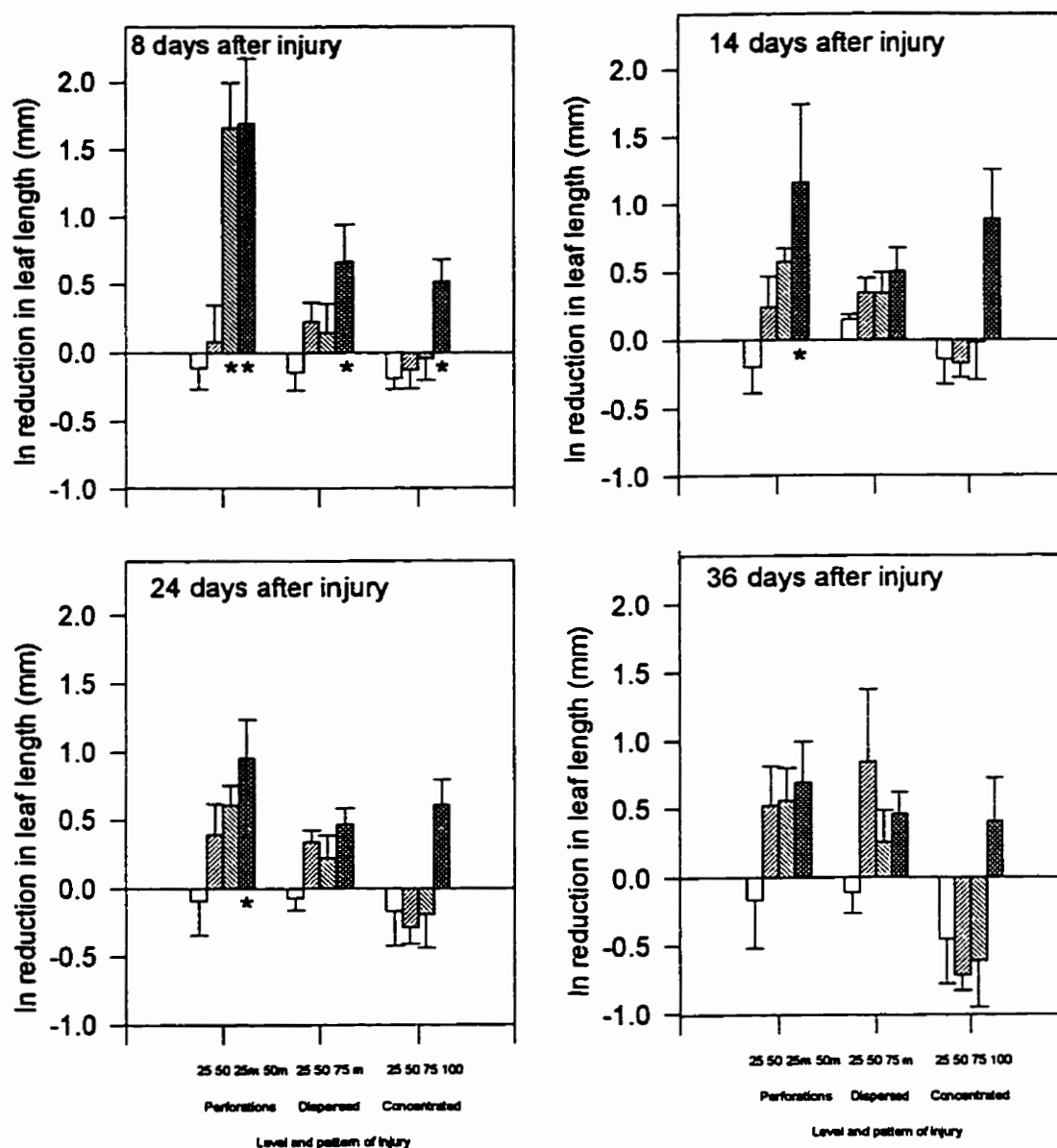


Fig. 9. Reduction in leaf length in *Sinapis alba* plants due to injury to seedlings by different types of artificial injury. *=Significantly different from control plants ($P < 0.05$; Dunnett's two-tailed t -test). Numbers for treatments indicate the % of the area of the cotyledons injured. m=injury to the apical meristem.

reduced at the highest level of injury. In *B. napus*, 25% or 50% injury by perforations resulted in a greater reduction than 25% or 50% concentrated injury at 8 ($t=2.33$; $df=44$; $P=0.0247$), 14 ($t=2.98$; $df=45$; $P=0.0047$), 24 ($t=2.69$; $df=45$; $P=.0100$), and 36 ($t=2.04$; $df=45$; $P=.0468$) days after the injury. In *S. alba* the pattern was similar except the injury usually caused less reduction in leaf length than for *B. napus*.

Injury to the apical meristem altered patterns of leaf and stem growth. Eleven of the 18 plants of *S. alba* that received one of the meristem injury treatments produced more than one main stem ($n=72$; $P<0.0001$). Only two of the 18 plants of *B. napus* that received meristem injury had multiple stem growth, however ($n=72$; $P=0.060$). Multiple stem growth did not occur unless treatments included meristem injury. Both *B. napus* and *S. alba* had injury to true leaves as a result of meristem injury, although this was more evident in *B. napus* than in *S. alba*. Eleven of 18 plants of *B. napus* had injury to the true leaves ($n=72$; $P<0.0001$), and four of 18 plants of *S. alba* showed true leaf injury ($n=72$; $P=0.0029$). Both *B. napus* and *S. alba* plants that received meristem injury treatments occasionally had leaves growing from the axils of the cotyledons. Cotyledon injury alone, regardless of the pattern, did not result in plants producing leaves from the axils of the cotyledons.

Meristem injury in addition to perforation injury reduced leaf length more than perforation injury alone. In *B. napus* a greater reduction in leaf length due to combining meristem injury and perforations occurred at 8 ($t=5.12$; $df=44$; $P<0.0001$), 14 ($t=3.38$; $df=45$; $P=0.0015$), 24 ($t=2.91$; $df=45$; $P=0.0055$), and 36 ($t=2.58$; $df=45$; $P=0.0131$) days after the injury. In *S. alba* the combination of perforations and meristem injury caused a greater reduction in leaf length at 8 ($t=8.34$; $df=44$; $P=0.0000$), 14 ($t=3.47$; $df=36$; $P=0.0014$), and

24 ($t=3.52$; $df=43$; $P=0.0010$) days after the injury. Injury to meristems can have a prolonged negative impact on leaf growth in *B. napus* and *S. alba*. This is further supported by *B. napus* plants with injury only to meristems having a reduced leaf length at 14, 24, and 36 days after the injury, and *S. alba* plants with only meristem injury having a reduced leaf length at 8 days after the injury.

Whether total leaf length completely recovers from feeding when seedlings of *B. napus* and *S. alba* are injured depends on the pattern and level of injury. Total leaf length never was reduced in *B. napus* due to any level of concentrated injury, and in *S. alba* complete removal of cotyledons was the only concentrated treatment to reduce total leaf length. For some injury treatments, there was a considerable delay between the time plants were injured and the time that a reduction in leaf length became significant. This was most noticeable in *B. napus*. At eight days after injury only treatments where perforation injury was combined with meristem injury had a reduced leaf length. It took 14 days for *B. napus* plants with only meristems removed, and 24 days for *B. napus* plants with 50% of the cotyledons removed by perforations or 75% dispersed injury to have significantly less total leaf length than control plants. Thirty-six days after the injury, treatments resulting in reduced leaf lengths at 24 days after the injury still resulted in reduced leaf lengths compared to control plants in *B. napus*.

Although *B. napus* plants with complete removal of cotyledons produced a leaf length equal to those of control plants quicker than did *S. alba*, *S. alba* regained leaf length quicker after perforation injury than did *B. napus*. For *S. alba*, all perforation injury treatments had fully compensated in leaf length by 36 days after injury, whereas in *B. napus* plants all

perforation injury treatments except 25% still had a reduction in leaf length 36 days after the injury.

Relative Growth Rate. Reduction in relative growth rate of leaves was similar for the different patterns of injury in *B. napus*. Reduction in relative growth rate was usually greater in *B. napus* than *S. alba*, regardless of pattern of injury or level of injury (Fig. 10 and 11). In *S. alba*, reduction in relative growth rate of leaves was greater 24-36 days after the injury for plants that received dispersed injury than plants that received concentrated injury ($t=3.03$; $df=42$; $P=0.0042$) (Fig. 11).

Perforation injury alone resulted in more of an increase in the reduction of relative growth rate of leaves initially after injury than combining meristem injury with perforation injury: in *B. napus* from 8-14 ($t=-3.95$; $df=44$; $P=0.0003$) days after the injury (Fig 10) and in *S. alba* from 8-14 ($t=-6.15$; $df=35$; $P=0.0000$) and 14-24 ($t=-2.71$; $df=34$; $P=0.0106$) days after the injury (Fig. 11). In spite of there being less of a reduction in relative growth rates when perforation and meristem injury were combined, compared with perforation injury alone, the reduction in total leaf length in *B. napus* continued to be greater in plants that received both perforation and meristem injury than in plants that only received perforation injury.

A reduced relative growth rate of the leaves may not appear until several weeks after seedling injury. Eight to 14 days after the injury there was no significant reduction in relative growth rate in leaves of *B. napus*. From 14-24 days after the injury, relative growth rate of leaves was decreased for plants at all levels of perforated or dispersed damage. From 24-36 days after the injury there was no significant reduction in relative growth rate of leaves.

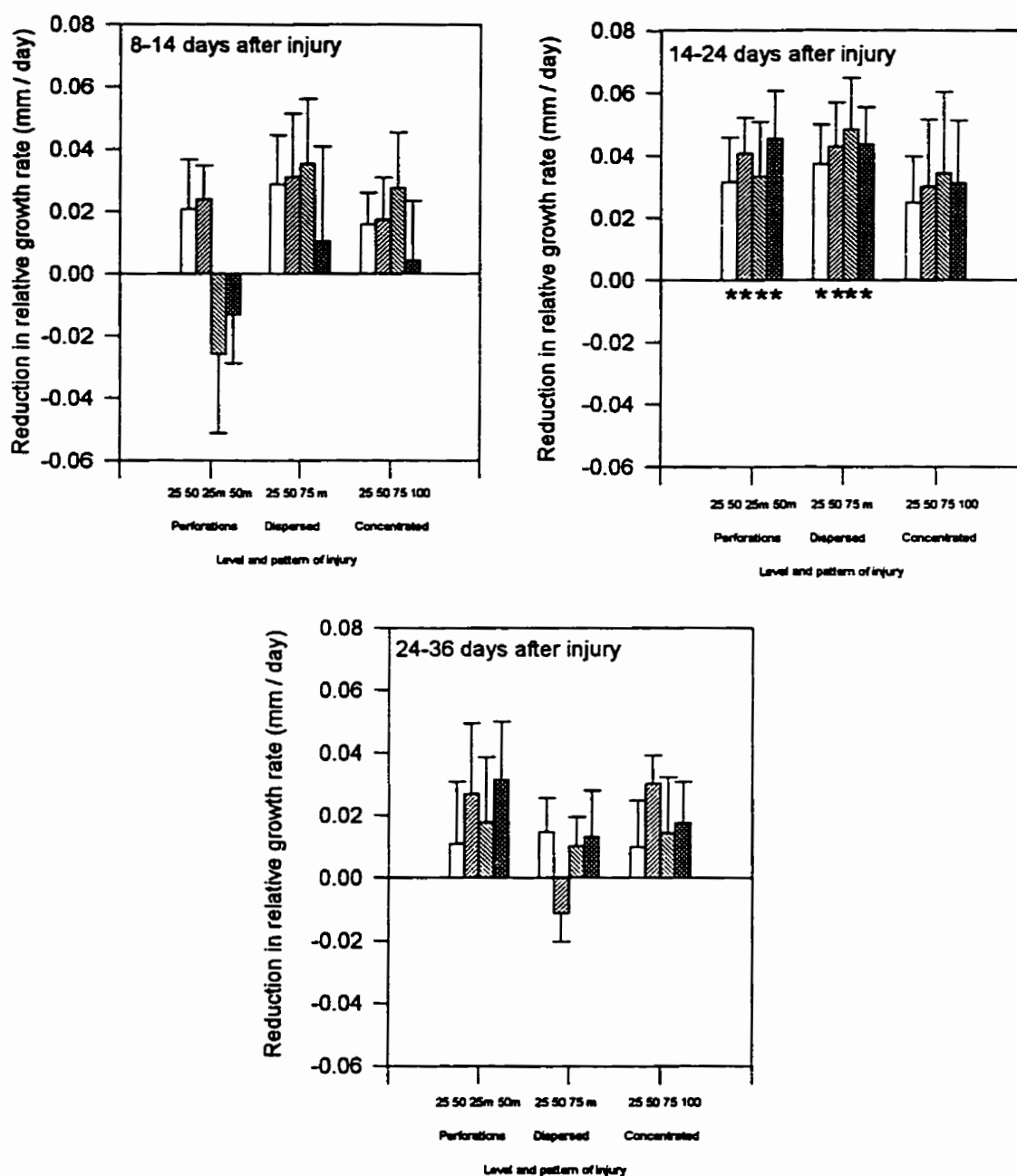


Fig. 10. Reduction in relative growth rates in leaves of *Brassica napus* plants due to injury to seedlings by different types of artificial injury. *=Significantly different from control plants ($P < 0.05$; Dunnett's two-tailed t -test). Numbers for treatments indicate the % of the area of the cotyledons injured. m=injury to the apical meristem.

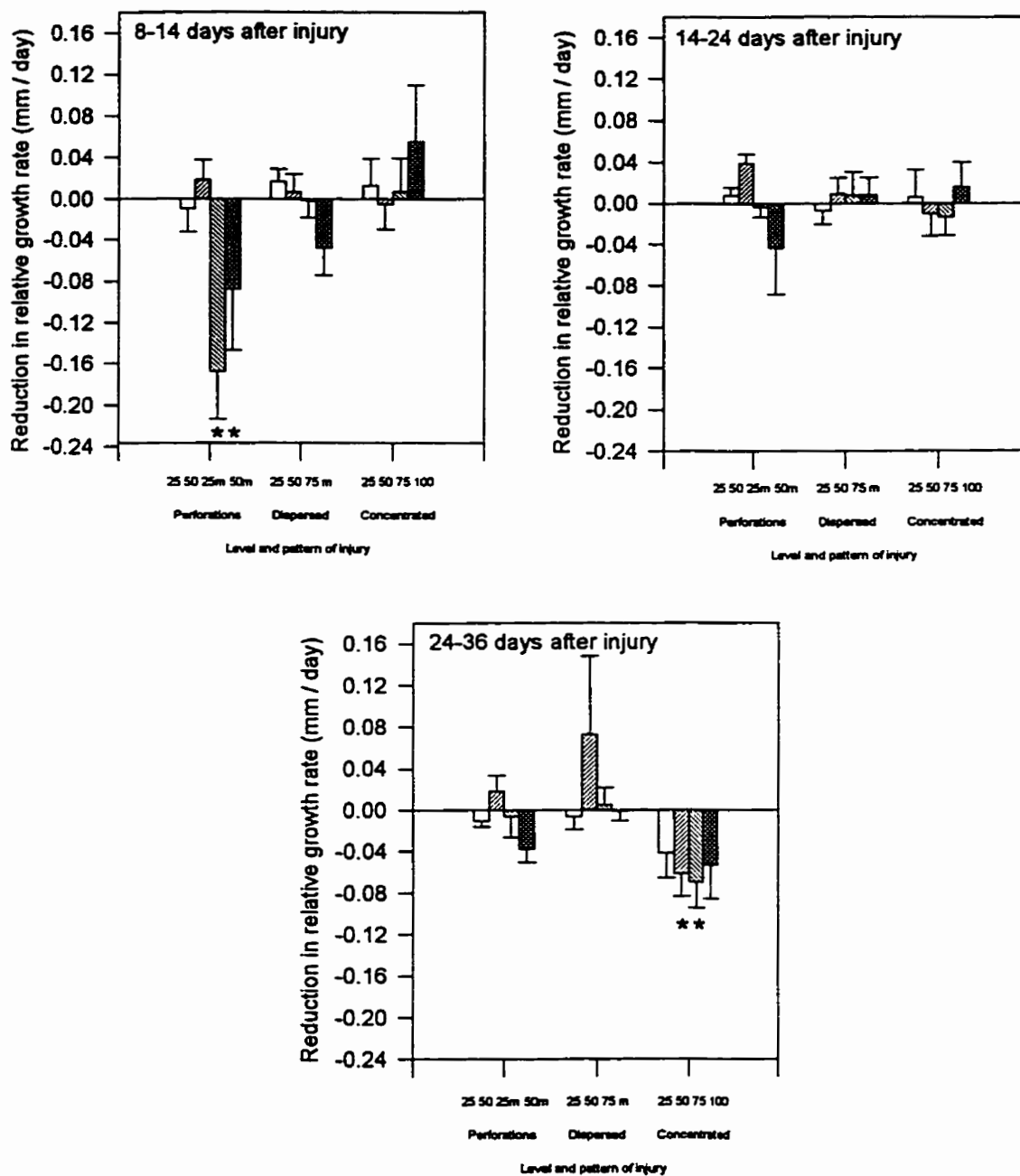


Fig. 11. Reduction in relative growth rates in leaves of *Sinapis alba* plants due to injury to seedlings by different types of artificial injury. * = Significantly different from control plants ($P < 0.05$; Dunnett's two-tailed t -test). Numbers for treatments indicate the % of the area of the cotyledons injured. m = injury to the apical meristem.

Artificial injury did not result in as great a reduction in relative growth rate of the leaves in *S. alba* as in *B. napus*. No significant reductions in relative growth rate occurred in the leaves of *S. alba*. *S. alba* increased the relative growth rate of its leaves when meristem injury was combined with 25% and 50% perforation injury 8-14 days after the injury, and in plants with 50% and 75% concentrated injury 24-36 days after the injury.

Growth Stage. Growth stage measurements were based on the number of true leaves expanded on the plant for *B. napus* at 8 and 14 days after the injury and for *S. alba* at 8 days after the injury (Figs. 12 and 13). During these periods there was a trend toward an increased number of leaves, although such an increase was only significant for meristem damaged plants in *S. alba*.

Fifty percent perforation injury combined with meristem injury resulted in a reduced growth stage in both *S. alba* and *B. napus* 24 days after the injury. Fifty percent perforation injury to cotyledons and 25% perforation injury combined with meristem injury also resulted in a significant reduction in growth stage in *B. napus* at 24 days after the injury. There were no significant differences in growth stage for any of the treatments at 70 days after the injury in either *B. napus* or *S. alba*.

Treatments of perforation injury combined with meristem injury resulted in a greater reduction in growth stage than treatments of only perforation damage in *S. alba* at 14 ($t=3.63$; $df=45$; $P=0.0007$), 24 ($t=5.83$; $df=44$; $P<0.0001$), and 70 ($t=2.62$; $df=42$; $P=0.0121$) days after injury, and in *B. napus* at 70 ($t=2.02$; $df=45$; $P=0.0488$) days after injury. Twenty five or 50% perforation injury caused a greater reduction in growth stage in *B. napus* than 25% or 50% concentrated injury at 24 days after injury ($t=3.06$; $df=45$; $P=0.0037$).

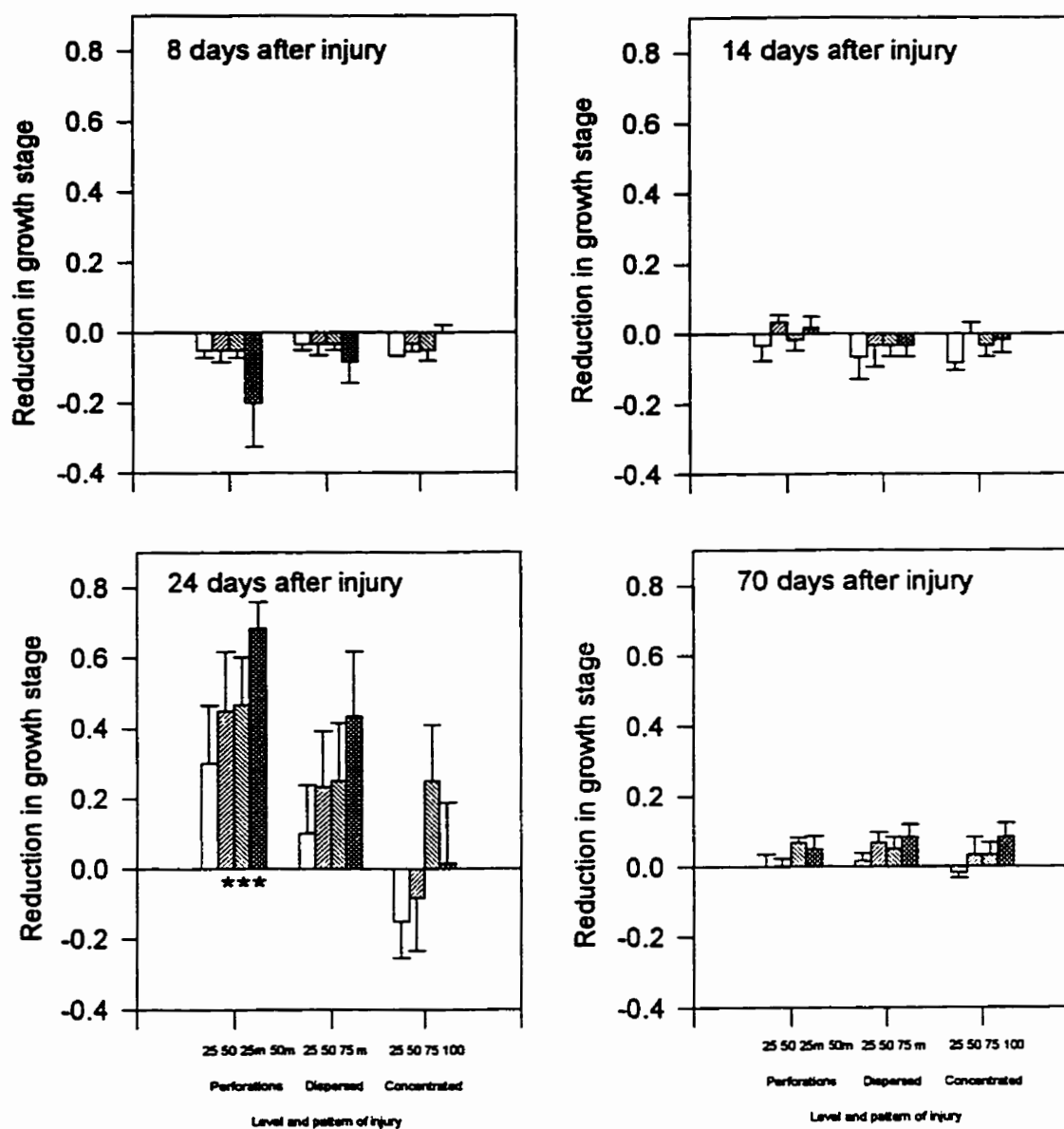


Fig. 12. Reduction in growth stage in *Brassica napus* plants due to injury to seedlings by different types of artificial injury. * = Significantly different from control plants ($P < 0.05$; Dunnett's two-tailed t -test). Numbers for treatments indicate the % of the area of the cotyledons injured. m = injury to the apical meristem.

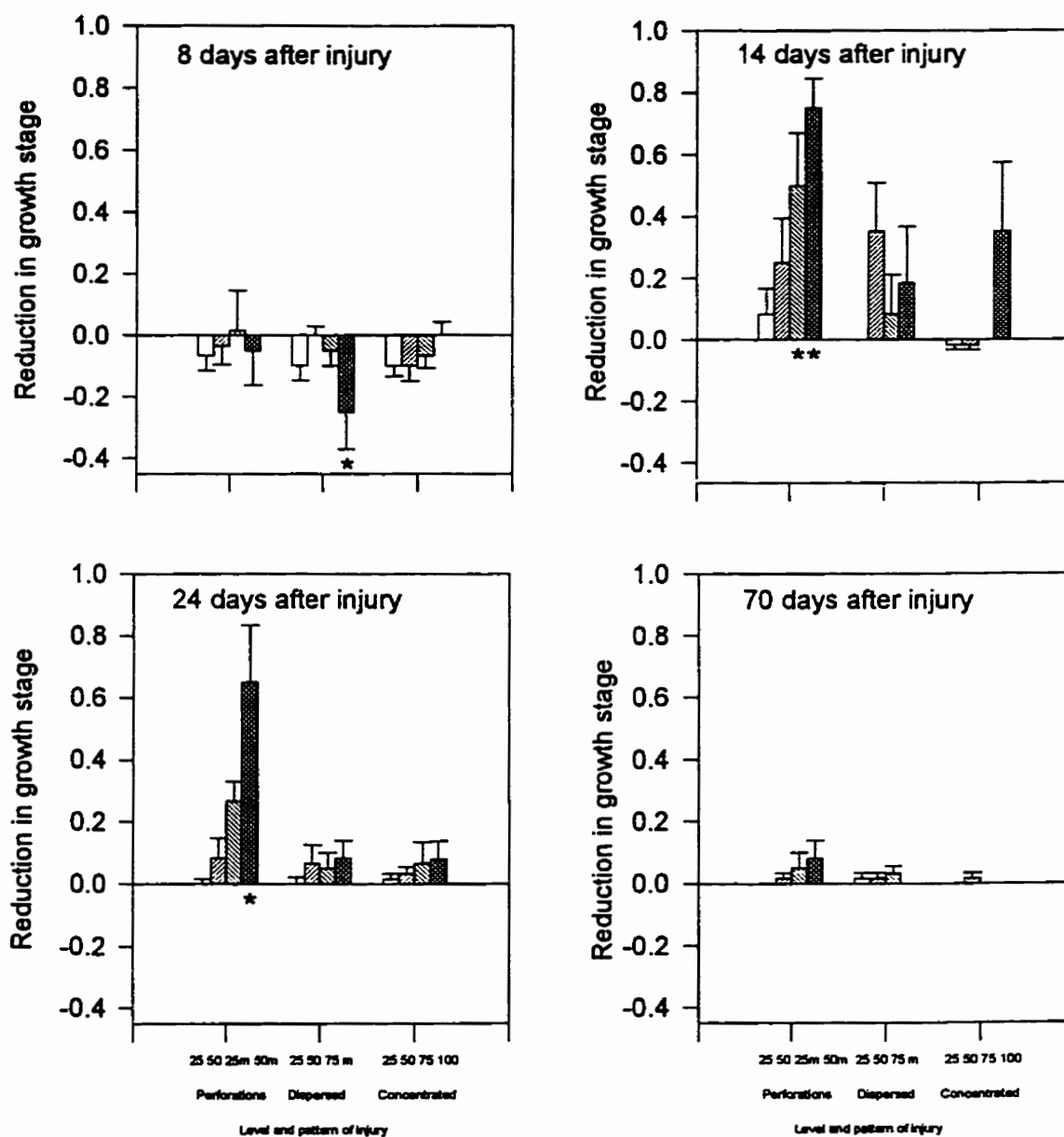


Fig. 13. Reduction in growth stage in *Sinapis alba* plants due to injury to seedlings by different types of artificial injury. *=Significantly different from control plants ($P < 0.05$; Dunnett's two-tailed t -test). Numbers for treatments indicate the % of the area of the cotyledons injured. m=injury to the apical meristem.

Number of Seeds, Seed Size and Germination. Pattern of injury to seedlings affected the number of seeds produced (Fig. 14). *S. alba* plants receiving concentrated injury produced more seeds than plants receiving dispersed injury ($t=2.60$; $df=43$; $P=0.0127$). Combining meristem injury with perforation injury reduced the number of seeds in *S. alba* more than perforation injury alone ($t=4.32$; $df=43$; $P=0.0001$).

Injury to the apical meristems alone resulted in a reduction in the number of seeds in both plant species (Fig. 14). *S. alba* plants where meristem injury was combined with 25% or 50% perforated injury to cotyledons, and *B. napus* plants with cotyledons completely removed or 75% dispersed injury, also had reductions in the number of seeds.

The average weight of seeds was not changed in *B. napus* or *S. alba* due to any treatment, although 25 or 50% perforation injury reduced average weight of seeds more than 25 or 50% dispersed injury in *B. napus* ($t=3.15$; $df=41$; $P=0.0031$). Germination was also not changed due to any of the treatments, although combining meristem injury with perforation injury reduced germination in *S. alba* more than perforation injury alone ($t=2.60$; $df=43$; $P=0.0128$), and 25 or 50% perforation injury reduced germination more than 25 or 50% dispersed injury in *B. napus* ($t=0.248$; $df=42$; $P=0.0172$).

Comparing Plant Response to Insect and Artificial Injury. Some patterns of artificial injury to cotyledons and meristems that appeared similar to specific types of insect injury resulted in a greater recovery in length of *B. napus* and *S. alba* leaves than did the corresponding insect injury (from Chapter 4). This is particularly noticeable for leaf length measurements at 8-9 days after the injury, where the compensatory leaf growth of artificially injured plants was often significantly greater than that of insect injured plants (Table 9).

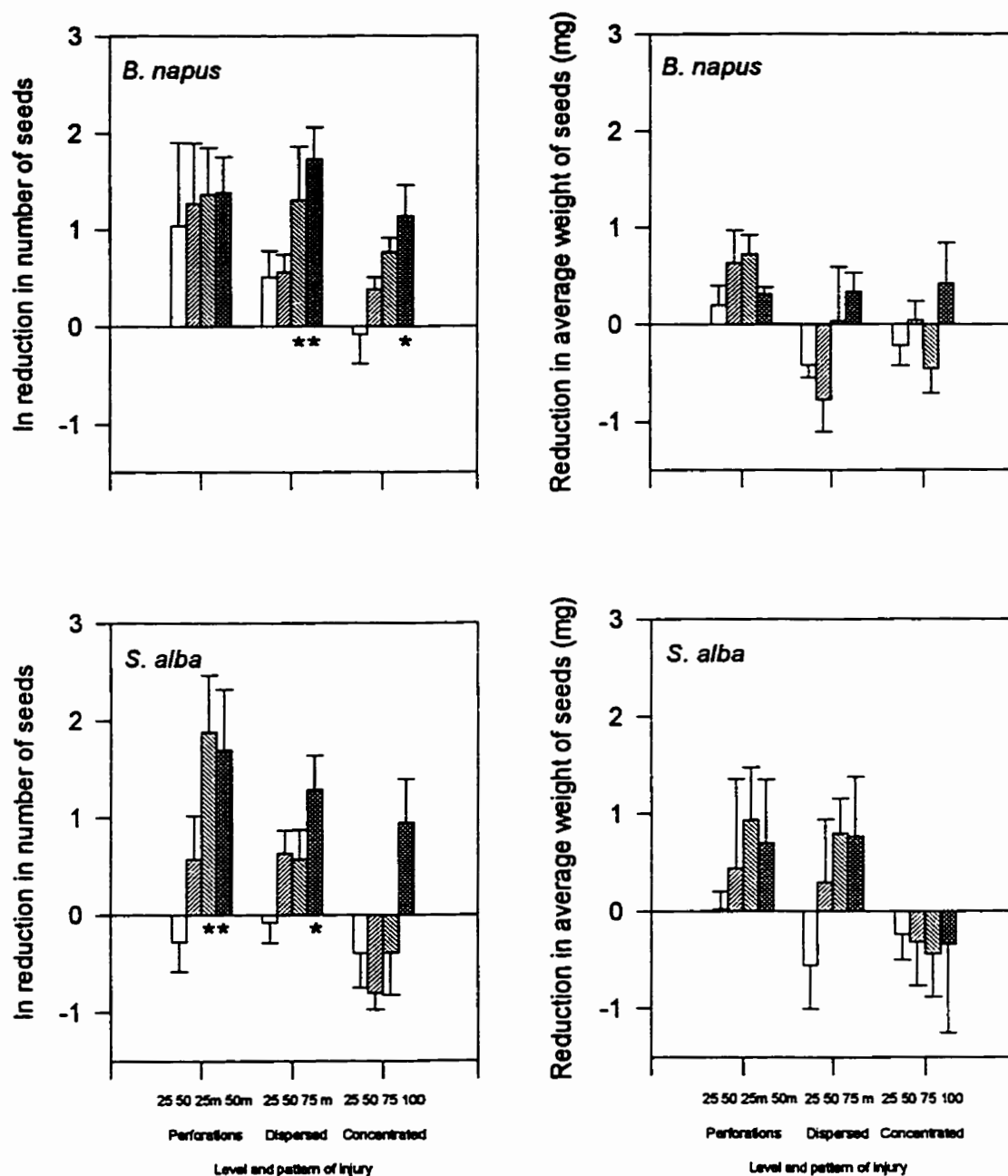


Fig. 14. Reduction in the number of seeds and average weight of seeds in *Brassica napus* and *Sinapis alba* plants due to injury to seedlings by different types of artificial injury. *=Significantly different from control plants ($P < 0.05$; Dunnett's two-tailed t -test). Numbers for treatments indicate the % of the area of the cotyledons injured. m=injury to the apical meristem.

TABLE 9. *t* and *P* - values from *t*-tests comparing the effect of insect injury and artificial injury to cotyledons and meristems on total leaf lengths in *Brassica napus* and *Sinapis alba*.

Injury		Days after the injury							
Insect	Artificial	8-9		14-16		24-25		31-36	
		<i>t</i> ^{1,2}	<i>P</i> ³	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>
<i>B. napus</i>									
'FB 25	Perf.25	6.11	0.001	2.96	0.014	1.61	0.139	2.00	0.074
FB 25	Perf.25m	2.97	0.014	1.42	0.186	0.41	0.692	1.06	0.316
FB 75	Perf.50	4.23	0.002	3.31	0.008	1.53	0.156	1.49	0.166
FB 75	Perf.50m	1.57	0.148	1.58	0.146	0.24	0.812	0.42	0.684
DBM 25	Disp.25	3.23	0.010	1.78	0.106	0.46	0.654	1.12	0.291
DBM 75	Disp.75	2.26	0.047	2.24	0.049	1.77	0.106	1.76	0.109
BA 25	Conc.25	4.52	0.001	3.07	0.021	1.85	0.094	1.50	0.164
BA 75	Conc.75	1.92	0.084	2.18	0.054	0.91	0.386	1.41	0.188
<i>S. alba</i>									
FB 25	Perf.25	1.56	0.167	2.11	0.064	1.14	0.298	0.60	0.571
FB 25	Perf.25m	-1.94	0.081	-1.20	0.261	-2.28	0.046	-1.86	0.092
FB 75	Perf.50	3.83	0.003	2.16	0.059	1.07	0.311	-0.10	0.924
FB 75	Perf.50m	-1.01	0.366	-0.52	0.627	-0.67	0.519	-0.52	0.616
DBM 25	Disp.25	2.33	0.042	0.71	0.498	0.43	0.674	-0.29	0.776
DBM 75	Disp.75	1.55	0.152	0.40	0.697	0.33	0.746	-0.53	0.609
BA 25	Conc.25	4.72	0.001	2.02	0.075	0.99	0.344	0.23	0.826
BA 75	Conc.75	2.35	0.041	1.48	0.174	2.01	0.080	1.46	0.178

¹Positive *t*-values indicate a longer total leaf length in plants with artificial damage than in plants with insect damage.

²Values of leaf length were transformed using natural logarithms and standardized by their control plants prior to analysis.

³Since 64 *t*-tests were performed, a test-wise α of 0.005 was used to determine significance to reduce the risk of a type I error.

⁴Injury treatments: FB= flea beetle, Perf.= perforations, DBM= diamondback moth, Disp.= dispersed, BA= bertha armyworm, Conc= concentrated, m= damage to apical meristem in addition to the cotyledon damage. Numbers for treatments indicate the % of the area of the cotyledons damaged.

Plant response to perforation injury to the cotyledons combined with meristem injury was more similar to flea beetle injury than perforation injury alone (Table 9). Compensatory leaf growth was greater for plants with only perforation injury than plants with a similar amount of flea beetle injury to the cotyledons 8-9 days after the injury at both the low and high levels of the injury in *B. napus*, and 8-9 days after the injury in *S. alba* plants with a high level of injury. At the high level of injury there was never any difference in the level of compensatory growth of leaves or average size of seeds or number of seeds between flea beetle injured plants and plants with perforation injury and meristem injury (Tables 9 and 10). At the low level of injury, *S. alba* plants with a combination of perforation and meristem injury had a reduction in the number of seeds compared with flea beetle injured plants.

A low level of injury by bertha armyworm larvae initially resulted in greater reductions in leaf length than concentrated injury, although by 14-16 days after the injury these differences no longer existed (Table 9). In general, insect injury initially reduced leaf length more than the corresponding artificial injury techniques, and these differences disappeared over time. The patterns of compensation were similar, however, regardless of whether insect or artificial injury was used. In both studies plants compensated best for injury by bertha armyworm larvae or simulation of this injury, and poorest for injury by flea beetles or simulation of this injury.

TABLE 10. *t* and *P* - values from *t*-tests comparing the effect of insect and artificial injury to cotyledons and meristems of *Brassica napus* and *Sinapis alba* on average weight of seeds and number of seeds.

Injury		Average weight of seeds ¹ (g)		Number of Seeds	
Insect	Artificial	<i>t</i> ²	<i>P</i>	<i>t</i>	<i>P</i>
<i>B. napus</i>					
4FB 25	Perf.25	0.75	0.471	0.10	0.925
FB 25	Perf.25m	-1.47	0.172	-0.40	0.698
FB 75	Perf.50	1.13	0.283	1.29	0.225
FB 75	Perf.50m	2.09	0.089	1.37	0.205
DBM 25	Disp.25	-0.51	0.619	-0.07	0.946
DBM 75	Disp.75	0.27	0.795	0.90	0.388
BA 25	Conc.25	2.16	0.057	1.41	0.188
BA 75	Conc.75	2.57	0.028	1.24	0.242
<i>S. alba</i>					
FB 25	Perf.25	-0.64	0.535	1.35	0.207
FB 25	Perf.25m	-2.06	0.079	-2.85	0.041
FB 75	Perf.50	1.00	0.342	1.00	0.342
FB 75	Perf.50m	0.86	0.415	-0.70	0.499
DBM 25	Disp.25	1.56	0.149	0.34	0.744
DBM 75	Disp.75	0.96	0.360	0.50	0.630
BA 25	Conc.25	0.49	0.634	0.22	0.828
BA 75	Conc.75	0.54	0.602	1.04	0.324

¹Total weight of seeds/number of seeds

²Positive *t*-values indicate larger seed weights and number of seeds in plants with artificial damage than in plants with insect damage.

³Values for number of seeds were transformed using natural logarithms, and average weight of seeds and number of seeds were standardized by their control plants prior to analysis.

⁴Injury treatments: FB= flea beetle, Perf.=perforations, DBM= diamondback moth, Disp.= dispersed, BA= bertha armyworm, Conc.= concentrated, m= injury to the apical meristem in addition to the injury to the cotyledon. Numbers for treatments indicate the % of the area of the cotyledons injured.

Discussion

The response of *B. napus* and *S. alba* to the different patterns of injury was dependent on the type of injury. Plants generally compensated best for concentrated injury, and least for injury by perforations and to the apical meristem. There were reductions in the number of seeds per plant even though injury was restricted to plants in the seedling stage.

Recovery of leaf growth was quicker in the controlled environment experiments than in the field experiment. For all the types of injury, recovery of leaf growth in controlled environment experiments was complete for *S. alba* by 14 days after the injury, and for *B. napus* by 20 days after the injury. By 36 days after the injury leaf growth was still reduced due to perforations and dispersed injury in the field, however. This may be because plants grown in a controlled environment had more ideal soil moisture and temperature conditions and had less competition from neighboring plants.

Perforation injury did not result in reductions in plant growth in *B. napus* in a controlled environment, and meristem injury was the most detrimental in these plants. Perforation injury was the most detrimental in *S. alba* plants in a controlled environment, however, while meristem injury did not result in reductions in plant growth. The reason for the different responses by *S. alba* and *B. napus* to the different forms of injury is not clear.

Differences in the circumference of injury for different types of injury is a possible reason for the varying levels of compensation. By simulating insect injury to apple leaves, Hall and Ferree (1976) found that photosynthesis was reduced more when leaf area was removed as small holes than when the same leaf area was removed as large holes. The

average circumference of leaf area removed was 2.8 times greater for small holes than for large holes. Thus the amount of exposed edge after feeding injury may be more important to the severity of injury than the amount of tissue removed. This mechanism may explain the reduced recovery of the crucifers from perforation injury than from the other patterns of injury.

Plants appear to compensate better when injury is dispersed among leaves, instead of concentrated on a few leaves (Lowman 1982, Mauricio *et al.* 1993). Yet in this study, where patterns of injury were often restricted to a pair of cotyledons, plants compensated better for concentrated patterns of injury than when injury was more dispersed. To determine whether plants compensate better for dispersed or concentrated injury it is important to distinguish between injury within a leaf and injury among all the plant foliage.

Whether plants compensate better for dispersed or concentrated injury may also depend on the feeding habits of the herbivore, and the stage of the plant attacked. For herbivores whose feeding kills more tissue than is consumed, such as through injury to petioles or vascular tissue, dispersed injury throughout the plant is likely more detrimental to the plant than concentrated injury. For feeding patterns where uneaten plant tissue is able to function normally, concentrated injury appears to be more detrimental than dispersed injury (Lowman 1982, Mauricio *et al.* 1993).

In Chapter 4 it was found that *B. napus* and *S. alba* compensated better for feeding injury by bertha armyworm larvae than for feeding injury by diamondback moth larvae. Injury by bertha armyworm larvae was also more concentrated than injury by diamondback moth larvae. Thus, pattern of injury is a likely reason for the differential response of *B. napus*

and *S. alba* to feeding by these two insects.

Injury to the apical meristem of plants can be beneficial in some plants and harmful in others. The thistle *Jurinea mollis* Ascherson produced multiple rosettes on individual roots after two unidentified species of lepidopteran larvae ate the central part of the basal rosette. Each of these rosettes eventually produces a flower stalk. Plants with multiple stalks can produce up to three times as many seeds as those without multiple stalks (Inouye 1982). On the other hand, removal of active meristems of the tussock grass *Agropyron desertorum* (Fisch. ex Link) Schult. resulted in much less regrowth than clipping patterns where active meristems were not removed (Gold and Caldwell 1989). Injury to the apical meristem was harmful to both *B. napus* and *S. alba*. Meristem injury resulted in reductions in leaf length and the number of seeds, in spite of the alterations in leaf and stem growth due to the injury.

Ehrlén (1995) tested the effects of meristem injury by mollusks, grazing by roe deer, and leaf injury by invertebrates on survival, growth, and fruit production in the perennial herb, *Lathyrus vernus* L. Meristem injury by mollusks had the most severe effects on the performance of *L. vernus* individuals. Grazing and leaf injury in *L. vernus* occurs late in the season, whereas meristem injury occurs early in the season. Growth is delayed by the need for activation of new shoot meristems, and the accumulation of resources during the most favourable period of the year is thus impossible. Thus the plant stage when meristem injury occurs may affect how the plant compensates for the injury.

In the cruciferous seedlings studied here, there was sometimes a considerable delay between the time plants were injured and the time that a reduction in leaf length became significant. This was most noticeable in *B. napus*. Thus, the timing of measurements for

compensatory growth in plants is important. Data from this experiment suggest that for cotyledon injury in *B. napus*, measurements be made for at least 24 days after injury to cotyledons to determine losses in leaf growth due to the injury.

Leaves of *B. napus* appear to be affected less by complete removal of cotyledons than leaves of *S. alba*, yet leaves of *S. alba* appear to be affected less by perforation injury to cotyledons than leaves of *B. napus*. This suggests that plant compensation is dependent on the type of injury, and is not just a general response to a particular level of injury. Dry weight measurements 6 days after injury to *B. napus* and *S. alba* seedlings demonstrated that seedlings of *S. alba* can tolerate low levels of flea beetle feeding injury to their cotyledons and continue to grow at almost the same rate as uninjured seedlings, whereas the growth of *B. napus* declines continually with the level of feeding injury (Bodnaryk and Lamb 1991). Yet this does not imply that *S. alba* will compensate better than *B. napus* for other defoliators of crucifer seedlings, or that there would be a recovery in yield.

This research supports the hypothesis that the pattern of injury to a leaf will affect the ability of the plant to compensate for the injury. Thus when simulating injury by herbivores it is important that the pattern of feeding of the herbivore be mimicked as closely as possible. If meristems are injured by the herbivore, this injury also must be mimicked for plant response to be as similar to the herbivore injured plant as possible.

In Chapter 4 it was found that plant response to insect herbivory is different for equal amounts of injury by different species of insects. The patterns of injury that plants compensated best for in this study are the patterns associated with the insect feeding that plants were observed to compensate best for in Chapter 4. This supports the hypothesis that

the important difference between how these insects affect plant growth is the pattern of their injury, and not other insect-specific factors associated with their feeding or excretion.

CHAPTER 6**GENERAL DISCUSSION**

Although a diverse fauna of insects may feed on the same plant, or even on the same plant tissue, the interaction between each herbivore and the plant are often thought to be unique. We often conclude that different herbivores have different impacts on the same plant. The factors which contribute to the uniqueness of the relationships between plants and their herbivores have not been thoroughly investigated, however. Therefore, we have little basis for generalization about these processes. In fact, an unequivocal demonstration of the uniqueness of such interactions has proved elusive. This research investigates the interaction between seven insect herbivores and two cruciferous plants, *B. napus* and *S. alba*. These plants were selected because they have similar growth habits and are known to respond differentially to herbivory by the flea beetle *Ph. cruciferae* (Bodnaryk and Lamb 1991, Brandt and Lamb 1994). These insects and plants were used to test three hypotheses: that the specific impact of an insect feeding on a plant varies for different insect herbivores; that plant compensation for defoliation by insects is specific to a particular herbivore, and is not a generalized response to a certain level of injury; and that the specificity of plant compensation to insect injury is a consequence of the pattern of defoliation, and not other insect-specific factors associated with their feeding or excretion.

The research presented in Chapter 3 supports the hypothesis that the specific impact of an insect feeding on a plant varies for different insect herbivores. This hypothesis was tested earlier and supported by Moran and Whitham (1990) and Meyer (1993), although different methodology for quantifying impact was used to test the hypothesis than in Chapter 3. The methodology used by these authors failed to discriminate between the type of herbivory and the amount of herbivory. No standard method for quantifying the impact of

insects on plants has been accepted. Insect populations, the size of individual insects, and the way they feed can vary widely, making it hard to develop standard methods which might be used comparatively. A method referred to as “specific impact” was adopted, which has been used previously to compare the impact of different aphid species on their host (Lamb and MacKay 1995, MacKay and Lamb 1996). This method relies on a comparison of the biomass conversion ratio to quantify the transfer of biomass from plant to insect. It overcomes the differences in size of individual insects and insect populations attacking a plant. It can be implemented relatively easily, and allows comparisons among diverse insects. This method also provides a good understanding of a number of aspects of the interaction between the insect and its host plant.

There are some limitations to using biomass conversion ratios as a measure of impact, however. This technique is easy to implement if young, and thus small, plants are used. Caging the insects on the plants and recovering the insects would be difficult on larger plants. A specific impact for bertha armyworm feeding on *B. napus*, calculated from studies using older plants (Bracken and Bucher 1977, Bailey 1976), was similar to specific impacts for bertha armyworms found in these studies, however. The effects of herbivory on seedling plants may not become apparent until weeks after the injury is inflicted (Chapters 4 and 5). Thus impacts, as measured through biomass conversion ratios, may depend on when the measurements were taken. Also, measurements of impact based on leaf and stem measurements may not be indicative of seed production in the plant (Chapter 4).

Measuring impact on a per plant basis, instead of on a population of plants, may at times be misleading. The injury in these studies, and the response of the plant, were all

measured at the individual plant level. Injury to a plant in a natural setting may reduce competition on neighbouring plants, enabling neighbouring plants to grow larger and produce more seeds. When the density of *B. napus* and *B. rapa* is reduced from 100-200 plants per m² to 40 plants per m² there is less than a 20% loss in seed yield (McGregor 1987). Compensation is mainly due to an increased number of pods on remaining plants, which increase from 20-90 to as many as 600. The effect of insect herbivory on the regrowth of neighbouring plants is still unclear. In some instances herbivores may selectively remove weakened plants, thus reducing competition with healthier plants and enhancing the fitness of the population. The increased growth of uninjured or less injured plants due to higher rates of herbivory on neighboring plants may also intensify the effect of herbivory on the injured plants. This was controlled in the studies in this thesis by eliminating competitive interactions in the laboratory, or equalizing competitive ability among neighboring plants by damaging groups of plants in the field.

In Chapter 3, the seven species of insect herbivores had different impacts on *B. napus* when impact measured as the plant biomass lost to herbivory is adjusted for the biomass gained by the herbivore. The impacts varied from 3-18 for larval defoliators, 11-16 for aphids, and was 80-150 for flea beetles. The impacts for the aphids in this study were high compared to impacts of about 3.0 for the aphids *Metopolophium dirhodum* (Walker) and *Rhopalosiphum padi* L. feeding on wheat and barley (Lamb and MacKay 1995), and impacts around 3.4 for five species of aphids feeding on barley (MacKay and Lamb 1996). No consistent differences in impacts were found between crucifer specialists and generalist feeders or between defoliators and phloem feeders in Chapter 3 of this thesis .

The age of plant tissue consumed may affect the impact the insect has on the plant. Some insects may selectively feed on older and less productive leaf tissue, thus reducing material that may act as a sink competing for nutrients with younger and more productive leaves. Leaves that become shaded as the plant grows have a decreased photosynthetic capacity (Mooney and Gulmon 1982), and defoliation of these older leaves may be less detrimental to the plant than defoliation of younger leaves. Size of the insect may also be a factor in determining impact, since smaller herbivores had the highest impacts on *B. napus* in Chapter 3 of this thesis. Other factors that may affect the impact of a herbivorous insect on a plant are: insect specific products associated with herbivory, such as saliva (Reardon *et al.* 1972, 1974), or excretory products that might affect plant growth either positively through fertilization (Owen and Weigert 1976, Owen 1978) or negatively by covering vegetation or encouraging pathogens or other herbivores; the type of tissue injured (Inouye 1982); the pattern of dispersal of injury on a tissue (Marquis 1992, Mauricio *et al.* 1993); the amount of tissue that is removed or injured but not ingested by defoliating herbivores (Lowman 1982); or plant compensatory responses specific to particular herbivores.

The research from Chapter 4 supports the hypothesis that one of the reasons that the specific impacts of different insects on a plant varies is that plant compensation varies for equal amounts of feeding by different insects. The insects with the highest specific impacts on the plants were also the insects for which the plants were found to compensate least. In Chapter 3, insects with very different mechanisms of feeding on the plants were used, whereas in Chapter 4 a subset of the insects from Chapter 3 were used, all of which bite and chew the plant tissues. Even among insects that feed by biting and chewing plant tissues, the

specificity of the compensatory responses of the plant for the different insects may cause the interactions between the insect and its host plant to be different. The different levels of compensation for feeding by different insects was more evident in *B. napus* than *S. alba*. *Sinapis alba* recovered better from the injury than *B. napus*.

A problem with studying the compensatory responses of plants for feeding by different herbivores is controlling the level of injury so insect specific effects on the plant can be separated from the effects of different levels of injury by the different insects. This experiment overcame this problem by using plants in the cotyledon stage, where the level of injury was easy to rate. The rating system used in the present experiments proved to be an effective method of estimating the actual level of injury to the plant. Similar studies using plants in a more advanced stage of development would be difficult because controlling and rating the level of injury to older plants would be a technical challenge.

Several studies have been performed using simulated herbivore injury to determine the effect of different types of injury on a plant's compensatory ability (Lowman 1982, Marquis 1992, Mauricio *et al.* 1993). Level of injury was able to be controlled in these studies. These studies determined that injury dispersed over the plant, such as over many leaves of a mature plant, results in improved compensation over more concentrated injury. Although these studies deal with how plants respond to different patterns of injury, they do not test the hypothesis that plant compensation for herbivory is herbivore dependant, since insect specific factors other than pattern of injury were not considered. The research in Chapter 4 is the first research to study the specificity of plant compensation for herbivory, without having factors such as the level of injury confound the results. Some of the reasons

that plant compensation for injury by these insects may have been insect specific are: the type of tissue injured; the pattern of dispersal of injury on a tissue; and insect specific products associated with herbivory, such as saliva or excretory products.

Artificial means of injury designed to simulate herbivory may enable the importance of pattern of injury on plant compensation to be tested, provided the artificial injury accurately simulates insect injury. Such techniques can demonstrate the response of the plant to the pattern of injury or type of tissue injured without the confounding effects that insect saliva or excretory products may have on the plant. Results from Chapters 4 and 5 demonstrate that insect injury to plants is difficult to simulate. Although plants sometimes compensated better for artificial injury than for insect injury during the first two weeks of measurements, the patterns of compensation were similar, however, regardless of whether insect or artificial injury was used. In both studies plants compensated best for injury by bertha armyworm larvae or simulation of this injury, and poorest for injury by flea beetles or simulation of this injury. Thus, the simulation techniques in this experiment enable a test to be performed on whether the important difference in how the insects used in Chapter 4 affect plant growth is due to the pattern of injury on the plant, or other insect specific factors such as saliva or excretory products.

The research in Chapter 5 supports the hypothesis that the level of compensation by a plant for insect injury is dependant on the pattern of injury. Plants compensated best for concentrated injury, less for injury that was dispersed, and even less for injury by perforations or injury to the apical meristem. It is hypothesized that the circumference of injury may be an aspect of pattern of injury that affects a plant's compensatory ability.

Increased circumference of area of apple, *Malus sylvestris* Mill, leaf area lost by simulated insect feeding increased the reduction in net photosynthesis (Hall and Ferree 1976). The circumference of injury may be why plants compensate worse for injury by flea beetles than for injury by diamondback moth larvae and bertha armyworm larvae, since the small perforations typical of feeding by flea beetles would have a higher circumference of injury than an equal amount of tissue removed as larger pieces by bertha armyworm larvae or diamondback moth larvae.

An insect's size and mobility may affect the pattern of feeding, and hence the circumference of injury an insect causes. For defoliating insects, an insect's size may affect the insect's feeding style. Small insects, such as flea beetles, may not be able to remove as great a volume of plant tissue in a bout of feeding as a larger insect, such as a late-instar bertha armyworm larva. This may result in more dispersed injury. Insects that are very mobile may also cause more dispersed injury.

To determine how plants respond to different patterns of dispersal of injury, a distinction must be made between pattern of dispersal on a single plant structure, such as a leaf, versus the pattern of dispersal where the unit of study is the whole plant. Injury dispersed over the whole plant, such as over many leaves of a mature plant, results in improved compensation over more concentrated injury (Lowman 1982, Marquis 1992, Mauricio *et al.* 1993). However, results from Chapter 5 show that plants compensate better for concentrated injury than dispersed injury when pattern of injury is assessed within the cotyledon of a plant.

This research demonstrates that injury to seedlings alone can affect the number of

seeds produced by *B. napus* and *S. alba*. In *S. alba*, all artificial injury treatments that reduced the number of seeds involved meristem injury. The only insect injury treatment to reduce the number of seeds in *S. alba* was a high level of flea beetle injury, which was shown to result in meristem injury. In *B. napus*, a high level of artificial injury to cotyledons alone, or a high level of injury by diamondback moth larvae or flea beetles, can cause a reduction in the number of seeds. This research is consistent with the fact that defoliation by flea beetles generally has a much greater impact on canola than defoliation by larvae of diamondback moth and bertha armyworm, unless the amount of defoliation the latter two insects do is very substantial. This research does not support the finding by Nowatzki and Weiss (1997), however, that when the growing point, stem, or petiole is not injured, *B. napus* seedlings have the ability to compensate with growth when 75% of the cotyledon is injured.

This research demonstrates that pattern of injury is very critical when attempting to simulate insect injury. Removing a certain level of plant tissue will result in different responses by the plant when the tissue is removed in different patterns. For example, attempts to simulate flea beetle injury by removing large areas of the cotyledons using scissors, as done by Lamb *et al.* (1993), would result in better compensation and recovery from the injury than a technique that more accurately simulates the perforation pattern of injury done by flea beetles. When feeding on seedlings, flea beetles often feed on the apical meristem as well as the cotyledons. Thus, perforation injury to cotyledons alone, as done by Nowatzki and Weiss (1997), may not simulate flea beetle injury as well as injury to both cotyledons and the apical meristem.

The uniqueness of these insect-plant interactions has implications for interpreting

herbivory in natural and agricultural ecosystems. This research provides a better understanding of the relative effect of herbivorous insects on plants. An understanding of the specificity by which plants compensate for insect injury and the factors which influence this specificity may aid attempts to identify crops or cultivars with tolerance to insects. Artificial injury can be a useful technique for studying some aspects of insect-plant interactions. However, great care must be taken to ensure that the pattern of simulation is as similar as possible to actual insect feeding. The pattern by which tissue is removed can affect how the plant responds to the injury.

Insect-plant interactions are unique, even for insects feeding on the same tissues on one plant. The specific impacts that insects have on plants varies for different insect herbivore. One of the reasons for these different specific impacts is that plant compensation for defoliation by insects is specific to the injury caused by a particular insect. The specificity of this compensation is determined by the type of tissue injured and the pattern of injury to a tissue. The compensatory responses for these specific types of injury can also be specific to a plant species. The techniques and results of this thesis help address some basic problems in understanding insect-plant interactions. Use of these techniques with other insect-plant systems would enable more generalizations about insect-plant interactions to be made.

CHAPTER 7**LIST OF REFERENCES**

- Alderfer, R.G. and C.F. Eagles. 1976. The effect of partial defoliation on the growth and photosynthetic efficiency of bean leaves. *Bot. Gaz.* 137: 351-355.
- Anderson, M.D., C. Peng, and M.J. Weiss. 1992. Crambe, *Crambe abyssinica* Hochst., as a flea beetle resistant crop (Coleoptera: Chrysomelidae). *J. Econ. Entomol.* 85: 594-600.
- Archer, S. and L.L. Tieszen. 1980. Growth and physiological responses of tundra plants to defoliation. *Arct. Alp. Res.* 12: 531-552.
- Archer, S.R. and L.L. Tieszen. 1986. Plant response to defoliation: hierarchical considerations. pp. 45-59. *In*: Gudmundsson, O. (Ed.), *Grazing research at northern latitudes*. Plenum Publishing Corporation.
- Bailey, C.G. 1976. A quantitative study of composition and utilization of various diets in the bertha armyworm, *Mamestra configurata* Wlk (Lepidoptera: Noctuidae). *Can. Entomol.* 108: 1319-1326.
- Bakhietia, D.R.C. and R.S. Sandhu. 1973. Differential response of *Brassica* varieties to the aphid (*Lipaphis erysimi* Kalt.) infestation. *Journal of Research.* 10: 272-279.
- Bakhietia, D.R.C., K.S. Brar, and B.S. Sekhon. 1984. Screening of some *Brassica* species and their strains for resistance to mustard aphid. *Journal of Oilseeds Research.* 1: 81-82.
- Baldwin, I.T. and J.C. Schultz. 1983. Rapid changes in tree leaf chemistry induced by damage: evidence for communication between plants. *Science* 221: 277-279.
- Bardner, R and K.E. Fletcher. 1974. Insect infestations and their effects on the growth and yield of field crops: a review. *Bull. entomol. Res.* 64: 141-160.
- Beck, S.D. 1965. Resistance of plants to insects. *Ann. Rev. Entomol.* 10: 207-232.
- Beirne, B.P. 1971. Pest insects of annual crop plants in Canada. I. Lepidoptera. II. Diptera. III. Coleoptera. *Mem. entomol. Soc. Can.* No. 78.
- Belsky, A.J. 1986. Does herbivory benefit plants? A review of the evidence. *Am. Nat.* 127: 870-892.
- Belsky, A.J. 1987. The effects of grazing: Confounding of ecosystem, community, and organism scale. *Am. Nat.* 129: 777-783.
- Belsky, A.J., W.C. Carson, C.L. Jensen and G.A. Fox. 1993. Overcompensation by plants:

herbivore optimization or red herring? *Evolutionary Ecology* 7: 109-121.

- Bilbrough, C. and J.H. Richards. 1991. Branch architecture of sagebrush and bitterbrush: use of a branch complex to describe and compare patterns of growth. *Can. J. Bot.* 69: 1288-1295.
- Bilbrough, C. and J.H. Richards. 1993. Growth of sagebrush and bitterbrush following simulated winter browsing: Mechanisms of tolerance. *Ecology* 74: 481-492.
- Birch, A.N.E., D.W. Griffiths, and W.H. MacFarlane-Smith. 1990. Changes in forage and oilseed rape (*Brassica napus*) root glucosinolates in response to attack by turnip root fly (*Delia floralis*). *J. Sci. Food Agric.* 51: 309-320.
- Blackman, R.L. and V.F. Eastop. 1984. Aphids on the worlds crops: An identification guide. John Wiley & Sons, New York.
- Bodnaryk, R.P. 1991. Developmental profile of sinalbin (p-hydroxybenzyl glucosinolate) in mustard seedlings, *Sinapis alba* L., and its relationship to insect resistance. *J. Chem. Ecol.* 17: 1543-1556.
- Bodnaryk, R.P. 1992. Effects of wounding on glucosinolates in the cotyledons of oilseed rape and mustard. *Phytochemistry* 31: 2671-2677.
- Bodnaryk, R.P. and R.J. Lamb. 1991. Mechanisms of resistance to the flea beetle, *Phyllotreta cruciferae* (Goeze), in mustard seedlings, *Sinapis alba* L. *Can. J. Plant Sci.* 71: 13-20.
- Bodnaryk, R.P. and P. Palaniswamy. 1990. Glucosinolate levels in cotyledons of mustard, *Brassica juncea* L. and rape, *B. napus* L. do not determine feeding rates of flea beetle, *Phyllotreta cruciferae* (Goeze). *J. Chem. Ecol.* 16: 2735-2746.
- Bokhari, U.G. 1977. Regrowth of western wheatgrass utilizing ¹⁴C-labeled assimilates stored in belowground parts. *Plant Soil* 48: 115-127.
- Bokhari, U.G. and J.S. Singh. 1974. Effects of temperature and clipping on growth, carbohydrate reserves, and root exudation of western wheatgrass in hydroponic culture. *Crop Sci.* 14: 790-794.
- Bracken, G.K. 1984. Within plant preferences of larvae of *Mamestra configurata* (Lepidoptera: Noctuidae) feeding on oilseed rape. *Can. Entomol.* 116: 45-49.
- Bracken, G.K., and G.E. Bucher. 1977. An estimate of the relation between density of bertha armyworm and yield loss on rapeseed, based on artificial infestations. *J. Econ.*

Entomol. 70: 701-705.

- Brandt, R.N. and R.J. Lamb. 1993. Distribution of feeding damage by *Phyllotreta cruciferae* (Goeze) (Coleoptera: Chrysomelidae) on oilseed rape and mustard seedlings in relation to crop resistance. Can. Entomol. 125: 1011-1021.
- Brandt, R.N. and R.J. Lamb. 1994. Importance of tolerance and growth rate in the resistance of oilseed rapes and mustards to flea beetles, *Phyllotreta cruciferae* (Goeze) (Coleoptera: Chrysomelidae). Can. J. Plant Sci. 74: 169-176.
- Briske, D.D. 1986. Plant response to defoliation: morphological considerations and allocation priorities. pp. 425-427. In Joss, P.J., P.W. Lynch and O.B. Williams (Eds.), Rangelands: a resource under seige. Proceedings of the 2nd International Rangeland Congress. Australian Acad. Sci, Canberra.
- Brues, C.T. 1924. The specificity of food plants in the evolution of phytophagous insects. Am. Nat. 58: 127-144.
- Bryant, J.P., F.S. Chapin III, and D.R. Klein. 1983. Carbon/nutrient balance of boreal plants in relation to herbivory. Oikos 40: 357-368.
- Buntin, G.D. and P.L. Raymer. 1994. Pest status of aphids and other insects in winter canola in Georgia. J. Econ. Entomol. 87: 1097-1104.
- Burgess, L. 1981. Winter sampling to determine overwintering sites and estimate density of adult flea beetle pests of rape (Coleoptera: Chrysomelidae). Can. Entomol. 113: 441-447.
- Burgess, L. and J.E. Wiens. 1980. Dispensing allyl isothiocyanate as an attractant for trapping crucifer-feeding flea beetles. Can. Entomol. 112: 93-97.
- Caemmerer, S. von and G.D. Farquhar. 1984. Effects of partial defoliation, changes of irradiance during growth, short-term water stress and growth at enhanced p(CO₂) on the photosynthetic capacity of leaves of *Phaseolus vulgaris* L. Planta 160: 320-329.
- Caldwell, M.M., J.H. Richards, D.A. Johnson, R.S. Nowak and R.S. Dzurec. 1981. Coping with herbivory: photosynthetic capacity and resource allocation in two semiarid *Agropyron* bunchgrasses. Oecologia 50: 14-24.
- Caloin, M., B. Clement, and S. Herrmann. 1990. Regrowth kinetics of *Dactylis glomerata* following defoliation. Ann. Bot. 66: 397-405.
- Chapin, F.S. 1980a. Nutrition allocation and responses to defoliation in tundra plants. Arct.

- Alp. Res. 12: 553-563.
- Chapin, F.S. 1980b. The mineral nutrition of wild plants. *Ann. Rev. Ecol. Syst.* 11: 233-260.
- Chapin, F.S. 1991. Integrated responses of plants to stress. *Bioscience* 41: 29-36.
- Chapin, F.S., III, D.A. Johnson, and J.D. McKendrick. 1980. Seasonal movement of nutrients in plants of differing growth forms in an Alaskan tundra ecosystem: implications for herbivory. *J. Ecol.* 68: 189-209.
- Chapin, F.S., III, J.D. McKendrick, and D.A. Johnson. 1986. Seasonal changes in carbon fractions in Alaskan tundra plants of differing growth form: implications for herbivory. *J. Ecol.* 74: 707-731.
- Chapin, F.S., III and M. Slack. 1979. Effect of defoliation upon root growth, phosphate absorption and respiration in nutrient-limited tundra graminoids. *Oecologia* 42: 67-79.
- Chapman, R.F. 1974. The chemical inhibition of feeding by phytophagous insects: a review. *Bull. entomol. Res.* 64: 339-363.
- Chew, F.S. 1988. Biological effects of glucosinolates. pp. 155-181 *In*: Cutler, H.G. (Ed.), *Biologically active natural products - potential use in agriculture*. American Chemical Society Symposium, Washington, D.C.
- Choudhury, D. 1984. Aphids and plant fitness: a test of Owen and Wiegert's hypothesis. *Oikos* 43: 401-402.
- Clarke, J.M. 1978. The effects of leaf removal on yield and yield components of *Brassica napus*. *Can. J. Pl. Sci.* 58: 1103-1105.
- Cochran, W.G. 1977. *Sampling techniques*, third edition. John Wiley & Sons, New York. 426pp.
- Coley, P.D., J.P. Bryant and F.S. Chapin. 1985. Resource availability and plant antiherbivore defense. *Science* 230: 895-899.
- Connell, J.H. 1971. On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forests. pp. 298-312, *In* Boer, P.J. den and G.R. Gradwell. (Eds.), *Dynamics of populations*. Pudoc, Wageningen.
- Coulson, R.N. and J.A. Witter. 1984. *Forest entomology: ecology and management*. John Wiley & Sons, New York. 669pp.

- Cox, C.S. and P.B. McEvoy. 1983. Effect of summer moisture stress on the capacity of tansy ragwort (*Senecio jacobaea*) to compensate for defoliation by cinnabar moth (*Tyria jacobaeae*). *J. appl. Ecol.* 20: 225-234.
- Crawley, M.J. 1983. *Herbivory: the dynamics of animal-plant interactions*. Blackwell Scientific Publications, Oxford.
- Crawley, M.J. 1988. Herbivores and plant population dynamics. pp. 367-392. *In* A.J. Davey, M.J. Hutchings and A.R. Watkinson (Eds.), *Plant population ecology*. Blackwell Scientific Publications, Oxford.
- Darwin, C. 1859. *The origin of species*. John Murray. London.
- Detling, J.K. and M.I. Dyer. 1981. Evidence for potential plant growth regulators in grasshoppers. *Ecology* 62: 485-488.
- Dilawari, V.K. and A.S. Atwal. 1987. Effect of cruciferous glucosinolates on probing pattern and feeding uptake by mustard aphid, *Lipaphis erysimi* (Kaltenbach). *Proc. Indian Acad. Sci.* 96: 695-703.
- Dirzo, R. 1984. Herbivory: a phytocentric overview. pp.141-165. *In* Dirzo, R. and J. Sarukhan (Eds.), *Perspectives on plant population ecology*. Sinauer Assoc., Sunderland, Mass.
- Downey, R.K. 1983. The origin and description of the *Brassica* oilseed crops. pp. 1-20. *In* Kramer, J.K.G., F.D. Sauer, and W.J. Pigden (Eds.), *High and low erucic acid rapeseed oils: production, usage, chemistry, and toxicological evaluation*. Academic Press, New York.
- Dreyer, D.L. and B.C. Campbell. 1987. Chemical basis of host-plant resistance to aphids. *Plant, Cell and Environment* 10: 353-361.
- Dyer, M.I. and U.G. Bokhari. 1976. Plant-animal interactions: Studies of the effects of grasshopper grazing on blue gamma grass. *Ecology* 57: 762-772.
- Edwards, P.J. and S.D. Wratten. 1983. Wound induced defences in plants and their consequences for patterns of insect grazing. *Oecologia* 59: 88-93.
- Ehrlén, J. 1995. Demography of the perennial herb *Lathyrus vernus*. I. Herbivory and individual performance. *J. Ecol.* 83: 287-295.
- Ehrlich, P.R. and P.H. Raven. 1964. Butterflies and plants: a study in coevolution. *Evolution* 18: 586-608.

- van Emden, H.F. 1973. Insect/plant relationships. Blackwell Scientific Publications, Oxford.
- van Emden, H.F., V.F. Eastop, R.D. Hughes, and M.J. Way. 1969. The ecology of *Myzus persicae*. Ann. Rev. Entomol. 14: 197-270.
- Engelbrecht, L., V. Orban and W. Heese. 1969. Leaf-miner caterpillars and cytokinins in the "green island" of autumn leaves. Nature 223: 319-321.
- Evans, P.S. 1971. Root growth of *Lolium perenne* L. II. Effects of defoliation and shading. NZ. J. Agr. Res. 14: 552-562.
- Feeny, P. 1976. Plant apparency and chemical defense. Recent Adv. Phytochem. 10: 1-40.
- Feeny, P. 1977. Defensive ecology of the cruciferae. Ann. Missouri Bot. Gard. 64: 221-234.
- Feeny, P., K.L. Paauwe and N.J. Demong. 1970. Flea beetles and mustard oils: host plant specificity of *Phyllotreta cruciferae* and *P. striolata* adults (Coleoptera: Chrysomelidae). Ann. Entomol. Soc. Amer. 63: 832-841.
- Fenwick, G.R., R.K. Heaney, and R. Mawson. 1989. Glucosinolates. pp. 1-41, In: Cheeke, P.R. (Ed.), Toxicants of plant origin, Vol. 2. CRC Press, Boca Raton, Florida. 277PP.
- Fitter, A.H. and R.K.M. Hay. 1987. Environmental physiology of plants, 2nd ed. Academic Press, New York. 423pp.
- Fowler, S.V. and J.H. Lawton. 1985. Rapidly induced defenses and talking trees: the devil's advocate position. Am. Nat. 126: 181-195.
- Gerber, G.H. 1978. Effect of burying the eggs in soil on survival in the red turnip beetle, *Entomoscelis americana* (Coleoptera: Chrysomelidae). Manitoba Entomol. 12: 49-51.
- Gerber, G.H. 1981. Cold-hardiness in the eggs of the red turnip beetle, *Entomoscelis americana* (Coleoptera: Chrysomelidae). Can. Entomol. 113: 795-800.
- Gerber, G.H. 1982. A pest management system for the red turnip beetle on rapeseed and canola. Can. Agric. 27: 8-11.
- Gerber, G.H. 1984. The distribution of the red turnip beetle, *Entomoscelis americana* Brown (Coleoptera: Chrysomelidae). The Can. Agric. Insect Pest Rev. 61: 39-47.
- Gerber, G.H. and R.J. Lamb. 1982. Phenology of egg hatching for the red turnip beetle, *Entomoscelis americana* (Coleoptera: Chrysomelidae). Environ. Entomol. 11: 1258-

1263.

- Gerber, G.H. and A.A. Obadofin. 1981. Growth, development, and survival of the larvae of the red turnip beetle, *Entomoscelis americana* (Coleoptera: Chrysomelidae), on *Brassica campestris* and *B. napus* (Cruciferae). Can. Entomol. 113: 395-406.
- Gilbert, L.E. 1971. Butterfly-plant coevolution: has *Passiflora adenopoda* won the selection race with heliconine butterflies? Science 172: 585-586.
- Gillett, J.B. 1962. Pest pressure, an underestimated factor in evolution. Syst. Assoc. Publ. 4: 37-46.
- Gold, W.G. and M.M. Caldwell. 1989. The effect of the spatial pattern of defoliation on regrowth of a tussock grass. I. Growth responses. Oecologia 80: 289-296.
- Gould, F. 1978. Resistance of cucumber varieties to *Tetranychus urticae*: genetic and environmental determinants. J. Econ. Entomol. 71: 680-683.
- Grime, J.P. 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. Am. Nat. 111: 1169-1194.
- Gulmon, S.L. and H.A. Mooney. 1986. Costs of defense and their effects on plant productivity. pp. 681-698. In Givnish, T.J. (Ed.), On the economy of plant form and function. Cambridge University Press, Cambridge.
- Hall, F.R. and D.C. Ferree. 1976. Effects of insect injury simulation on photosynthesis of apple leaves. J. Econ. Entomol. 69: 245-248.
- Harcourt, D.G. 1957. Biology of the diamondback moth, *Plutella maculipennis* (Curt.) (Lepidoptera: Plutellidae), in Eastern Ontario. II. Life-history, behavior, and host relationships. Can. Entomol. 89: 554-564.
- Harper, F.R. and B. Berkenkamp. 1975. Revised growth-stage key for *Brassica campestris* and *B. napus*. Can. J. Plant Sci. 55: 657-658.
- Haynes, R.L., and C.M. Jones. 1975. Wilting and damage to cucumber by spotted and striped cucumber beetles. HortSci. 10: 256-266.
- Hodgkinson, K.C. 1974. Influence of partial defoliation on photosynthesis, photorespiration and transpiration by lucerne leaves at different ages. Aust. J. Plant Physiol. 1: 561-578.
- Holtzer, T.O., T.L. Archer, and J.M. Norman. 1988. Host plant suitability in relation to water

- stress. pp. 111-137, *In*: Heinrichs, E.A. (Ed.), Plant stress-insect interactions. John Wiley and Sons, New York.
- Horber, E. 1972. Plant resistance to insects. Agric. Sci. Rev. Coop. State Res. Serv. U.S. Department of Agriculture, Vol. 10.
- Howe, H.F. and L.C. Westley. 1988. Ecological relationships of plants and animals. Oxford University Press, New York. 273pp.
- Hoxie, R.P., S.G. Wellso and J.A. Webster. 1975. Cereal leaf beetle response to wheat trichome length and density. *Environ. Entomol.* 4: 365-370.
- Hsiao, T.C. 1973. Plant responses to water stress. *Ann. Rev. Plant Physiol.* 24: 519-570.
- Inglis, I.R., J.T. Wadsworth, A.N. Meyer and C.J. Feare. 1992. Vertebrate damage to 00 and 0 varieties of oilseed rape in relation to SMCO and glucosinolate concentration in the leaves. *Crop Protection* 11: 64-68.
- Inouye, D.W. 1982. The consequences of herbivory: a mixed blessing for *Jurinea mollis* (Asteraceae). *Oikos* 39: 269-272.
- Iwasa, Y. and Roughgarden, J. 1984. Shoot/root balance in plants: optimal growth of a system with many vegetative organs. *Theor. Popul. Biol.* 25: 78-105.
- Janzen, D.H. 1979. New horizons in the biology of plant defenses. pp. 331-350. *In* Rosenthal, G.A. and D.H. Janzen (Eds.), *Herbivores: their interaction with secondary plant metabolites*. Academic Press, New York.
- Jermy, T. 1993. Evolution of insect-plant relationships - a devil's advocate approach. *Entomol. exp. appl.* 66: 3-12.
- Kennedy, J.S., M.F. Day and V.F. Eastop. 1962. A conspectus of aphids as of *Myzus persicae* (Sulzer). *Ann. vectors of plant viruses*. Commonwealth Inst. Entomol., London.
- Knerer, G. and C.E. Atwood. 1973. Diprionid sawflies: polymorphism and speciation. *Science* 179: 1090-1099.
- Kogan, M. and E.F. Ortman. 1978. Antixenosis - A new term proposed to define Painter's "nonpreference" modality of resistance. *Entomological Society of America Bulletin* 24: 175-176.
- Koritsas, V.M., J.A. Lewis, and G.R. Fenwick. 1989. Accumulation of indole glucosinolates

in *Psylliodes chrysocephala* L. -infested or -damaged tissues of oilseed rape (*Brassica napus* L.). *Experientia* 45: 493-495.

Koritsas, V.M., J.A. Lewis, and G.R. Fenwick. 1991. Glucosinolate responses of oilseed rape, mustard, and kale to mechanical wounding and infestation by cabbage stem flea beetle (*Psylliodes chrysocephala*). *Ann. appl. Biol.* 118: 209-221.

Lamb, R.J. 1980. Hairs protect pods of mustard (*Brassica hirta* 'Gisilba') from flea beetle feeding damage. *Can. J. Plant Sci.* 60: 1439-1440.

Lamb, R.J. 1988. Susceptibility of low- and high-glucosinolate oilseed rapes to damage by flea beetles, *Phyllotreta* spp. (Coleoptera: Chrysomelidae). *Can. Entomol.* 120: 195-196.

Lamb, R.J. 1989. Entomology of oilseed *Brassica* crops. *Ann. Rev. Entomol.* 34: 211-229.

Lamb, R.J. and P.A. MacKay. 1995. Tolerance of antibiotic and susceptible cereal seedlings to the aphids *Metopolophium dirhodum* and *Rhopalosiphum padi*. *Ann. appl. Biol.* 127: 573-583.

Lamb, R.J., P. Palaniswamy, K.A. Pivnick, and M.A.H. Smith. 1993. A selection of oilseed rape, *Brassica rapa* L., with resistance to flea beetles, *Phyllotreta cruciferae* (Goeze) (Coleoptera: Chrysomelidae). *Can. Entomol.* 125: 703-713.

Lamb, R.J., W.J. Turnock, and H.N. Hayhoe. 1985. Winter survival and outbreaks of bertha armyworm, *Mamestra configurata* (Lepidoptera: Noctuidae), on canola. *Can. Entomol.* 117: 727-736.

Larsen, L., J.K. Nielsen, A. Ploger, and H. Sorensen. 1985. Responses of some beetle species to varieties of oilseed rape and to pure glucosinolates. pp. 230-244, *In*: Sorensen, H. (Ed.), *Advances in the production and utilization of cruciferous crops*. Martinus Nijhoff/Dr. W. Junk Publishers, Dordrecht.

Larsen, P.O. 1981. Glucosinolates. pp. 501-525, *In*: Conn E.E. (Ed.), *The biochemistry of plants*, vol.7. Academic Press, New York.

Lee, T.D. and F.A. Bazzaz. 1980. Effect of defoliation and competition on growth and reproduction in the annual plant *Abutilon theophrasti*. *J. Ecol.* 68: 813-821.

Levin, D.A. 1973. The role of trichomes in plant defense. *Q. Rev. Biol.* 48: 3-15.

Levin, D.A. 1976. The chemical defenses of plants to pathogens and herbivores. *Ann. Rev. Ecol. Syst.* 7: 121-159.

- Llewellyn, M. 1972. The effect of the lime aphid, *Eucallipterus tiliæ* L. (Aphididae) on the growth of the lime, *Tilia x vulgaris* Hayne. J. appl. Ecol. 9: 261-282.
- Lowman, M.D. 1982. Effects of different rates and methods of leaf area removal on rainforest seedlings of coachwood (*Ceratopetalum apetalum*). Aust. J. Bot. 30: 477-483.
- Lyman, J.M. and C. Cardona. 1982. Resistance in lima beans to a leafhopper, *Empoasca kraemeri*. J. Econ. Entomol. 75: 281-286.
- MacGibbon, D.B. and E.J. Beuzenberg. 1978. Location of glucosinolates in *Brevicoryne brassicae* and *Lipaphis erysimi* (Aphididae). N.Z. J. Sci. 21: 389-392.
- MacKay, P.A. and R.J. Lamb. 1996. Dispersal of five aphids (Homoptera: Aphididae) in relation to their impact on *Hordeum vulgare*. Environ. Entomol. 25: 1032-1044.
- Major, D.S., J.B. Bole, and W.A. Charnetski. 1978. Distribution of photosynthates after $^{14}\text{CO}_2$ assimilation by stems, leaves and pods of rape plants. Can. J. Pl. Sci. 58: 783-787.
- Malik, R.S., I.J. Anand, and D. Srinivasachar. 1983. Effect of glucosinolates in relation to aphid (*Lipaphis erysimi* Kalt.) fecundity in crucifers. Int. J. Trop. Agric. 1: 273-278.
- Marquis, R.J. 1992. A bite is a bite? Constraints on response to folivory in *Piper arieianum* (Piperaceae). Ecology 73: 143-152.
- Martens, B. and J.T. Trumble. 1987. Structural and photosynthetic compensation for leafminer (Diptera: Agromyzidae) injury in lima beans. Environ. Entomol. 16: 374-378.
- Maschinski, J., and T.G. Whitham. 1989. The continuum of plant response to herbivory: the influence of plant association, nutrient availability, and timing. Am. Nat. 134: 1-19.
- Mattson, W.J. 1980. Herbivory in relation to plant nitrogen content. Ann. Rev. Ecol. Syst. 11: 119-161.
- Mattson, W.J. and N.D. Addy. 1975. Phytophagous insects as regulators of forest primary production. Science 190: 515-522.
- Mauricio, R., M.D. Bowers, and F.A. Bazzaz. 1993. Pattern of leaf damage affects fitness of the annual plant *Raphanus sativus* (Brassicaceae). Ecology 74: 2066-2071.
- McGregor, D.I. 1987. Effect of plant density on development and yield of rapeseed and its

- significance to recovery from hail injury. *Can. J. Plant Sci.* 67: 43-51.
- McLeod, D.G.R. and D.T. Lowery. 1989. Aphids infesting crucifers in Canada. Agriculture Canada Publication 1835/E.
- McNaughton, S.J. 1983. Physiological and ecological implications of herbivory. pp. 657-677 *In*: Lange, O.L., Nobel, P.S., Osmond, C.B., and Zeigler, H. (Eds.), *Physiological plant ecology III: Responses to the chemical and biological environment*, New Series, vol. 12c. Springer, Berlin.
- McNaughton, S.J. and F.S. Chapin, III. 1985. Effects of phosphorus nutrition and defoliation on C₄ graminoids from the Serengeti plains. *Ecology* 66: 1617-1629.
- Meijden, E. van der, M. Wijn and H.J. Verkaar. 1988. Defence and regrowth, alternative plant strategies in the struggle against herbivores. *Oikos* 51: 355-363.
- Mendoza, A., D. Pinero, and J. Sarukhan. 1987. Effects of experimental defoliation on growth, reproduction and survival of *Astrocaryum mexicanum*. *J. Ecol.* 75: 545-554.
- Meyer, G.A. 1993. A comparison of the impacts of leaf- and sap-feeding insects on growth and allocation of goldenrod. *Ecology* 74: 1101-1116.
- Miles, P.W. 1968. Studies on the salivary physiology of plant-bugs: experimental induction of galls. *J. Insect Physiol.* 14: 97-106.
- Milford, G.F.J., J.K. Fieldsend, A.J.R. Porter, C.J. Rawlinson, E.J. Evans and P.E. Bilsborrow. 1989. Changes in glucosinolate concentrations during the vegetative growth of single- and double-low cultivars of winter oilseed rape. pp. 83-90, *In*: Dale, M.F.B., A.M. Dewar, R.J. Froud-Williams, T.J. Hocking, D. Gareth Jones, and B.L. Rea. (Eds.), *Production and protection of oilseed rape and other brassica crops*. Association of Applied Biologists, Wellesbourne. 475pp.
- Mithen, R. 1992. Leaf glucosinolate profiles and their relationship to pest and disease resistance in oilseed rape. *Euphytica* 63: 71-83.
- Mittler, T.E. 1958. Studies on the feeding and nutrition of *Tuberolachnus salignus* (Gmelin) (Homoptera, Aphididae). II. The nitrogen and sugar composition of ingested phloem sap and excreted honeydew. *J. exp. Biol.* 35: 74-84.
- Mooney, H.A. and S.L. Gulmon. 1982. Constraints on leaf structure and function in reference to herbivory. *BioScience* 32: 198-206.
- Moran, N.A. and T.G. Whitham. 1990. Interspecific competition between root-feeding and

leaf-galling aphids mediated by host-plant resistance. *Ecology* 71: 1050-1058.

Narang, D.D. and A.S. Atwal. 1985. Effect of leaf extracts of glucosinolates of cruciferous plants on feeding response and population build up of *Lipaphis erysimi*. *Indian J. Ecol.* 12: 104-108.

Nielsen, J.K. 1977. Host plant relationships of *Phyllotreta nemorum* L. (Coleoptera: Chrysomelidae). I. Field studies. *Z. angew. Entomol.* 84: 396-407.

Nielsen, J.K. 1988. Crucifer-feeding Chrysomelidae: Mechanisms of host plant finding and acceptance. pp. 25-40, *In*: Jolivet, P., E. Petipierre, and T.H. Hsiao. (Eds.), *Biology of Chrysomelidae*. Kluwer Academic Publishers, Dordrecht.

Nooden, L.D. and A.C. Leopold. 1988. Senescence and aging in plants. Academic Press, New York, USA.

Norris, D.M. and M. Kogan. 1980. Biochemical and morphological bases of resistance. pp. 23-61, *In*: Metcalf, R.L. and W. Stumm. (Eds.), *Breeding plants resistant to insects*. John Wiley and Sons, New York.

Nowak, R.S. and M.M. Caldwell. 1984. A test of compensatory photosynthesis in the field: implications for herbivory tolerance. *Oecologia* 61: 311-318.

Nowatzki, T.M., and M.J. Weiss. 1997. Effects of simulated and flea beetle injury to cotyledons on growth of drought-stressed oilseed rape, *Brassica napus* L. *Can. J. Plant Sci.* 77: 475-481.

Osborne, D.J. 1973. Mutual regulation of growth and development in plants and insects. pp. 33-42, *In*: van Emden, H.F. (Ed.), *Insect/plant relationships*. Symp. R. Entomol. Soc. London: Number Six.

Owen, D.F. 1978. Why do aphids synthesize melezitose? *Oikos* 31: 264-267.

Owen, D.F. and R.G. Wiegert. 1976. Do consumers maximize plant fitness? *Oikos* 27: 488-492.

Owens, J.C. 1975. An explanation of terms used in insect resistance to plants. *Iowa St. J. Res.* 49: 513-517.

Painter, R.H. 1951. *Insect resistance in crop plants*. MacMillan, New York. 520pp.

Painter, R.H. 1968. Crops that resist insects provide a way to increase world food supply. *Kans. Agric. exp. Sta. Bull.* 22: 520.

- Palaniswamy, P. and R.P. Bodnaryk. 1994. A wild *Brassica* from Sicily provides trichome-based resistance against flea beetles, *Phyllotreta cruciferae* (Goeze) (Coleoptera: Chrysomelidae). *Can Entomol.* 126: 1119-1130.
- Palaniswamy, P. and R.J. Lamb. 1993. Wound-induced antixenotic resistance to flea beetles, *Phyllotreta cruciferae* (Goeze) (Coleoptera: Chrysomelidae), in crucifers. *Can. Entomol.* 125: 903-912.
- Pathak, M.D. 1970. Genetics of plants in pest management. pp. 138-157, *In*: Rabb, R.L. and F.E. Guthrie (Eds.), Concepts of pest management. North Carolina State University, Raleigh.
- Pedigo, L.P., S.H. Hutchins, and L.G. Higley. 1986. Economic injury levels in theory and practice. *Ann. Rev. Entomol.* 31: 341-368.
- Petelle, M. 1980. Aphids and melezitose: a test of Owen's 1978 hypothesis. *Oikos* 35: 127-128.
- Philip, H. and E. Mengersen. 1989. Insect pests of the prairies. University of Alberta Press, Edmonton, Alta. 122pp.
- Ponti, O.M.B. de. 1982. Plant resistance to insects: a challenge to plant breeders and entomologists, pp. 337-347. *In* J.H. Visser and A.K. Minks (Eds.), Insect-plant relationships. Centre for agricultural publishing and documentation. Wageningen.
- Rawlinson, C.J., K.J. Doughty, C.H. Bock, V.J. Church, G.F.J. Milford and J.K. Fieldsend. 1989. Diseases and response to disease and pest control on single- and double-low cultivars of winter oilseed rape. pp. 393-400, *In*: Dale, M.F.B., A.M. Dewar, R.J. Froud-Williams, T.J. Hocking, D. Gareth Jones, and B.L. Rea (Eds.), Production and protection of oilseed rape and other brassica crops. Association of Applied Biologists, Wellesbourne. 475pp.
- Reardon, P.O., C.L. Leinweber and J.L.B. Merrill. 1972. The effect of bovine saliva on grasses. *J. Anim. Sci.* 34: 897-898.
- Reardon, P.O., C.L. Leinweber and J.L.B. Merrill. 1974. Response of sideoats grama to animal saliva and thiamine. *J. Range Manage.* 27: 400-401.
- Rhoades, D.F. 1983. Herbivore population dynamics and plant chemistry. pp. 155-220. *In* R.F. Denno and M.S. McClure (Eds.), Variable plants and herbivores in natural and managed systems. Academic Press, New York.
- Rhoades, D.F. and R.G. Cates. 1976. Towards a general theory of plant antiherbivore

- chemistry. Recent Adv. Phytochem. 19: 168-213.
- Richards, J.H. and M.M. Caldwell. 1985. Soluble carbohydrates, concurrent photosynthesis and efficiency in regrowth following defoliation: a field study with *Agropyron* species. J. appl. Ecol. 22: 907-920.
- Riedell, W.E. 1990. Tolerance of wheat to Russian wheat aphids: nitrogen fertilization reduces yield loss. J. Plant Nutrition 13: 579-584.
- Rodman, J.E. 1981. Divergence, convergence, and parallelism in phytochemical characters: The glucosinolate-myrosinase system. pp. 43-79, *In*: Young, D.A. and D.A. Seigler (Eds.), Phytochemistry and angiosperm phylogeny. Praeger, New York.
- Ronquist, F. and I. Åhman. 1990. Reproductive rate of the Indian mustard aphid (*Lipaphis erysimi pseudobrassicae*) on different *Brassica* oilseeds: comparisons with Swedish strains of mustard (*Lipaphis erysimi erysimi*) and cabbage aphid (*Brevicoryne brassicae*). Ann. Appl. Biol. 116: 425-430.
- Rosenthal, J.P. and P.M. Kotanen. 1994. Terrestrial plant tolerance to herbivory. Trends Ecol. Evol. 9: 145-148.
- Roundy, B.A. and G.B. Ruyle. 1989. Effects of herbivory on twig dynamics of a Sonoran desert shrub *Simmondsia chinensis* (Link) Schn. J. appl. Ecol. 26: 701-710.
- Ruess, R.W. 1988. The interaction of defoliation and nutrient uptake in *Sporobolus kertrophyllus*, a short-grass species from the Serengeti Plains. Oecologia 77: 550-556.
- Ryle, G.J.A. and C.E. Powell. 1975. Defoliation and regrowth in the graminaceous plant: the role of current assimilate. Ann. Bot. 39: 297-310.
- SAS Institute Inc. 1989. SAS/STAT® User's Guide, Version 6, Fourth edition, Volume 1, Cary, NC:SAS Institute Inc. 943 pp.
- Saxena, R.C. 1987. Antifeedants in tropical pest management. Insect Sci. Applic. 8: 731-736.
- Sernyk, J.L. and B.R. Stefansson. 1983. Heterosis in summer rape (*Brassica napus* L.). Can. J. Plant Sci. 63: 407-413.
- Slansky, F. 1982. Towards a nutritional ecology of insects. pp. 253-259. *In* Proceedings of the fifth international symposium of insect-plant relationships. Pudoc, Wageningen.
- Stapledon, R.G. 1928. Cocksfoot grass (*Dactylis glomerata* L.) ecotypes in relation to the

- biotic factor. *J. Ecol.* 16: 71-104.
- Steenbergh, W.F. and C.H. Lowe. 1969. Critical factors during the first years of life of the saguaro (*Cereus giganteus*) at Saguaro National Monument, Arizona. *Ecology* 50: 825-834.
- Stewart, D.B. 1973. The red turnip beetle, *Entomoscelis americana* Brown (Coleoptera: Chrysomelidae), biology and plant relationships. M. Sc. Thesis, Univ. of Alberta, Edmonton. 86pp.
- Stickler, F.C. and A.W. Pauli. 1961. Leaf removal in grain sorghum. I. Effects of certain defoliation treatments on yield and components of yield. *Agron. J.* 53: 99-102.
- Strauss, S.Y. 1991. Direct, indirect, and cumulative effects of three native herbivores on a shared host plant. *Ecology* 72: 543-558.
- Swank, W.T. J.B. Waide, D.A. Crossley Jr., and R.L. Todd. 1981. Insect defoliation enhances nitrate export from forest ecosystems. *Oecologia* 51: 297-299.
- Ta, T.C., F.D.H. Macdowall, and M.A. Faris. 1990. Utilization of carbon and nitrogen reserves of alfalfa roots in supporting N₂-fixation and shoot regrowth. *Plant Soil* 127: 231-236.
- Tahvanainen, J.O. and R.B. Root. 1972. The influence of vegetational diversity on the population ecology of a specialized herbivore, *Phyllotreta cruciferae* (Coleoptera: Chrysomelidae). *Oecologia* 10: 321-346.
- Talekar, N.S. and A.M. Shelton. 1993. Biology, ecology, and management of the diamondback moth. *Ann. Rev. Entomol.* 38: 275-301.
- Taylor, W.E. and R. Bardner. 1968. Effects of feeding by larvae of *Phaedon cochleariae* (F.) and *Plutella maculipennis* (Curt.) on the yield of radish and turnip plants. *Ann. appl. Biol.* 62: 249-254.
- Tingey, W.M. 1981. The environmental control of insects using plant resistance. pp. 175-197. *In* D. Pimental (Ed.), *Handbook of pest management in agriculture*. CRC Press, Boca Raton, Florida.
- Tripathi, N.L.M., G.C. Sachan, S.K. Verma, and P.K. Pathak. 1986. Developmental behaviour of *Lipaphis erysimi* (Kaltenbach) on different parts/stages of *Brassica campestris* var. Toris. *Indian J. Entomol.* 48: 295-300.
- Trumble, J.T. 1982. Within-plant distribution and sampling of aphids (Homoptera:

- Aphididae) on broccoli in southern California. *J. Econ. Entomol.* 75: 587-592.
- Trumble, J.T., Kolodny-Hirsch, D.M. and I.P. Ting. 1993. Plant compensation for arthropod herbivory. *Ann. Rev. Entomol.* 38: 93-119.
- Tsunoda, S. 1980. Eco-physiology of wild and cultivated forms in *Brassica* and allied genera. pp. 109-120. *In* Tsunoda, S., K. Hinata and C. Gómez-Campo (Eds.), *Brassica crops and wild allies: Biology and breeding*. Japan Scientific Society Press, Tokyo.
- Turnock, W.J. and H.G. Philip. 1977. The outbreak of bertha armyworm *Mamestra configurata* (Noctuidae: Lepidoptera) in Alberta, 1971 to 1975. *Manitoba Entomol.* 11: 10-21.
- Underhill, E.W. 1980. Glucosinolates. pp. 493-511, *In*: Bell, E.A. and B.V. Charlwood (Eds.), *Secondary plant products*. Springer-Verlag, New York.
- Velusamy, R. and E.A. Heinrichs. 1986. Tolerance in crop plants to insect pests. *Insect Sci. Applic.* 7: 689-696.
- Via, S. 1990. Ecological genetics and host adaptations in herbivorous insects: The experimental study of evolution in natural and agricultural systems. *Ann. Rev. Entomol.* 35: 421-446.
- Welch, B.L. and E.D. MacArthur. 1979. Feasibility of improving big sagebrush (*Artemisia tridentata*) for use on mule deer winter ranges, pp. 451-473. *In* Goodin, J.R. and D.K. Northington (Eds.), *Aridland plant resources. Proceedings of the International Arid Lands Conference on Plant Resources*, Texas Tech. University, Lubbock.
- Welter, S.C. 1989. Arthropod impact on plant gas exchange. pp. 135-150, *In* E.A. Bernays (Ed.), *Insect-plant interactions*. CRC, Boca Raton, Florida, USA.
- Westdal, P.H. and W. Romanow. 1972. Observations on the biology of the flea beetle, *Phyllotreta cruciferae* (Coleoptera: Chrysomelidae). *Manitoba Entomol.* 6: 35-45.
- White, T.C.R. 1984. The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. *Oecologia* 63: 90-105.
- Whitham, T.G. Maschinski, J., Larson, K.C., and K.N. Paige. 1991. Plant response to herbivory: the continuum from negative to positive and underlying physiological mechanisms. pp. 227-256. *In* P. W. Price, T.W. Lewinsohn, G.W. Fernandes, and W.W. Benson (Eds.), *Plant-animal interactions: Evolutionary ecology in tropical and temperate regions*. John Wiley & Sons, Inc. New York.

- Williams, I.H., K.J. Doughty, C.H. Bock and C.J. Rawlinson. 1991. Incidence of pests and diseases and effects of crop protection on double- and single-low winter rape cultivars. pp. 518-523, *In*: McGregor, D.I. (Ed.), Rapeseed in a changing world, Proceedings of the Eighth International Rapeseed Congress, Vol.2. Saskatoon, Saskatchewan.
- Wilson, J.B. 1988. A review of evidence on the control of shoot: root ratio, in relation to models. *Ann. Bot.* 61: 433-449.
- Wiseman, B.R. 1985. Types and mechanisms of host plant resistance to insect attack. *Insect Sci. Applic.* 6: 239-242.

Appendix A. Means of untransformed and unadjusted measurements for undamaged control plants of *Sinapis alba* and *Brassica napus* in field and laboratory experiments, ($n=18$).

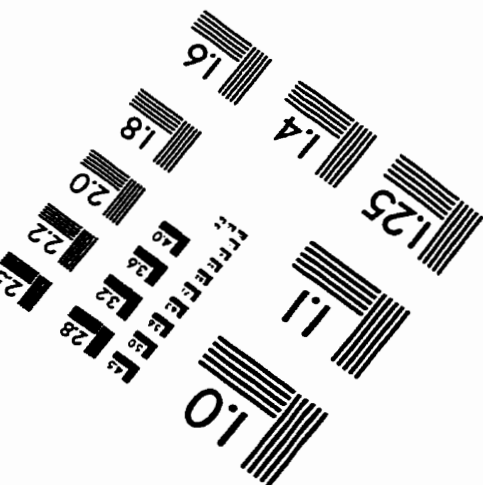
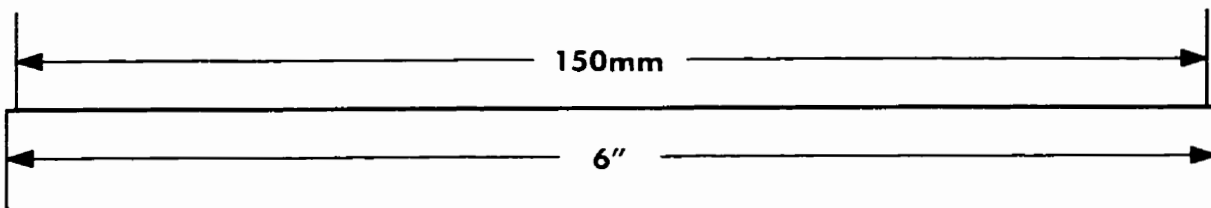
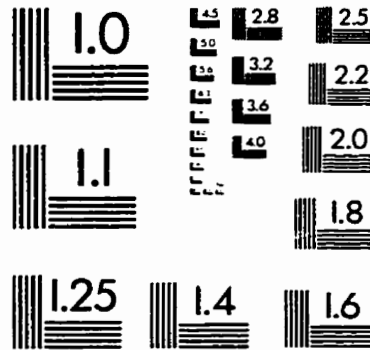
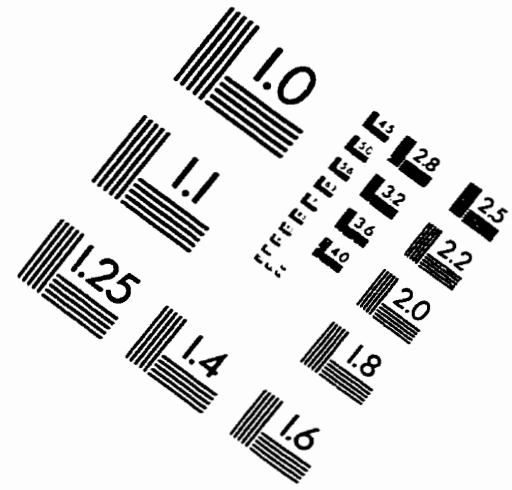
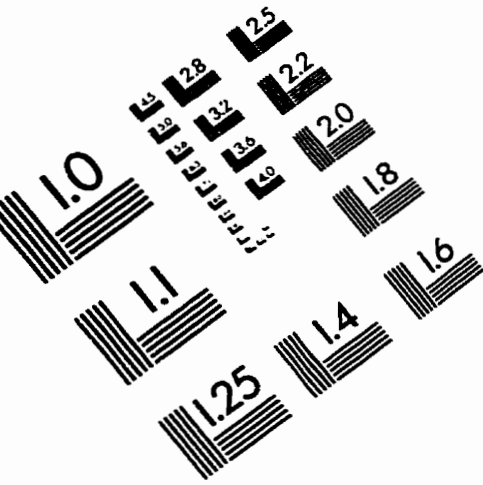
Days after planting	<u>Growth Stage¹</u>		<u>Field Experiment</u>		<u>Seed traits²</u>	
	<i>S. alba</i>	<i>B. napus</i>	<u>Total leaf length (mm)</u>		<i>S. alba</i>	<i>B. napus</i>
			<i>S. alba</i>	<i>B. napus</i>		
21	2.4	2.2	217 ± 14	83 ± 5	Number of seeds	
28	3.1	2.5	669 ± 59	311 ± 14	436 ± 69	1756 ± 282
37	4.2	3.1	1155 ± 128	831 ± 50		
43	4.3	3.3	1153 ± 156	1147 ± 81	Average dry weight of	
57	5.1	4.3			individual seeds (mg)	
64	5.1	5.1			4.9 ± 0.2	3.0 ± 0.2
70	5.1	5.1				
77	5.3	5.2			Avg. germination (%)	
85	5.3	5.3			99.3 ± 0.6	83.5 ± 4.6

Days after planting	<u>Dry Weight of</u>		<u>Laboratory Experiment</u>		<u>Dry Weight of Roots</u>	
	<u>Leaves (mg)</u>		<u>Dry Weight of Support</u>		<u>(mg)</u>	
	<i>S. alba</i>	<i>B. napus</i>	<i>S. alba</i>	<i>B. napus</i>	<i>S. alba</i>	<i>B. napus</i>
7	15 ± 1	7 ± 1	3 ± 0	1 ± 0	4 ± 0	2 ± 0
14	142 ± 7	92 ± 10	29 ± 1	16 ± 2	32 ± 4	19 ± 2
21	727 ± 97	810 ± 77	433 ± 60	199 ± 23	222 ± 37	215 ± 27
28	1266 ± 8	2853 ± 131	2226 ± 240	964 ± 87	423 ± 44	816 ± 85
35	1672 ± 94	5174 ± 298	5180 ± 246	2944 ± 419	771 ± 49	1367 ± 76

¹ Based on the modification of Harper and Berkenkamp's 1975 key to the growth stages described in the Materials and Methods.

² *S. alba* was harvested 91 days and *B. napus* was harvested 98 days after planting.

IMAGE EVALUATION TEST TARGET (QA-3)



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