VARYING FOOD QUALITY AND ITS INFLUENCE ON SPRUCE BUDWORM GROWTH, DEVELOPMENT, AND FECUNDITY

by

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ABSTRACT

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Keywords: *Abies balsamea,* artificial diet, balsam fir, black spruce, casein, cellulose,*Choristoneura fumiferana,* fitness, food quality, indigestible fibre, leaf toughness, nitrogen, performance, *Picea glauca*, *Picea mariana,* spruce budworm, water, white spruce, McMorran diet

These studies assess how changing food quality influences spruce budworm, *Choristoneura fumiferana* (Clem.), in terms of the performance characteristics of survival, development, growth, and fecundity. The components of food quality that were investigated were nitrogen (N), water, indigestible fibre (IF), and leaf toughness. I believe that budworm feeding, at a given defoliation intensity triggers a relative increase of foliar IF in comparison to the other nutritional constituents that are essential to the growth and development of spruce budworm It was my hypothesis that there is a threshold IF:nutrient above which performance and fitness declines. Four experiments were conducted to test this hypothesis.

In experiment 1, foliar samples of balsam fir (Bf), *Abies balsamea* (L.), white spruce (Sw), *Picea glauca* (Moench) Voss, and black spruce (Sb), *Picea mariana* (Mill.) B.S.P., were chemically analyzed for levels of N and IF to determine the seasonal variation in these components and to quantify the physiological bounds of IF:N in the natural budworm diet. As well, water content was assessed. Foliar N levels were initially high and decreased rapidly in the early growing season and then gradually for the remainder of the season. Little difference was observed among species and crown positions. IF followed a trend of low levels in the swollen buds, a rapid increase to about early July and then a general tapering off. There was slight variability among crown positions. At the start of budworm feeding early in the growing season, IF:N was about 20:1 for all species. By early July, when feeding stops, the IF:N ratio was about 50:1 for Sw and 30:1 for Bf and Sb. Foliar water content followed a trend of high levels at growth initiation that increased soon to seasonal peaks. Water content then decreased from about mid-June to late August. Little difference was observed between crown positions for all species.

In experiment 2 spruce budworm were reared on early- and late-season foliage of Bf, Sw, and Sb to test the hypothesis that budworm can perform better on foliage with low IF:N (early-season foliage) than on foliage with high IF:N (late-season foliage). Spruce budworm showed superior development, growth and fecundity on early-season compared to late-season foliage. Development times were shorter and pupal weights and number of eggs laid were greater on

early-season than on late-season foliage. The early- and late-season, current-year needles of Bf, Sw and Sb were collected and measured with a penetrometer for leaf toughness in order to quantify the seasonal variation of this food quality component. Early-season needles were significantly softer than late-season needles for all species, although toughness measurements were highly variable. Among early-season measurements, Bf foliage was significantly softer than the two spruces, which themselves did not differ. Among late-season measurements, species differed significantly from each other with Sb being the toughest and Bf being the softest. It is interesting that the two spruces differed significantly considering that they had similar IF levels. It appears that a component other than IF may be contributing to leaf toughness for Sb.

In experiment 3, to test the hypothesis that below some level of dietary N budworm performance would be low, casein was added to the standard McMorran diet at 12, 18, 25 and 100 percent casein in the standard diet. This lower level would then be included into the design of experiment 4. As well, components of the standard diet were analyzed for sources and levels of N and IF. Spruce budworm reared on the 12 percent casein diet showed the poorest performance; survival, weights at sixth instar and pupa, and number of eggs laid were lowest while development times in sixth instar were longest. No clear association between pupal weight and fecundity occurred; fecundity was greatest on diets with 25 percent casein although female pupal weights were significantly lighter than those on 100 percent casein. It may be that there are inherent problems with the weight/fecundity relationship or that the standard diet is too N-rich and not optimally nutrient balanced for egg production. These findings have implications for optimizing the standard McMorran diet.

To test the hypothesis that there is a threshold IF:N above which spruce budworm performance declines, experiment 4 was conducted with 12, 18, 25, and 100 percent casein in the standard diet and varying cellulose:casein N. Cellulose:casein N ratios of 3:1 (standard McMorran diet), 15:1, and 50:1 were included to imitate the physiological bounds of IF:N in host foliage diets. Budworm performed well on all diets with few significant differences. The reason for these unexpected results, in comparison to experiment 3, was probably because budworm were initially reared on the standard diet before being transferred, whereas in experiment 3, the insects were reared from second instar to pupa on treatment diets. The standard diet probably gave the larvae sufficient protein reserves to support their growth when reared on the deemed protein-deficient diets. As well, the inclusion of powdered cellulose as the primary source of fibre had little effect on performance, probably because it passed through the larval gut easily without interfering with digestion. An exception was the positive effect that increasing cellulose to 15:1 in the 100 percent diet had on egg production, compared to the standard diet which was 3:1, cellulose:casein N. Females on the former diet had 14 percent heavier pupal weights and laid 19 percent more eggs than those on the latter. It appears that the standard diet could be improved through the addition of fibre. From these studies, it is still inconclusive that there is an interaction between N and indigestible fibres which leads to diminished budworm performance.

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INTRODUCTION

There is a great variety of insects that feed on foliage of forest trees and yet the majority of trees manage to stay green. One of the reasons is that trees are well equipped with defence mechanisms that resist insect attack (Rhoades 1979; Berryman 1988). One such defensive tactic is varying the nutritional quality of tree tissue so that it becomes a less favourable food source for insect consumption (Scriber and Slansky 1981; Neuvonen and Haukioja 1984).

Food quality can be assessed by three components - nutrients, allelochemicals and physical attributes (Slansky and Scriber 1985). Nutrients refer to the presence and relative amounts of digestible proteins, carbohydrates, fats, water, minerals, vitamins, etc. which are used for normal growth, development, and health (Reese 1979). Allelochemicals refer to non-nutritional chemicals such as tannins, terpenes and fibres which are not required by insects but detrimentally affect their growth, health, behaviour, or population biology (Reese 1979). Physical attributes refer to leaf toughness and surface hairiness (Slansky and Scriber 1985). These three components are seldom static in a food source and can change over a single growing season, thereby resulting in variable food quality (Slansky and Scriber 1985).

Spruce budworm, *Choristoneura fumiferana* (Clem.), is a pest of considerable economic importance to the Canadian forest industry and society. Its primary hosts are the commercially valuable species white spruce, *Picea glauca* (Moench) Voss, black spruce, *Picea mariana* (Mill.) B.S.P. and balsam fir, *Abies balsamea* (L.) Mill.. Outbreaks of spruce budworm devastate vast tracts of forested land (MacLean 1985). For Ontario in 1991, 9.1 million ha

were moderately to severely defoliated (Canadian Council of Forest Ministers 1993). From 1982 to 1987 the average annual mortality and growth loss from spruce budworm was estimated to be 8.7 million m^3 (Hall and Moody 1994).

Spruce budworm populations are cyclical and severe outbreaks occur approximately every 30 years (Royama 1984). Many factors contribute to the regulation of budworm numbers, among them host-plant interactions (Mattson 1985). Little is known concerning the role host-plant interactions play in budworm population dynamics especially concerning the dietary relationship that spruce budworm has with its host trees.

This study moves in this direction by investigating components of food quality provided by the host trees of spruce budworm.

Nitrogen (N) is a limiting element in the growth of organisms (Mattson 1980). It plays a central role in all metabolic processes, cellular structure, and genetic coding (Mattson 1980). When fed artificial diets with various N levels, female spruce budworm were reported to develop faster (Harvey 1974) and increase in weight with increasing N levels (Mattson *et al.* 1983). Similarly, when raised on intact foliage of balsam fir and black spruce trees receiving N fertilization treatments, female budworm increased in weight gain with increasing N levels (Mattson *et al.* 1983).

Dietary water can also have a major influence on larval performance (Scriber and Slansky 1981). Faster larval development (Scriber 1977) and improved growth efficiencies were associated with increased food moisture content for various Lepidoptera (Scriber 1977; Scriber and Feeny 1979; Hough and Pimental 1978; Mattson 1980). The increase in food water content was reported to bring about an improvement in the ability of insects to utilize N (Scriber 1977, 1978, 1979). However, Clancy (1991a) reported decreasing larval performance and no improvement in N utilization with increasing diet

moisture levels for western spruce budworm (*Choristoneura occidentalis* Freeman).

Plant water and N were used very successfully as indices of food quality for predicting insect herbivore performance (Slansky and Scriber 1985). Clancy (1991a) recently challenged the predictive power of plant moisture and N and argued that these components were known to be strongly correlated with other tissue characteristics (Mattson and Scriber 1987). Clancy suggested that insect performance was probably not responding directly to foliar water and N but to factors that were linked to these components.

One such factor may be the content of indigestible fibre (IF) composed of cellulose, hemicellulose and lignin which are not digested once ingested by insects. Fibres play a dual role in the life of plants because they are the primary components of plant cell walls and provide fundamental, mechanical strength to plant structure (Rhoades 1979; Raven *et al.* 1986). They also play a defensive role by protecting plants against herbivorous insect attack by interfering with the digestive processes of some species of insects (Rhoades 1979) which results in increased larval mortality and smaller pupal weight (Feeny 1976; Baltensweiler *et al.* 1977; Bauce and Hardy 1988).

Indigestible fibre has always been considered as a constitutive chemical defense that exist within trees independently of insect feeding (Feeny 1976; Rhoades and Cates 1976). However, recent studies (Bauce and Hardy 1988; Beaudette 1986) suggested that IF was a dynamic defense that was stimulated into production as a direct response to insect feeding. Relative increases in IF levels were observed in defoliated over nondefoliated white spruce and balsam fir trees by Beaudette (1986) and Bauce and Hardy (1988) respectively. Spruce budworm feeding tests with foliage with the higher relative IF levels have correspondingly shown decreased pupal weights,

increased mean development times, and increased mortality (Bauce and Hardy 1988).

These results fail to prove conclusively that IF is responsible for decreased performance of the spruce budworm because the fibres were not isolated in the feeding experiments. Foliage contains many chemical components in varying concentrations which could affect insect performance. The relative proportions of nutrients were shown to be important for insects (House 1969), and altering the proportion of nutrients and allelochemicals affected larval performance (Scriber and Slansky 1981). A relative increase in IF levels, as observed by Bauce and Hardy (1988) and Beaudette (1986), indicated a relative decrease in other food components of importance to budworm nutrition.

I believe that budworm feeding, at a given defoliation intensity, triggers a relative increase of foliar IF to the other nutritional constituents that are essential to the growth and development of spruce budworm. It is my hypothesis that there is a threshold IF:nutrient ratio above which budworm performance declines. Above this threshold, the ability of budworm to contribute to future generations decreases.

To test the hypothesis, I conducted the following experiments with their corresponding objectives.

Experiment 1. Foliar samples of balsam fir, white spruce and black spruce were analyzed for levels of N, water and IF to determine the seasonal variation in these components and to quantify the physiological bounds of IF:N in the budworm diet.

Experiment 2: Early- and late-season foliage of balsam fir, white spruce and black spruce was measured for leaf toughness to determine the seasonal variation of this component of food quality. Spruce budworm were reared

on early- and late-season foliage of balsam fir, white spruce and black spruce to confirm that budworm perform better on foliage with low IF:N (early-season foliage) than on foliage with high IF:N (late-season foliage).

Experiment 3: Various casein levels were tested to determine a lower limit below which spruce budworm failed to survive. This lower limit was included into the design of experiment 4.

Experiment 4: Various cellulose:casein N levels were tested in artificial diets to assess the effect on spruce budworm performance (development time, larval weight, pupal weight, survival, number of eggs laid and percentage of hatch).

LITERATURE REVIEW

SPRUCE BUDWORM

Distribution

Spruce budworm is a native insect which occurs primarily in the northern boreal forest from Newfoundland west to the McKenzie River near 66° N (Mattson *et al.* 1988a). Its primary host species are balsam fir, white spruce, black spruce, and red spruce, although it sometimes feeds on other genera in the family Pinaceae (Mattson *et al.* 1988a).

Life Cycle

Spruce budworm is univoltine. Moths emerge in late June to late July and mating occurs shortly thereafter (Mattson *et al.* 1988a). Females lay an average of 200 eggs, in clusters of about 20, over a period of several days (Royama 1984). The eggs hatch within 10-14 days and the first instars (L1) disperse within the tree or stand, or are carried beyond by wind (Royama 1984). First-instar larvae spin hibernacula without feeding, molt to the second instar (L2), enter diapause and overwinter (Mattson *et al.* 1988a). Overwintering sites occur between bark scales, bark fissures, staminate flower bracts, or lichens (Mattson *et al.* 1988a).

In mid-May, second-instar larvae emerge and move to branch tips where they can be dispersed by wind. Old needles, unopened vegetative buds and staminate flowers are mined until buds flush and shoots expand, at which time the budworm begin feeding on the new, succulent foliage (Mattson *et al.*

1988a). Spruce budworm molt through six instars and pupate in late June to early July.

Host Specialization

Although it has a wide host range, spruce budworm is considered to be a specialist on white spruce and balsam fir (Mattson *et al.* 1988a). Mattson *et al.* (1988a) suggested that the reason for this specialization might be that the feeding cycle of the spruce budworm has coevolved to most closely match the phenology of these two species. Second-instar budworm emerge shortly before bud flush of balsam fir and white spruce and feed while shoots are elongating. Also, they pupate at a time when balsam fir and white spruce have nearly finished their annual shoot elongation (Mattson *et al.* 1988a).

The phenology of black spruce, on the other hand, is not as closely synchronized with budworm feeding (Prévost and Laing 1986). Black spruce buds flush up to 13 days later than those of white spruce and balsam fir (Greenbank 1963). Third-instar (L3) larvae which leave the mined needles of black spruce in search of new foliage commonly disperse or die because their preferred food source is not available (Morris 1963). In this way, late flushing allows black spruce foliage to be relatively undamaged by feeding and thereby escape from serious budworm outbreaks, similar to some resistant Douglas-fir *Pseudotsuga menziesii* (Mirb.) Franco against western spruce budworm (Clancy *et al.* 1993)

Growth and Development

Spruce budworm are voracious feeders. They consume a greater amount of food and eat it more rapidly than most other leaf-feeding insects, probably because immature foliage is easy to digest (Koller and Leonard 1981). The combination of an above average consumption rate and fast growth rate leads to rapid development of the budworm (Thomas 1989; Koller

and Leonard 1981). Fifth (L5) and sixth instars (L6) consume the majority (90-95%) of food (Miller 1977; Retnakaran 1983), although considerable damage can by done by early instars which feed and grow concomitantly with elongating shoots (Morris 1963; Régnière and You 1991).

Male and female sex differences occur with budworm (Koller and Leonard 1981; Thomas 1989) as with insects in general (Scriber and Slansky 1981). Females develop more slowly than males (McGugan 1954), consume more food, (Thomas 1983, 1987, 1989) and have pupal weights which are about twice as heavy as males (Koller and Leonard 1981; Mattson *et al.* 1983). They also emerge as adults about three days later than males (Koller and Leonard 1981).

Host Trees and Insect Performance

Although spruce budworm feeds on the foliage of white spruce and balsam fir , it is not clear which, if either, of the two hosts support superior growth and development. More rapid development on white spruce has frequently been reported (McGugan 1954; Thomas 1989; Lavallée and Hardy 1988; Lysyk 1989) yet Koller and Leonard (1981) recorded a shorter development time on balsam fir. Results of larger (Koller and Leonard 1981; Thomas 1983) or at least equal (Blais 1957; Greenbank 1963) insect growth have been reported on diets of white spruce rather than balsam fir. However, Mattson *et al.* (1983) noted the opposite situation of superior growth of spruce budworm on balsam fir compared to white spruce. The findings are confusing and it is difficult to draw conclusions or compare results because of the different circumstances under which the experiments were conducted.

It is generally agreed that spruce budworm fitness is poorest when larvae feed on a diet of black spruce (Thomas 1989; Mattson *et al.* 1983),

although, upland black spruce appears to support better growth than lowland black spruce (Mattson *et al.* 1983).

Height class of the host trees has been shown to have an effect on spruce budworm fitness. Clear differences in budworm growth and fecundity between small (1-5 m) and large (10-15 m) trees were reported by Mattson *et al.* (1983). Larger trees produced larger and more fecund insects than did smaller trees. This may be an effect due to tree age which affects the chemical makeup, i.e. nutrients and allelochemicals, in current-year growth (Mattson 1985).

Spruce budworm fitness characteristics such as survival, development, growth and fecundity are known to decline with increasing age of the shoots being fed upon. Although sixth-instar larvae can feed successfully and complete their development on the old foliage of both white spruce and balsam fir (Greenbank 1963), they suffer increased larval mortality and reduced size and fecundity of surviving adults (Blais 1953). These effects are also felt when larvae are forced to feed on current-year shoots which are increasing in age over the course of a single growing season.

Variable Host Defoliation

Even though spruce budworm feeds avidly on its host trees, the trees are not equally defoliated. White spruce experiences less percent defoliation in the field than balsam fir (Greenbank 1963; Régnière *et al.* 1989; Lysyk 1989). Through simulation modelling of spruce budworm feeding Régnière and You (1991) determined that white spruce suffered about 25 percent less defoliation than balsam fir given equal values of budworm density, survival rates and shoot density.

The reason for this variable defoliation is not because of differential consumption of the hosts; spruce budworm larvae consume almost identical

amounts of white spruce and balsam fir foliage (Koller and Leonard 1981; Régnière and You 1991). Rather, it is mainly due to the faster growth and heavier shoots of white spruce that it thereby suffers less defoliation than balsam fir (Lavallée and Hardy 1988; Régnière and You 1991). Balsam fir shoots elongate more slowly than those of white spruce (Greenbank 1963); thereby allowing defoliation to outpace shoot expansion (Koller and Leonard 1981).

FOOD QUALITY

Nutritional Composition

Nitrogen

Importance

Nitrogen is considered to be critical to animal growth (Mattson 1980). It is one of the elements of amino acids which then make up proteins which are the major structural and functional components of cells and tissues (Curtis 1975). Mattson (1980) summarized the importance of N as " play[ing] a central role in all metabolic processes as well as in cellular structure and genetic coding. "

Protein is required for both maintenance and growth of animals (Dadd 1985). The protein requirement for maintenance is "that intake needed to repair the continual normal wear and tear of tissue protein structures and enzymes so as to keep the animal just in nitrogen balance" and the protein requirement for growth is "that needed over and above the maintenance requirement for production of new cellular substance and reproduction of progeny" (Dadd 1985).

Nitrogen is regarded as a limiting factor to growth and animals experience a relative shortage of usable or metabolizable N during critical growth periods (Mattson 1980). The growth requirement for proteins is high during insect larval growth and abated in the non-feeding adult (Dadd 1985). Nevertheless, when the whole life cycle is considered, protein is always a prime dietary necessity and, in adults, it is supplied by larval stores (Dadd 1985).

Artificial Diets

To test for the importance of a diet component such as N, artificial diets have been developed for many insects and animals. With these diets, all nutrient variables are controllable and this feature allows for the testing of individual components. The McMorran diet (McMorran 1965) is an artificial diet which is routinely used for standardized rearing and nutritional studies of spruce budworm.

Nitrogen Influence

Many studies (e.g. Mattson 1980; Scriber and Slansky 1981; Scriber 1984) have explored the effects of increasing dietary N and shown a positive correlation with insect performance. In a review of over 200 investigations regarding the response of insects to N fertilization of plants, Scriber (1982) reported that increases in insect growth, fecundity and population density were shown in the majority of studies. Research by Mattson *et al.* (1983) in which white spruce and balsam fir foliar concentrations of mineral elements were analyzed revealed that N was the only variable consistently and positively related to growth, i.e. dry weight of fifth- and sixth-instar spruce budworm larvae. Bauer and Nordin (1988) noted increased pupal weights and reduced development times for spruce budworm reared on artificial diets with 4.5 percent N than those on 2.5 percent N diets.

Males appear to have a lower N dietary requirement than females. Mattson *et al.* (1983) reported that female larvae reared on artificial diets responded to increasing N levels (2 to 4 percent) with increasing weight gains whereas males exhibited only weak responses. Similar trends were found with larvae feeding on intact foliage of balsam fir and black spruce trees that were receiving a range (0.47 to 2.05 percent) of N fertilization treatments. In fact, male weight gains showed little tendency to increase with foliar N levels above 1.5 percent (Mattson *et al.* 1983). The difference between males and females may be due to the female requirement for additional protein intake for oogenesis (Mattson *et al.* 1983).

A N-rich diet obtained early in their development is thought to optimize performance factors later in the life cycle of spruce budworm. Using transfer experiments and two artificial diets with levels of N that mimicked early (high N) and late (low N) season foliage, Harvey (1974) determined that budworm that were started (as second- and third-instar larvae) on the early diet and then transferred (as late third- and early fourth-instar larvae) to the late diet developed more quickly than those that were started on the late diet and then transferred to the early diet, although the final weights average out to be about the same. Mattson *et al.* (1983), using transfer experiments with artificial (high N) and foliage (lower N) diets, reported that budworm started (as second to fourth instars) on the artificial diet and transferred to balsam fir foliage diets were significantly larger than foliage to foliage reared insects. They observed that "the early diet ration seemed to "bump up" the final adult weights. However, budworm transfers from the artificial to white spruce foliage diets resulted in reduced insect growth on the diet to foliage experiment compared to the foliage to foliage reared insects (Mattson *et al.* 1983). To explain this, they suggested that early season white spruce foliage may be superior to the

artificial diet or that there may have been a transfer shock going from diet to foliage. This shock may have been due to higher metabolic costs associated with operating the mixed-function oxidase (MFO) system on spruce than on fir (Mattson *et al.* 1983). The MFO system is a system of enzymes which deactivate and eliminate plant allelochemicals from insect bodies (Brattsten 1979).

Although N has been believed to be a key nutrient which determines herbivore performance (e.g. Mattson 1980; Scriber and Slansky 1981), controversy exists concerning its singular importance in isolation from other diet components (Clancy 1992). Changes in levels of N in plant tissue are known to coincide with changes in levels of other nutrients, water, fibre and numerous allelochemicals (Mattson 1980; Clancy *et al.* 1988). It may be that a combination of diet constituents including N is responsible for determining herbivore performance. An investigation by Clancy (1992) with western spruce budworm illustrates this point. She examined the performance effects of artificial diets containing varying levels of N and minerals and determined that the ratio of zinc to N was the best predictor of budworm fitness and not the actual N content of the diet. Clancy (1992) argues that it is a proper balance of many different nutrients and not simply N that is the most important factor in determining the performance of insect herbivores.

Nitrogen Variation

Foliar N varies seasonally, with leaf age (ontogenetically) and among species (Mattson 1980). Nitrogen concentrations are highest in the spring when young tissues are actively growing and expanding, drop sharply when growth wanes and then gradually decline further through the course of a growing season until tissue senescence (Mattson 1980). Levels of N at the beginning and end of the growing season for current-year foliage of black

spruce have been reported to be 3.6 percent and 1.2 percent dry weight, respectively (Prévost and Laing 1986). Similarly for balsam fir, values between 2.5 percent and 4.4 percent dry weight in the early season and 0.88 percent and 0.9 percent dry weight in the late season have been noted (Shaw and Little 1972; Shaw *et al.* 1978).

Prévost and Laing (1986) determined that, at the initiation of the growing season, current-year bud foliage of black spruce contained 3.6 percent dry weight N while one-year old foliage had about 1.0 percent and two-year old foliage had about 0.8 percent. For balsam fir, changes in N concentration with leaf age have been published as 1.46 percent in current-year foliage, 0.97 percent in one-year old foliage and declining to 0.76 percent dry weight in five-year old foliage (Mattson and Scriber 1987).

The host trees of spruce budworm also vary in levels of foliar N. It was observed that white spruce contained lower N concentrations in mid-June than both black spruce (Thomas 1989) and balsam fir (Mattson *et al.* 1983). Thomas (1989) reported 1.30 percent in white spruce compared to 1.68 percent for black spruce; Mattson *et al.* (1983) reported 1.24 percent for white spruce compared to 1.52 percent dry weight N for balsam fir. Clancy *et al.* (1988 and references therein) compiled values from seven sources which showed that the range of N values for current-year foliage of balsam fir were 0.88 to 4.4 percent while that for black spruce were 0.7 to 1.0 percent dry weight. Commenting on the changing concentrations of N, Clancy *et al.* (1988) suggested that spruce budworm has evolved the ability to cope with a wide range of nutrient levels between " individual trees, populations and host species. " They stated that the " variability between trees is probably matched or exceeded by seasonal and ontogenetic changes that occur within individual trees."

Water

Importance

Dietary water, like N, is a key nutrient for all organisms (Mattson and Scriber 1987). Serving many functions (Wharton and Arlian 1972), water is integral and of primary importance to the structural conformation and metabolic activities of insects (Wharton 1985).

The major water source for insect larvae is from the ingestion of moist food (Martin and Van't Hof 1988) but other sources include drinking, absorption of vapour from the air and that made by metabolic activity (Wharton 1985).

Levels of plant water and N are positively correlated and these variables are strongly linked to insect growth and food utilization (Slansky and Scriber 1985; Mattson and Scriber 1987). As indices of food quality, plant water and N have been used to predict insect herbivore performance (Slansky and Scriber 1985). It has been shown that insect growth is best on tissue high in foliar water and N and poorest on tissue low in foliar water and N (Mattson and Scriber 1987).

Water Influence

The association of plant water and N has prompted investigators to question the relative importance of water given the putative importance of N. Studies manipulating dietary water levels while keeping N levels constant have repeatedly shown that with reduced food moisture content larval growth is reduced and development time extended due to a lowered efficiency of conversion of digested food to larval tissue (Scriber 1977; Reese and Beck 1978; Timmins *et al.* 1988; Martin and Van't Hof 1988; Paul *et al.* 1992).

Especially interesting is the fact that larvae on low-water diets may consume more food and ingest and assimilate more N but still remain smaller compared to larvae on high-water diets (Scriber 1977; Martin and Van't Hof

1988). The N is being assimilated but not being used to produce more new tissue (Martin and Van't Hof 1988). Moreover, larvae on low-water diets excrete greater amounts of uric acid, the principal nitrogenous waste product of insects (Cochran 1985), than insects on high-water diets (Martin and Van't Hof 1988). This suggests that on low-moisture diets larval growth is suppressed because of a reduced efficiency in using assimilated N for growth (Scriber 1977; Martin and Van't Hof 1988). Martin and Van't Hof (1988) further concluded that dietary water, rather than N, is a limiting nutrient to growth. They stated that "the upper limit to the amount of new tissue that can be synthesized is set by the amount of water that larvae can extract from their food." Likewise Schroeder (1976, 1986) and Schroeder and Malmer (1980) showed that N was not a growth-limiting nutrient for larval Lepidoptera and Hymenoptera fed on leaves with greater than 3 percent (dry weight) N. Schroeder (1986) and Martin and Van't Hof (1988) suggested that with limited water, surplus N became a liability rather than an asset because of the increased energy expended on excretion.

To provide further confirmation of the importance of water, Martin and Van't Hof (1988) described the adaptive mechanisms that larvae of *Manduca sexta* (L.) developed to enhance the retention and use of water. When faced with a low-water diet, larvae had the ability to retain water by resorbing it from the contents of the rectum. The faeces were much drier than the food. This is important because faecal excretion is the major source of water loss (Martin and Van't Hof 1988). Larvae also can tolerate lower levels of tissue hydration and reduce hydration levels in response to reduced water intake. Martin and Van't Hof (1988) reported that *M. sexta* larvae reared on low-water diets synthesized 70 percent more new, fully hydrated tissue from a given amount of water than larvae reared on high-water diets .

Paul *et al.* (1992) agree that water is a limiting factor to larval growth based on their investigation of dietary moisture and larvae of the Oriental silkworm, *Bombyx mori* L. With decreasing food moisture content they found decreases in nutritional indices and larval growth. In addition, larval duration was prolonged with no increase in leaf consumption. Paul *et al.* (1992) theorized that a voraciously feeding silkworm larva should consume a greater amount of food during an extended compared to a normal life span and that a longer life span would mean a higher maintenance cost for the larva. Therefore, the extended life and higher costs should force the insect to consume greater quantities of food. In their investigation, just the opposite was found. Relative to larvae reared on the control diet, those feeding on low-water diets consumed 20 percent less food in spite of prolongation of the feeding period. Paul *et al.* (1992) stated that these results implied that " water imposes a limiting factor for food utilization and its efficient conversion to larval biomass."

In contrast, Clancy (1991a) determined that dietary water in the range of 72 to 88 percent was not limiting to the performance of western spruce budworm and that performance was generally negatively associated with food moisture content. Moreover, increased dietary water failed to improve N utilization. Clancy (1991a) varied both water and N levels in artificial diets and reported that budworm survival, pupal biomass increase, and rapid development time were best on diets with low N and low water or high N and low water. This led to the conclusion that water seemed to act simply as a diluent. Clancy (1991a) explained that "as the water content of the diets was increased, the fresh biomass concentration of the nutrient was diluted and budworm performance decreased. Conversely, when dietary moisture was

decreased, the fresh biomass concentration of all nutrients was increased and budworm performance was improved, particularly on the low-nitrogen diets."

Clancy (1991a) had expected to find that diets high in water and N would support the best larval growth as predicted by food quality (indexed by water and N) as presented by Slansky and Scriber (1985). Instead, the opposite outcome occurred. This led Clancy (1991a) to deduce that there may not be a direct cause-and-effect relationship of plant water and N on insect herbivore performance. These two variables are known to be strongly correlated with many other tissue characteristics (Mattson and Scriber 1987). Clancy (1991a) asserts that the "predictive power of plant N and water appears to be related to other factors" that are linked to N and water.

Water Variation

Tree leaves are among the poorest sources of foliar water (45-75 percent water) while terrestrial annual and biennial forbs are among the best (75-95 percent water) (Slansky and Scriber 1985). Levels of foliar water vary between and among species, seasonally, with leaf age and within individuals (Scriber and Slansky 1981). Leaves of deciduous trees generally have higher water contents than those of coniferous trees (Larcher 1983); leaf water (percent fresh weight) generally declines through a growing season; mature leaves are generally lower in water than juvenile leaves (Scriber and Slansky 1981).

Hough and Pimental (1978) measured foliar water contents (percent fresh weight) over an eight-week period for several hardwood species and reported an initial increase early in the growing season (early-May) and then a steady decline through the rest of the collection period (mid-May to late-June). Peak values ranged from ≈70-80 percent and lows from ≈58-63 percent. Water content of old leaves (≥1 yr) remained constant around 50 percent. Feeny

(1970) reported varying water contents within individuals of oak (*Quercus robur* L.) depending on aspect and crown level. Moisture contents declined through the growing season and leaves from the south side of the tree crown ("upper sun leaves") had lower foliar moisture than those from the northern branches at the base of the tree ("lower shade leaves"). The peaks and lows for the upper sun leaves and lower shade leaves were ≈71-50 percent and 74-55 percent, respectively.

Trends in foliar moisture patterns are similar for coniferous trees. Prévost and Laing (1986) measured the change in seasonal moisture content for 35-year-old black spruce and reported an initial water content of ≈60 percent (fresh weight) in late May which peaked to 76 percent in June and declined to ≈52 percent in September. A steady, seasonal decline in foliar moisture was described by Pharis (1967) for two-year-old seedlings of ponderosa pine, *Pinus ponderosa* Dougl. ex Laws, and sugar pine, *Pinus lambertiana* Dougl.; seasonal highs in May were ≈300 percent (dry weight) and lows in September were ≈190 percent . On the other hand, levels of foliar moisture for two-year-old seedlings of grand fir, *Abies grandis* (Dougl.) Lindl., and Douglas fir declined from the beginning of the growing season to mid- or late-summer and then inclined through to early-fall (Pharis 1976). Grand fir moisture levels were ≈320 percent (dry weight) in May, ≈130 percent in August and ≈160 percent in September. Douglas fir moisture levels were ≈400 percent in May, ≈185 percent in July, ≈205 in August and ≈195 percent in September (Pharis 1967). For other conifers, Koller and Leonard (1981) determined that moisture content varied little for understory white spruce and balsam fir through the season of spruce budworm development; white spruce moisture content held steady at 80±3 percent and balsam fir at 73±3 percent (fresh weight).

Allelochemical Composition

Types of Chemical Defences

Constitutive Defences

Constitutive defences are chemicals which act as quantitative barriers to insects, i.e. the quantity of chemical contained in the tissue acts as the barrier to feeding (Price 1984). These defences are effective against all insects at high concentrations and act by reducing the digestibility of plant tissue, thereby, decreasing the insects' reproductive contribution (Price 1984).

Tannins and IF are examples of constitutive defences which characteristically occur within plants in naturally high concentrations. Tannins occur in concentrations of 60 percent based on plant dry weight (Rhoades 1979) and IF occur in concentrations from 24-52 percent based on plant dry weight (Dickson 1979; Prévost and Laing 1986). Other constitutive defences include resin, terpenoids, alkaloids, other phenolics, and their many derivatives (Berryman 1988).

Constitutive defences are characteristic of late successional (woody) plant species which tend to grow in pure stands of relatively low density and for long periods of time (Rhoades 1979; Mattson *et al.* 1988b). These are referred to as apparent plants which means that their tissue is both predictable and available to herbivores (Rhoades and Cates 1976). Because of this apparency, a nearly universally effective chemical defence has developed through selective pressure to protect the tissue against all the insects that feed on them (Price 1984).

Inducible Defences

Inducible defences are responses which are activated by an external stimulus such as insect attack. Defensive responses in host plants depend on

pest density (Haukioja and Neuvonen 1987) and include both the production of new structures and chemical constituents, as well as changes in structures or concentrations of existing compounds (Harvell 1990). Harvell (1990) comments that in this way, compounds present at low levels can be sequestered and become deterrents simply through an increase in concentration. Increases in IF (Benz 1974; Baltensweiler *et al.* 1977; Bauce and Hardy 1988), tannins and other phenolics (Thielges 1968; Haukioja 1980; Wagner and Evans 1985; Wagner 1988) and resin (Lewinsohn *et al.* 1991) are examples of such deterrents. In addition, decreases in concentrations of nutrients such as N (Baltensweiler *et al.* 1977; Tuomi *et al.* 1984), sugars, and starches (Webb and Karchesy 1977; Valentine *et al.* 1983; Ericcson *et al.* 1985) also act as deterrents to insect feeding. Finally, phenological characteristics such as timing of budbreak (Du Merle 1988; Clancy 1991b) and leaf abscission (Faeth *et al.* 1981; Williams and Whitham 1986) are inducible by insect activity as are alterations in morphology (Batzer 1973; Piene and Percy 1983; Myers and Bazely 1990).

An insect herbivore which feeds on plant tissue that employs inducible defences changes the quality of that plant tissue for subsequent feeding. When, as a result of increased pest density, there is a decrease in food quality or poor synchrony between budbreak and initiation of feeding, a negative feedback is created which consequently has the potential to regulate herbivore density (Haukioja and Neuvonen 1987).

A herbivore-induced response may occur rapidly or be delayed. If the response is rapid and lasts for a shorter time than the generation time of the herbivore, the effects are felt by the generation doing the damage (Haukioja and Neuvonen 1987). If the response is delayed, the effects occur after the generation that brought about the damage, and the effects negatively impact

the performance of succeeding generations (Haukioja and Neuvonen 1987). It is to this category that the response of changing concentrations of foliar IF and N seems to belong.

Induced alterations in food quality may act on the stability of insect populations because they appear to function in a density-dependent manner (Haukioja and Neuvonen 1987). Rapidly induced responses are thought to act as stabilizing agents because the negative effects are felt by the generation doing the damage (Haukioja and Neuvonen 1987). With the delayed response, food quality can be changed for many years and the effects felt by insects even after an outbreak subsides, i.e. at low densities. In this way, Haukioja and Neuvonen (1987) found that a delayed inducible response produces a "negative feedback and a time-lag component into the population dynamics of the pest which tends to destabilize the density of herbivore populations." Haukioja and Neuvonen (1987) suggested that the "delayed inducible defence offers a simple mechanism both for the decline in density (even before apparent food shortage) and for the long intervals between successive peaks in density." Delayed inducible defences are the responses found more commonly in woody plants and especially in deciduous trees (Wagner 1988).

Evidence for the effects of delayed induced responses on population cycling rests on two insect species and their host trees, the larch budmoth, *Zeiraphera diniana* Guenée, on European larch, *Larix decidua* Mill., and the autumnal moth, *Epirrita autumnata* Borkhausen, on mountain birch, *Betula pubescens spp. tortuosa* Ehrh (Haukioja 1990). Once a threshold density was attained, both host species exhibited delayed induced responses of decreased foliar N levels and increased levels of IF in larch needles and tannins and phenolics in mountain birch leaves (Benz 1974; Baltensweiler *et al.* 1977;

Haukioja 1980; Neuvonen and Haukioja 1984; Haukioja and Neuvonen 1985). Feeding tests with leaves of the defoliated trees showed significant performance reductions with both insects. In each case, the induced response took about four years to relax after which the species multiplied for four or five generations under favourable conditions (Benz 1974). Once a threshold herbivore density is attained, the food quality is altered so that resistance to the insect species is high for another four or five generations (Benz 1974). In this way, a regular nine- to ten-year cycle is produced (Haukioja 1990).

The preceding explanation of the inducible defence hypothesis provides a basic conceptual framework. It is, however, generally accepted that inducible defences are considerably more complex and poorly understood (Karban and Myers 1989; Harvell 1990; Haukioja 1990). It is unknown whether herbivore-induced responses provide simple resistance to herbivory (in those instances when herbivore performance is negatively affected by changes) or if they are, in fact, true defences. To be a true defence, it is thought that the benefits of producing a response must outweigh the metabolic costs and result in increased plant fitness (Karban and Myers 1989; Haukioja 1990). To date, little research has been conducted into the costs and benefits of producing inducible defences; these evolutive parameters are difficult to measure in long-lived trees (Wagner 1988; Haukioja 1990) .

Because of the ambiguity of the true nature of herbivore-induced responses, it is desirable to use the term "resistance" rather than "defence" (Haukioja 1990). It is thought that a defensive function is only one possible reason for the induction of the response; herbivore-induced responses need not be of a defensive nature at all, even if they detrimentally affect insect performance (Haukioja 1990). Haukioja (1990) points out that an induced

response may represent something other than a true defence; for example, it may be a 'by-product' of the repair process that takes place once tissue damage has occurred. Also, herbivore-induced response may be directed at something other than herbivores (Haukioja 1990). In some studies (Niemelä *et al.* 1984; Wagner and Evans 1985; Roland and Myers 1987) defoliation actually improves the quality of the foliage for subsequent feeding (Haukioja 1990).

In addition to variability of insect response, there is variability of plant response to insect damage. Variation exists among species, between individuals, within a single individual, with site condition and across environmental gradients (Neuvonen and Haukioja 1990). There may also be genetic variability as well as a temporal variation in induced responses (Haukioja 1990).

Because of the variability of herbivore performance and plant response, few generalities can be made concerning the role of induced plant responses on insect population dynamics (Karban and Myers 1989; Haukioja 1990). The examples of European larch and larch budmoth (Benz 1974; Baltensweiler and Fischlin 1988) and autumnal moth and mountain birch (Haukioja 1980; Neuvonen and Haukioja 1984; Haukioja and Neuvonen 1985) provide largely circumstantial evidence for the importance of delayed induced resistance in regulating populations (Haukioja 1990). Karban and Myers (1989) contend that to explain regional synchrony of population fluctuations of forest Lepidoptera, there would have to be predictability of host tree response to insect attack, but it appears that there are inconsistencies in the manner of response. On the other hand, Karban and Myers (1989) stated that "changes in the fecundity and survival of fluctuating populations of forest Lepidoptera often show consistent patterns through the cycle, even when caterpillars feed

on different species of host plant, in different areas, and following different histories of attack." This suggests that food quality may not be important in driving insect population cycles.

Although induced resistance may not drive insect cycles, it is possible that this factor plays a role in the regulation of insect populations (Haukioja 1990). Rather than placing emphasis on single regulative factors, combinations of factors should be explored such as the possibility that induced compounds might entice predators (Haukioja 1990). The relative importance of induced plant resistance compared with other ecological factors that may regulate insect populations should be also assessed (Karban and Myers 1989; Haukioja 1990).

One of the difficulties in studying induced chemical responses is determining which chemical constituent to focus upon. Karban and Myers (1989) assert that in order to understand the chemical mechanisms of induced resistance, it is necessary to consider all of the chemicals within a plant with potential activity against herbivores and not just a particular subset that is easy to work with or considered to be important. Each constituent should be varied singly by using artificial diets to collect the evidence and this procedure should ensue for all probable mechanisms (Karban and Myers 1989). Clearly this would be the ideal situation from an ecological point of view and would entail long-term and multidimensional studies (Karban and Myers 1989). Until that situation should occur, there is a need for studies that investigate dietary components that have not yet been looked at but for which importance has been indicated.

On the other hand, it must be remembered that results from experiments that are considered to be "highly artificial" cannot be easily extrapolated to the natural environment (Karban and Myers 1989). In the field,

the effects of plant chemicals on herbivores may be quite different than in the laboratory due to the likely importance of interactions and synergisms (Karban and Myers 1989). Plants do change in response to herbivory but, as stated by Karban and Myers (1989), probably "no single mechanism will explain all of these diverse plant responses."

Another complication in the study of induced resistance has been the difficulty in determining the relative importance of allelochemicals versus nutritive factors on insect performance (Haukioja 1990). In the seasons following defoliation, concentrations of some foliar allelochemicals and nutrients correlate negatively (Haukioja 1990). For example, in foliage produced subsequent to insect attack, higher concentrations of phenols and IF and lower concentrations of N, amino acids, and sugars have been found compared to concentrations found prior to feeding (Benz 1974; Baltensweiler and Fischlin 1988; Bauce and Hardy 1988; Valentine *et al.* 1983). This makes it difficult to determine the absolute importance of one dietary component over the other. However, it may be possible to view this situation in another light. Rather than being an "either-or" situation perhaps it is the balance of the two allelochemic:nutrient components that is important. Maybe there is a threshold ratio of allelochemic:nutrient above which effects on insect performance are experienced. Clancy (1992) determined that it was the proper balance of many different nutrients and not just N that impacted the fitness of western spruce budworm. It is possible that a similarity exist for allelochemicals and nutrients.

Elicitation of Induced Response

The mechanisms that trigger induced responses in plants are poorly understood (Karban and Myers 1989) but it is presumed that biochemical signals are responsible for communication with appropriate cells and tissues

(Wagner 1988). Biochemicals that elicit responses could be natural compounds (Wagner 1988) such as components of insect frass (Haukioja *et al.* 1985) or insect saliva (Hartley and Lawton 1990). Chitosan, which is a constituent of the cell wall of many fungi, has been implicated as an elicitor of localized plant responses, such as increased resin production in the conductive tissue (tracheids or sieve cells) of conifers (Lieutier and Berryman 1988). Plant cell wall fragments have been demonstrated to induce a systemic response in herbaceous plants (Lieutier and Berryman 1988). Fragments released by insect chewing or as a consequence of cell penetration by microorganisms (Lieutier and Berryman 1988) may be translocated to other parts of the plant where they induce proteinase inhibitors (Lieutier and Berryman 1988; Karban and Myers 1989). In tomato plants, proteinase inhibitors toxic to some caterpillars accumulate in vacuoles of uninjured cells of injured plants (Broadway *et al.* 1986). These are possible mechanisms that elicit rapid responses. Mechanisms that may elicit delayed responses, such as increases in IF, are generally not understood (Karban and Myers 1989).

Plant Defence Theories

Early Theories

Numerous theories have been formulated in the last twenty years to explain the evolution of plant defences but, as yet, no single theory has been successful in explaining and predicting defensive occurrences in all insect/herbivore situations and in all ecosystems (Berryman 1988; Tuomi *et al.* 1988; Scriber and Ayres 1988; Haukioja 1990). The plant apparency theory developed by Feeny (1976) predicted that plants that were easily found or 'apparent' to generalist insects invested heavily in costly constitutive defences. "Unapparent" plants utilized less costly inducible defences against generalist insects that fed upon them and relied upon non-detection or escape from

specialist herbivores. This theory provided a constructive focus for years of research but gradually lost favour because of difficulties in empirically assessing "apparency" (Scriber and Ayers 1988) and because of its inability to explain the continuum between constitutive and induced defences (Coley *et al.* 1985). In addition, investigations revealed that specialist and generalist herbivores failed to behave as the theory predicted (Coley *et al.* 1985).

The "optimal defence theory" suggested by Rhoades (1979) and Berryman (1988) was based on the assumptions that: 1) defensive adaptations evolve to maximize the inclusive fitness of the individual organism, and 2) all defensive traits have a cost in terms of individual fitness because energy and/or nutrients are diverted from other essential functions such as growth and reproduction. It extends from these assumptions that a particular trait will evolve only if the benefit to fitness exceeds its cost and that the benefit of a particular trait depends upon the impact of a particular herbivore on plant tissue (Berryman 1988).

Later Theories

Ideas by Feeny (1976) and Rhoades (1979), along with those from other early researchers, set the groundwork for succeeding theories which incorporated many of the important elements (Scriber and Ayers 1988). The resource availability hypothesis, advanced by Coley *et al.* (1985), proposed that either inducible or constitutive defences will develop depending on site quality. Slow growing plants which are adapted to nutrient-impoverished sites will invest most heavily in constitutive defences because of the high cost of replacing lost tissue. Furthermore, these plants will have carbon-based allelochemicals (tannins, other phenolics, lignins, terpenes) rather than N-based allelochemicals (alkaloids, hydrogen cyanide and other glycosides, cardenolides and mustard oils) because nutrients are often more limiting than

available sunlight. Rapidly growing species, on the other hand, which grow on nutrient-rich sites will employ less costly inducible defences because these plants are efficient at replacing tissue. The allelochemicals produced by species growing on rich sites will frequently be N based because these plants are usually more carbon-stressed than N stressed due to competition by shading for light rather than for nutrients.

Another theory advanced by Lorio (1986) suggested that plants may be either growth- or differentiation-dominated, having been evolved for more rapid growth or for more powerful defence, respectively. Herbaceous plants which invest little in chemical defence production are growth-dominated, whereas woody plants which invest highly in chemical defences are differentiation dominated.

The above theories are similar in some respects and in many cases their predictions are congruent (Scriber and Ayers 1988). Scriber and Ayers (1988) contend that each of the apparency, resource availability, and growth-differentiation balance theories account for trends of chemical defence from early-successional (inducible and N based) to late-successional (constitutive and carbon-based) plant communities. Scriber and Ayers (1988) indicate that each theory "can account for some differences in leaf nutritional quality between plant taxa of different growth forms (e.g. forbs vs shrubs vs trees)." However, they believe that the resource availability hypothesis appears to have greater strength in situations when predictions differ, as when comparing equally "apparent" plant species in resource-rich and resource-poor environments.

All of these theories fail to explain adequately the primarily carbon-based inducible defences found in woody species of late-successional communities. Models which advocate passive rather than active mechanisms

of resistance offer alternative explanations. The difference between the two is that "active responses involve *de novo* synthesis or energetically costly enzymatic processes, whereas passive responses involve only the consequences of tissue removal" (Karban and Myers 1989). The carbon/nutrients balance hypothesis by Bryant *et al.* (1983) describes the passive alteration of a plant's carbon/nutrient balance induced by severe defoliation. This model has also been termed nutrient stress by Tuomi *et al.* (1984, 1988) and passive deterioration by Myers and Williams (1984).

Carbon/nutrients balance

Carbohydrates are produced by plants through the reduction of atmospheric carbon dioxide during photosynthesis and are the raw materials used in the synthesis of further biochemicals (Waring and Schlesinger 1985). Total carbon gain from the photosynthetic process is thought to be distributed between two pools: 1) carbon used in growth (cellulose and metabolic machinery) and 2) carbon used in storage and resistance (phenolics, lignin, terpenes and starch) (Tuomi *et al.* 1988). Waring and Schlesinger (1985) defined this carbon allocation more precisely as a hierarchical sequence of: 1. formation of buds and new foliage; 2. production of new roots; 3. storage reserves; 4. diameter growth and 5. protective chemicals. These ideas differ from earlier ones in that defensive chemicals are produced only after basic growth requirements are met.

Implicit is the assumption that photosynthesis (hence carbon gain) takes place most efficiently when the plant has sufficient nutrients for growth. Macronutrients (e.g. N, phosphorus, and potassium) and micronutrients (e.g. iron, copper, and zinc) are essential to plant growth; consequently, total carbon gain and growth are functions of plant nutrient reserves (Tuomi *et al.* 1988). It is assumed that plants will preferentially allocate carbon to growth whenever

there are sufficient mineral nutrients to construct new cells. Only when growth requirements have been met will surplus carbon (carbon accumulated above levels required for growth) be allocated to storage and/or carbon-based allelochemicals (Tuomi *et al.* 1988).

Accumulation of surplus carbon and allelochemicals

All plants have an optimum balance of carbon and nutrient reserves which are required for maximum growth (Tuomi *et al.* 1988). Changes in the environment can disrupt this balance so that either carbon or nutrients become suboptimal for growth. When this occurs, the accumulation of secondary compounds is influenced in the following manner. When nutrients or water are the limiting condition in a soil environment, plants are unable to grow to their maximum potential (Tuomi *et al.* 1988). Photosynthesis is affected under these conditions, however, growth is more severely affected, i.e. the decline in growth with nutrient stress is generally greater than the decline in photosynthesis (Bryant *et al.* 1983). Consequently, photosynthesis proceeds at a faster rate than growth so that carbohydrates are produced in amounts greater than required for growth. This results in a surplus of carbon which is then utilized for storage and/or the production of carbon-based allelochemicals (Tuomi *et al.* 1988).

The accumulation of carbon-based secondary products (e.g. phenolics, polyphenols, tannins, lignin, terpenes) and starch and other carbon-stores is well documented under conditions where growth is more limited than photosynthesis (Tuomi *et al.* 1988). Tuomi *et al.* (1988) suggest that these observations support the hypothesis that the "production of carbon-based secondary compounds is supported by resource surplus accumulated above and beyond the requirements of plant primary metabolism."

Induced responses to the removal of foliage

By employing the model of the carbon/nutrient balance, the process by which plant responses are induced by herbivore attack can be explained. When defoliation occurs, the capacity of a plant to gain carbon is reduced in two ways: 1. by the removal of leaf area upon which photosynthesis takes place and 2. by the removal of any carbohydrate and nutrient pools that are stored in the leaves (Harper 1977). This foliage removal can therefore influence the carbon/nutrient balance which, in turn, can modify the accumulation of carbon-based secondary compounds (Tuomi *et al.* 1988). How this accumulation is affected depends on whether leaf removal reduces more strongly the carbon or nutrient reserves of trees.

Deciduous trees have large carbohydrate reserves in their stems and roots (Bryant *et al.* 1983). Removal of foliage, therefore, removes much of the nutrient capital but does not affect the carbon reserves in the roots. Consequently, defoliation should lead to a carbon surplus relative to nutrients, and thus the amount of carbon available for allelochemical production may increase in the season following defoliation (Tuomi *et al.* 1988). This has been shown to occur with mountain birch (Haukioja 1980, Neuvonen and Haukioja 1984; Haukioja and Neuvonen 1985) and European larch (Benz 1974; Baltensweiler *et al.* 1977). Increased concentrations of leaf phenols or IF and decreased leaf size and concentrations of N occurred after insect attack and insect performance declined as a result of feeding on such foliage.

Coniferous trees typically store carbon in the leaf tissue, particularly the previous year's needles, and little in the roots and stem (Wilson 1970; Dickmann and Kozlowski 1969). As a result, defoliation reduces the carbon reserves more strongly than the nutrient reserves which should improve the quality of foliage in the next growing season. Increases in foliar N (Piene 1980;

Gazelius *et al.* 1981; Ericsson *et al.* 1985; Wagner and Evans 1985) and decreases in leaf carbohydrates (Webb and Karchesy 1977; Ericsson *et al.* 1985) were reported for various conifers in the season following defoliation. Moreover, pine sawfly, *Neodiprion sertifer* Geoffroy, larvae were shown to grow better on Scots pine, *Pinus sylvestris* L., foliage in the season following defoliation (Niemelä *et al.* 1984).

Predictions from the carbon/nutrient balance hypothesis are not always met with either deciduous or coniferous trees. Increases in phenolic compounds after defoliation were observed in ponderosa pine (Wagner and Evans 1985) and Scots pine (Thielges 1968). In addition, pine sawfly, *Neodiprion autumnalis* Smith, feeding on previously damaged foliage of ponderosa pine performed significantly poorer (Wagner 1986). Defoliated birch trees which received strong fertilization failed to reduce their resistance as assayed by autumnal moth caterpillars (Haukioja and Neuvonen 1985) indicating that the poor quality of foliage was not caused by a shortage of nutrients. These examples point to inadequacies in the carbon/nutrient balance theory for predictions of induced defences. Alternate strategies apparently exist but, as yet, no single theory has been developed to explain all situations.

Indigestible Fibres

The characteristics of fibres that make them protective to plants from insect feeding are as follows: 1) providing a physical toughness that makes mastication difficult; 2) combining with nutrients (proteins, carbohydrates and nucleic acids) which renders them unavailable for intestinal absorption; and 3) combining with midgut enzymes which are required for digestion by the insect.

The outcome of feeding on sources high in IF may be that an insect suffers from malnutrition due to the removal of nutrients for absorption. It would have to chew more food and produce more digestive enzymes to try to make up for nutrient losses thereby leading to increased metabolic costs. Moreover, increased feeding time could leave the insect more susceptible to mortality agents (Price *et al.* 1980). Increased mortality from predation as a direct result from compensatory feeding on food plants low in N was shown for the cabbage white butterfly, *Pieris rapae* (L.) (Loader and Damman 1991).

Plant cell walls are composed of a matrix of cellulose, hemicellulose and lignin. Cellulose, a polysaccharide, is indigestible to many organisms and few microorganisms and animals produce the enzyme cellulase that is required for cellulose breakdown (Curtis 1975). Hemicelluloses are a mixture of polysaccharides which form part of the cross-linked matrix that encloses the cellulose microfibrils (Raven *et al.* 1986). This fraction was found to be only partially digestible by some insects (Terra *et al.* 1987). Lignins are phenolic heteropolymers (Rhoades 1979) which play two roles in terrestrial plants. They impart rigidity and strength to cell walls (Lewis *et al.* 1989) and serve a defensive role against pathogens (Rhoades 1979; Lewis *et al.* 1989) and herbivores (Rhoades 1979). Unlike cellulose, lignins are highly indigestible, if at all, to herbivores and most microorganisms (Rhoades 1979).

The defensive properties of IF are provided by the individual and combined characteristics of the cell wall components. Owing to the strength of chemical bonds, plant cell walls are not readily broken down in either the biotic or abiotic environment (Duchesne and Larson 1989). Strong hydrogen bonds occur between parallel cellulose chains (Duchesne and Larson 1989), between polysaccharides at the microfibrillar-matrix interface and between components of the matrix (Northcote 1989). Long cellulose molecules bundle

together to form microfibrils which are enclosed in an impermeable matrix of lignin, hemicelluloses and other cell wall components bound by covalent bonds (Northcote 1989). The tensile strength of the cell walls is provided by the microfibrils and its rigid structure is provided by the encasing lignified matrix (Northcote 1989). The accretion of fibre in cell walls leads to increasing leaf toughness which makes chewing by insects difficult.

The defensive role of lignins against fungal attack is well established and they are known to function as both constitutive (Swain 1979) and induced defences to fungal pathogens (Vance *et al.* 1980). By contrast, the role of lignins in providing resistance against feeding by insects has received limited attention (Wainhouse *et al.* 1990). Lignins, because of their structure as phenolic heteropolymers (Rhoades 1979), combine with nucleic acids and cell wall proteins and carbohydrates (Walker 1975; Swain 1979). Insoluble bonds are formed with these compounds because of the existence of a large number of hydroxyl groups in the lignin molecules (Walker 1975; Swain 1979). The result of the formation of insoluble complexes is thought to be a reduced availability of proteins and carbohydrates for insect consumption (Rhoades 1979).

Lignins are also thought to combine with and denature a wide variety of enzymes in a manner similar to tannins (Swain 1979). The implication of these formations is further disruption of insect digestion due to the immobilization of gut enzymes which are required for the breakdown of food into simpler compounds (Berenbaum 1983). Studies by Mole and Waterman (1987) with tannic acid have challenged this idea of enzyme immobilization. They questioned whether the observed action on the proteolytic system by a tannin relates to the direct action of the tannin on the enzymes (proteases) or to the binding of that tannin to the substrate proteins or to both these potential effects.

They found that with an excess of protein substrate, tannin failed to combine with the enzyme trypsin and that the enzyme remained able to act as a protease without any loss of activity. It was concluded that the result of mixing tannins and proteins in the gut depended on their relative concentrations. Nonetheless, complexing with proteins in the gut still removed those nutrient for absorption. These findings may be equally relevant to lignins.

The defensive properties of phenolics have also been questioned by Mattson *et al.* (1983) who points out that previous studies on the effects of phenolics and tannins on growth performance have almost all drawn the same conclusion that there is little or no negative effect, except perhaps at extremely high tannin levels. Moreover, Coley (1983) determined that for mature tropical tree leaves, all measures of fibre (both neutral-detergent and acid-detergent) content were negatively correlated with herbivory, but lignin was the least so. On the other hand, as suggested by Mattson *et al.* (1983), the long-term effects may exist due to chelation of tannins and/or phenols with micro-nutrients which would render them less available for digestion. These effects would have to be monitored over two or more successive generations to determine impacts on performance (Mattson *et al.* 1983).

Regardless of the defensive mode of action, various levels of IF have been reported to affect negatively insect performance (Benz 1974; Baltensweiler *et al.* 1977; Bauce and Hardy 1988). IF was also identified as a method of resistance that requires further investigation (Coley 1983).

Indigestible Fibre Influence

Feeding trials with foliage holding high IF levels and low N levels have shown negative effects on lepidopteran larval growth and development. Reduced larval and pupal weights and survival rates were reported (Benz 1974; Baltensweiler *et al.* 1977) for larch budmoth, *Zeiraphera diniana*

(Guenée), when reared on needles of European larch with high IF and low N levels. Similar results were found for spruce budworm when reared on comparable balsam fir foliage (Bauce and Hardy 1988). Longer mean development times, reduced pupal weights and increased mortality rates were attributed directly to feeding on branches with increased IF levels (Bauce and Hardy 1988).

The results of these studies concerning the defensive role of IF are equivocal due to the inability to control this variable in the foliar diets. Foliage fed to the budmoth and budworm contains many other components which could not be held constant or eliminated in these feeding trials. As a result, it is questionable that the reduced insect performance was directly attributable to the changes in IF and N levels. The observed performance may have been caused by another unidentified component of the tissue.

To test for the effects of IF on insect performance, the cell wall components should be included and controlled in artificial diets. A number of feeding trials (McGinnis and Kasting 1967; Peterson *et al.* 1988; Slansky and Wheeler 1991) have been conducted which incorporated a cellulose component of IF into artificial diets. Generally, the results of these trials showed that powdered cellulose acted as a diluent to nutrients in the diet and appeared to cause decreased digestibility and increased consumption rates. Growth rates were not affected however and it was concluded that cellulose additions did not affect nutrient digestion or absorption. These results elicited cautionary statements from their authors (Peterson *et al.* 1988; Slansky and Wheeler 1991) concerning their applicability to natural systems. Slansky and Wheeler (1991) stated that while their results did not point to a reduction in the availability or digestibility of nutrients, they also did not rule it out. They warned that the cell wall is a very tightly bound matrix of interlocking structures that is

impossible to mimic with the addition of powdered cellulose. Peterson *et al.* (1988) explained that in plant tissue, cellulose and other cell wall components are "likely to increase tissue toughness which could interfere with consumption and decrease nutrient availability which could further reduce digestibility."

An investigation into the role of lignin as a defence against spruce bark beetle, *Dendroctonus micans* Kugelann, and its effect on larvae and adults (Wainhouse *et al.* 1990) showed a dose-dependent reduction in larval survival, growth rate, and weight when reared on lignified bark tissue.

Indigestible Fibre Variation

IF levels are known to vary within a single growing season, with leaf age and among species (Scriber and Slansky 1981) but few studies have quantified these differences. Prévost and Laing (1986) determined that IF levels of current-year foliage of 35-year-old, upland black spruce increased through the growing season from 22-52 percent. One- and two-year old foliage varied little and contained approximately 44 percent and 50 percent IF, respectively (Prévost and Laing 1986).

Bauce and Hardy (1988) reported that previously defoliated, 65-year-old balsam fir located on well-drained sites maintained a higher (3.9 percent) IF content during the entire growing season in contrast to non-defoliated trees growing on comparable sites. Similar increases were reported for European larch after defoliation (Benz 1974; Baltensweiler *et al.* 1977; Baltensweiler and Fischlin 1988).

Physical Attribute

Leaf Toughness

Leaf toughness is a physical attribute of food quality which has been shown to deter herbivores (Williams 1954; Tanton 1962; Feeny 1970; Rausher

1981; Coley 1983; Raupp 1985; Nichols-Orians and Schultz 1990; Pennings and Paul 1992) and, yet, has received inadequate attention (Coley 1983; Raupp 1985). The possession by plants of tough, nutrient-poor leaves (known as sclerophylly) (Loveless 1961, 1962) is thought to serve three functions: 1) protection from herbivory; 2) protection from drought stress; and 3) conservation of mineral nutrients by plants growing in N-poor soils (Rausher 1981 and references therein). Sclerophylly is generally thought to serve a primary function as a defence against herbivore attack (Rausher 1981). Coley (1983) reported that when compared to levels of herbivory, leaf toughness showed the highest negative correlation of several chemical and physical leaf characteristics .

Toughness increases in leaves over a growing season (Feeny 1970; Scriber and Slansky 1981) as cell walls develop structurally and accrue cellulose, hemicelluloses, lignins and other materials (Raven *et al.* 1986). Strongly associated with the seasonal development of leaf toughness are decreasing concentrations of foliar N and water (Hough and Pimental 1978; Scriber and Slansky 1981; Schultz *et al.* 1982; Coley 1983). Feeny (1970) suggested that the loss of foliar water may contribute to toughness through a reduction of leaf succulence.

Feeding trials with Lepidoptera larvae on young and mature, current-year leaves report reduced performance and fitness on the tougher, mature leaf diets. When feeding on mature leaves of their host trees, winter moth, *Operophtera brumata* L., experienced reduced weights (Feeny 1970), gypsy moth, *Lymantria dispar* L., suffered increased mortality, slower growth and reduced fecundity (Hough and Pimental 1978), while the pipevine swallowtail butterfly, *Battus philenor* (L.) also sustained reduced larval growth (Rausher 1981).

Toughness acts as a defence by reducing the suitability of leaves as a food source in three ways. The first is by providing a physical toughness to tissue through which it is difficult for some mandibulate insects to chew (Rausher 1981). Williams (1954) observed that young grasshoppers fed only on the tips of bamboo grass blades supposedly because of an inability to insert their "small jaws" into the thicker parts of the blades. This behaviour disappeared in later instars. Rausher (1981) questioned the ability of early-instar pipevine swallowtail butterfly to penetrate through mature host leaves because of their "weaker mandibular muscles." Tanton (1962) surmised the existence of a threshold leaf toughness which matched the "mandibulate power" of the majority of mustard beetle, *Phaedon cochlearidae* Fab. larvae.

Increasing tissue toughness forces mandibulate herbivores to chew through a firm cellular matrix before extracting nutrients. With age, angiosperm leaves additionally develop tough vascular tissue (Wood 1933, Raven *et al.* 1986) which forces insects to chew through a network of hardened leaf veins in order to consume more nutritious interveinal tissues (Raupp 1985).

A study with leaf cutter ants, *Atta cephalores* (L.) revealed interesting food preferences when ants were forced to choose between young leaf diets with high levels of allelochemicals, especially tannins, and mature diets with high levels of leaf toughness (Nichols-Orians and Schultz 1990). Results showed that ants harvested more from the the less suitable young leaves because of their inability to chew through the more suitable mature leaves. Chewing difficulties lead to reduced rates of ingestion (Rausher 1981) and consumption by various insect taxa (Feeny 1970; Williams 1954; Tanton 1962; Rausher 1981).

Secondly, toughness reduces nutrient availability to larvae. It is unclear whether decreases in N and other nutrient concentrations reflects a simple

dilution by the extra IF or represent an actual decrease in intracellular concentration as well (Rausher 1981). As discussed, N is regarded as a limiting factor to growth (Mattson 1980) and, as a central component of proteins, N is required in high levels during larval growth (Dadd 1985). As noted, feeding on mature leaves which are high in IF and low in nutrients, such as N and water, has resulted in reduced fitness characteristics and higher mortality for many insects. Larvae feeding on young leaves are able to grow faster because they convert a greater fraction of young leaf biomass to larval tissue than they do old leaf biomass (Rausher 1981).

The availability of nutrients such as proteins, nucleic acids, and carbohydrates may be further reduced due to the formation of insoluble complexes with lignin (Walker 1975, Swain 1979, Rhoades 1979). Insects may compensate for reduced nutrient content by increased feeding rates (Slansky and Feeny 1977; Koller 1987) but plants with high IF may minimize this potential by decreasing the rate at which larvae can ingest leaf material (Rausher 1981). Also, as mentioned, additional feeding may endanger larvae (Feeny 1976; Price *et al.* 1980).

The third mechanism by which leaf toughness can act as a defence against herbivores is by wearing down mandibles (Raupp 1985). Feeding trials with the leaf beetle, *Plagiodera versicolora* Laich., on young and mature *Salix* leaves indicated higher cutting surface erosion of the jaw when feeding on mature leaves which resulted in reduced consumption rates (Raupp 1985). Raupp (1985) also investigated the effect of changing consumption rate (due to changing diet toughness) on fecundity. His study revealed a strong linear relationship which prompted him to suggest that mandibular wear may have a "direct effect on reproductive success because of a strong relationship between consumption rates and fecundity in *P. versicolora*.."

Certain dietary levels of IF are probably as important to insects as they are to mammals (Burkitt *et al.* 1974; Heaton 1983). Researchers investigating grasshoppers reported that diets of fresh plant material containing high levels of water had to be supplemented with fibre in order to obtain adequate growth and reproduction (McKinlay 1981; Hagen *et al.* 1984) suggesting that sufficient bulk was required in the artificial diets (Mattson and Scriber 1987).

Leaf toughness is frequently estimated with a penetrometer which was described by Feeny (1970) after a design by Williams (1952) and modifications by Tanton (1962). This device operates by applying a measurable weight, such as sand, to drive a small punch through a leaf. The weight of the sand provides a relative measure of leaf toughness.

Although the penetrometer and its variations have been used in several investigations (Feeny 1970; Schultz *et al.* 1982; Raupp 1985; Nichols-Orians and Schultz 1990), other techniques are also employed to determine the toughness of leaves. Hough and Pimental (1978) used an Instrom Universal Testing Machine adapted to measure the texture of food materials (Bourne *et al.* 1966). Lucas and Pereira (1990) designed a new device which measured fracture toughness which they state is a "well defined... fundamental material property" (Atkins and Mai 1985). The advantage of this measure of toughness is that it is an absolute measurement which can be used to compare with other materials, biological and non-biological (Lucas and Pereira 1990). This would be an excellent device for comparative material studies but for non-comparative investigations relative measurements of toughness such as those obtained by the penetrometer could suffice.

EXPERIMENT 1

SEASONAL TRENDS IN FOLIAR MOISTURE, NITROGEN, AND INDIGESTIBLE FIBRE

PURPOSE

The purpose of this experiment was to analyze chemically foliar samples of balsam fir, white spruce, and black spruce for levels of moisture, N, and IF in order to determine the seasonal variation in these components and quantify the physiological bounds of IF:N in the natural budworm diet.

METHODS

Foliage Collection

Foliage was collected from balsam fir, white spruce, and black spruce trees located on an upland site in Marks Township near Kakabeka Falls, Ontario. Selected trees were 30-year-old white and black spruce growing in plantations and 25- to 45-year-old balsam fir growing wild on neighbouring private property. Measurements for height and diameter at breast height were taken by a Suunto Clinometer and calipers, respectively. Age was determined by counting growth rings in an increment core sample. Foliage was collected for determinations of levels of foliar water, N, and IF. Twelve trees were sampled in total, four from each of balsam fir, white spruce, and black spruce (Table 1). Selected trees appeared healthy with minimal insect feeding damage, had well foliated south-facing aspects and were located either on plantation edges or in stand openings.

For each species, sampling was initiated in 1990 when buds were swollen and continued at intervals until September. There were eight collections of both balsam fir and white spruce and six of black spruce.

The tree crown was divided into thirds and one approximately 45 cm branch tip was selected from the upper- and lower-crown positions per tree per collection day and one mid-crown branch was selected for two trees only per collection day. The branches were immediately cut, bagged in plastic, identified and placed in a dry ice chest for transport to the laboratory.

Table 1. Description of trees sampled near Kakabeka Falls, Ontario.

Species	Tree No.	Height (m)	Diameter (cm)	Age (years)
Black spruce	1	10.08	16.0	30
Black spruce	2	9.00	14.0	30
Black spruce	3	7.77	14.0	30
Black spruce	4	9.38	15.0	30
White spruce	1	10.60	17.5	30
White spruce	2	8.80	16.0	30
White spruce	3	10.17	21.5	30
White spruce	4	11.20	21.5	30
Balsam fir	1	14.80	20.0	44
Balsam fir	2	13.28	21.5	39
Balsam fir	3	15.48	21.0	42
Balsam fir	4	15.60	26.0	27

Foliar Water and N Determinations

Foliar moisture content of current-year foliage was determined at the laboratory by weighing fresh samples upon arrival from the field and then drying them at 100^{o}C for 18 hours, . Percent moisture content of foliage was calculated as: wet weight - dry weight /wet weight.

Dried foliar and stem samples were prepared for N analysis using a Wiley mill with a 40-mesh screen. The ground tissue was stored in nalgene vials in a desiccator at -20°C. Subsamples were sent to the Lakehead University Instrumentation Laboratory to be analyzed for N with a Control Equipment Corporation 240-AX Elemental Analyzer.

Foliar samples for each collection date were analyzed individually for moisture, N, and IF levels but data were pooled before analysis.

Foliar Indigestible Fibre Determinations

I determined IF levels according to the method of Goering and Van Soest (1970). A neutral detergent solution (Appendix I) was used to chemically digest all foliar constituents except the cell wall which comprises cellulose, hemicellulose and lignin. This was called the neutral detergent fibre. Summarized, the method involved weighing an oven-dried foliar sample which was then chemically digested, filtered, oven-dried and reweighed. The difference in weight before and after digestion was the IF content.

Apparatus for the digestion comprised a LABCONCO Micro-Kjeldahl Digestor (Model No. 60300) connected by rubber vacuum tubing to a water aspirator and operated through a voltage regulator (Figure 1 and Appendix II). The vacuum filtering system consisted of a filtering unit, a three-arm distilling flask, a cold trap and a vacuum pump; all parts were connected by nalgene vacuum tubing (Figure 2).

Subsamples of the foliage for N determinations were used for IF determinations. The tissue was oven-dried overnight at 100°C and 0.10 g to 0.15 g was added to a 100 mL Kjeldahl flask along with 0.125 g sodium sulfite, 1.0 mL decahydronaphthalene and 25 mL neutral-detergent solution. The flasks on the heating mantle were brought to a boil in one to two minutes. The heat was then reduced to avoid foaming and adjusted (with the voltage

Figure 1. Micro-Kjeldahl Digestor used for processing micro-samples of nitrogen-containing material.

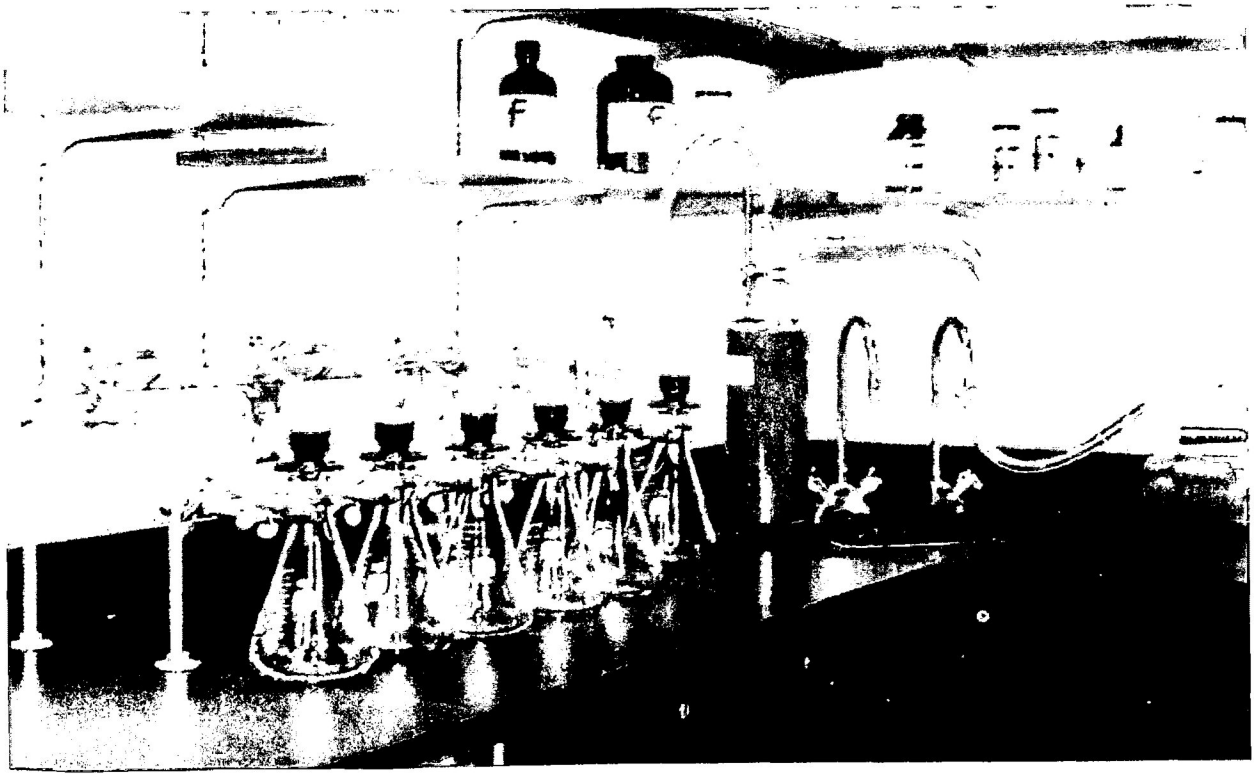


Figure 2. Vacuum filtering unit with six filter stations.

regulator) to achieve an even boil. The solution was refluxed for one hour, timed from the onset of boiling (Goering and Van Soest 1970) (Appendix III).

After digestion, the flask contents were filtered through Gooch crucibles. Although Goering and Van Soest (1970) suggested using hot (90°-100°C) water for rinsing the crucibles, distilled water at tap temperature was more effective because of reduced foaming. After filtering was complete, the crucibles were oven-dried overnight at 100°C and the dry-weight determined. Percent IF was calculated as: predigested weight - post digested weight/ predigested weight. Gooch crucibles were acid-cleaned, oven-dried, and weighed before each use.

RESULTS

Foliar Moisture

Foliar moisture followed a general trend of high levels at growth initiation, increased soon after to seasonal peaks and then decreased to late-August; except for black spruce in which moisture levels declined to late-July and inclined by late-August (Figure 3 and Appendix IV). Through the early growing season, foliar moisture was greatest for white spruce, followed by black spruce and balsam fir. Little difference in percent moisture was observed between crown positions for all species (Figure 4).

Foliar water levels of balsam fir (Figure 4a) were initially about 72 percent on June 1 (JD 152) and varied little (upper-crown) or increased slightly until June 21 (JD 172) at which time lower- and mid-crown positions had highs of 74 percent and 73 percent respectively. A gradual decrease then occurred

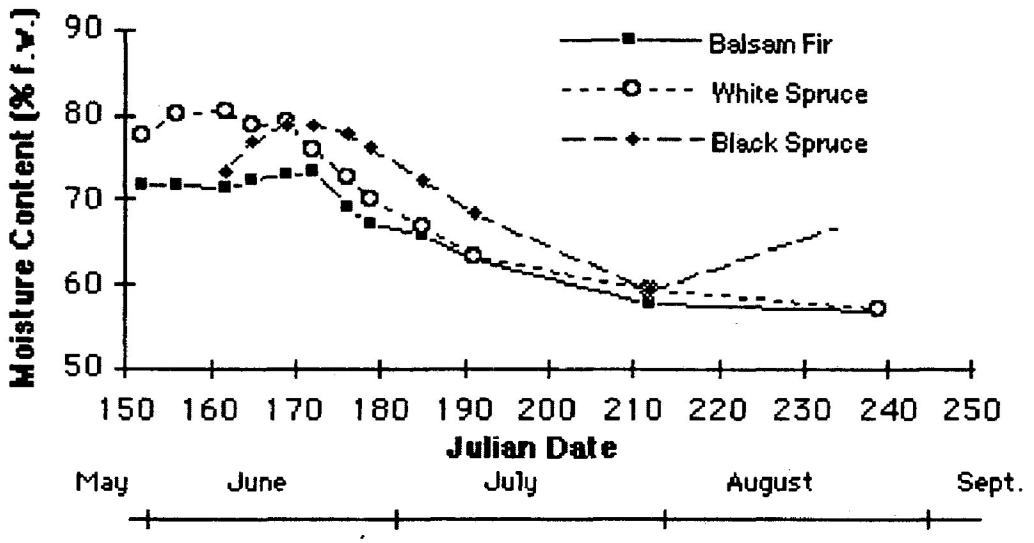


Figure 3. Mean seasonal values of moisture (% fresh weight) in current-year foliage of balsam fir, white spruce, and black spruce.

through July for all crown positions. Percent water reached lows of about 57 percent in August. For white spruce (Figure 4b), water levels for all crown positions started at about 77 percent on June 1 (JD 152), increased to about 80 percent by June 5 (JD 156) and plateaued until June 18 (JD 169). Levels decreased rapidly through July and then more slowly to seasonal lows of about 57 percent by August 27 (JD 239). The moisture content of black spruce needles in all crown positions (Figure 4c) was about 73 percent on June 11 (JD162), increased to highs of about 79 percent by June 21 (JD 172) and gradually decreased to lows of about 59 percent by July 31 (JD 212). Then, unlike balsam fir and white spruce, levels increased to about 68 percent by August 27 (JD 239).

The change in percent foliar moisture from early- to late-season was consistent among crown levels within species. Percent moisture of the swollen buds was about 1.3 times higher than for hardened foliage on August 27 (JD 239) for all crown levels of balsam fir; for white spruce, the change was about 1.4, and for black spruce, about 1.1.

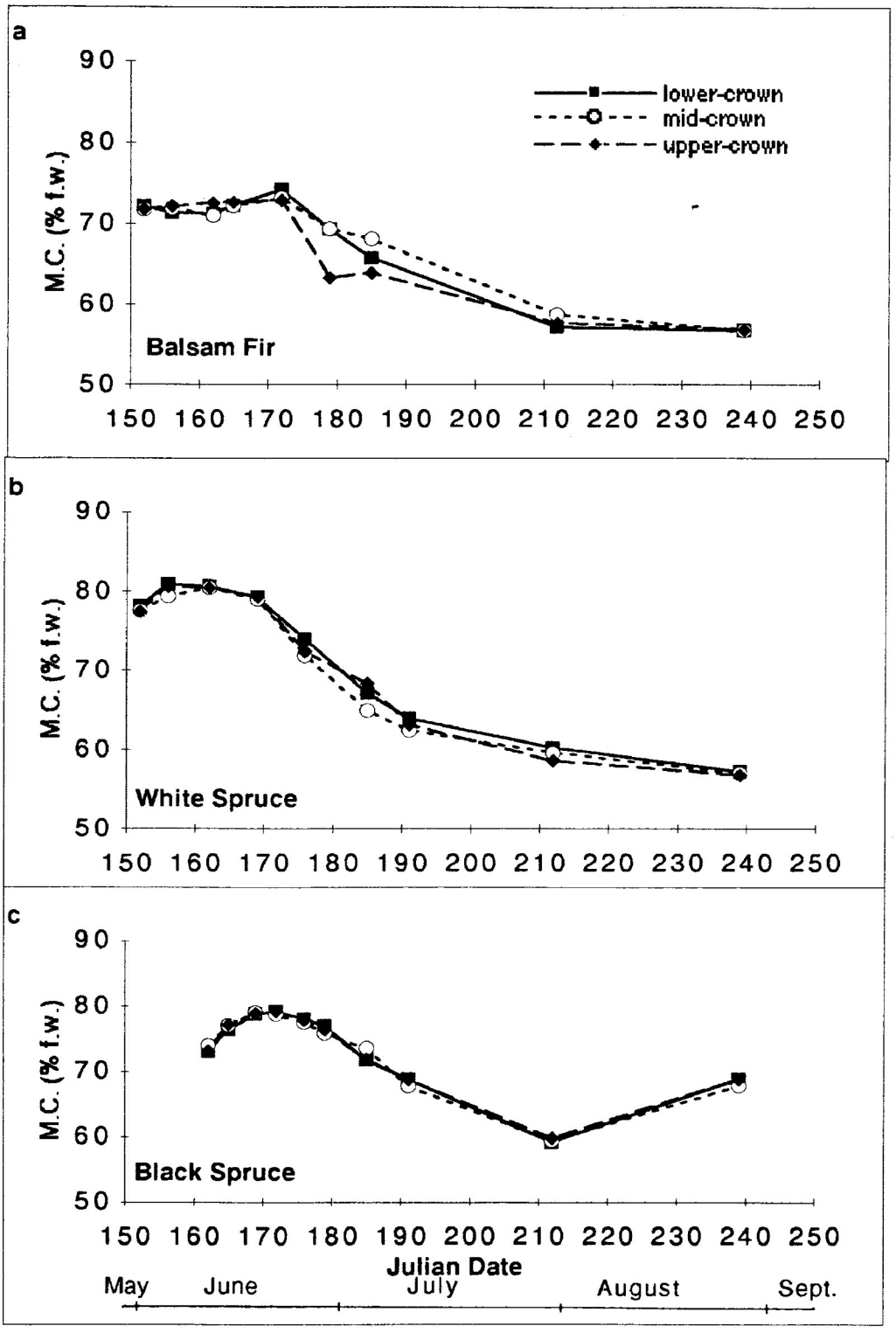


Figure 4. Mean (n=7) foliar moisture content (M.C.) (% fresh weight) from June to August, 1990 for a) balsam fir, b) white spruce, and c) black spruce foliage sampled near Kakabeka Falls, Ontario. First samples for each species were collected when vegetative buds were swollen.

Foliar Nitrogen Content

Foliar N levels decreased rapidly in the early-season and then gradually for the remainder of the growing season for balsam fir, white spruce, and black spruce. (Figure 5 and Appendix V). Little difference was observed between species, although balsam fir maintained the highest level and white spruce the lowest level from mid-June to late-August. There was little difference in levels of N among crown positions (Figure 6).

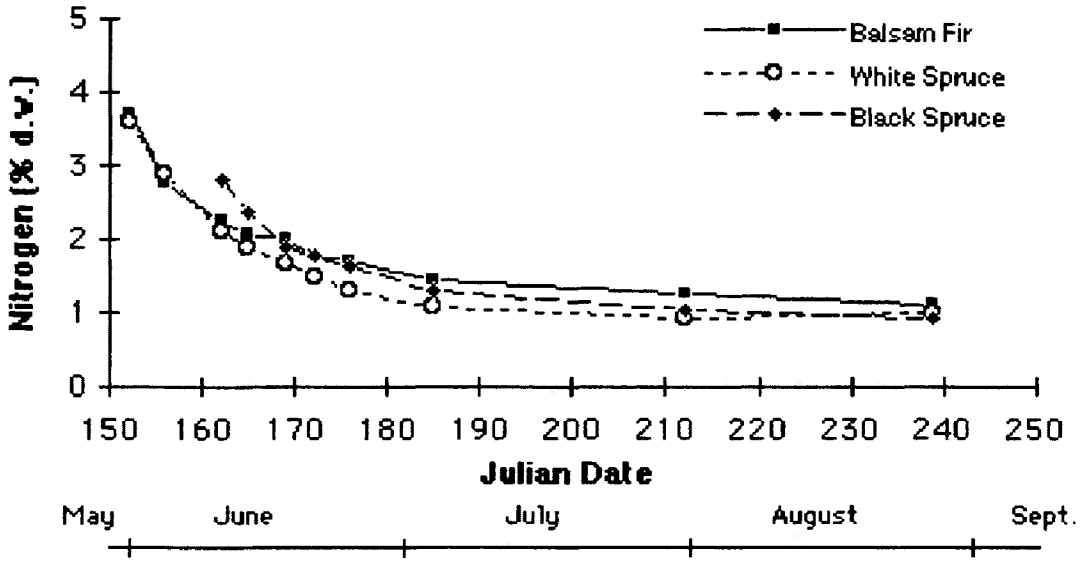


Figure 5. Mean seasonal values of nitrogen (% dry weight) in current-year foliage of balsam fir, white spruce, and black spruce.

Foliar N levels in balsam fir were between 3.6 percent and 3.9 percent on June 1 (JD 152) (Figure 6a). Rapid and then more gradual declines occurred until July 4 (JD 185) after which N concentrations slowly descended to lows of between 1.0 and 1.3 percent. Similarly for white spruce (Figure 6b), on June 1 (JD 152) N levels were between 3.5 and 3.9 percent and dropped to lows of about 1 percent by August 27 (JD 239). Slightly more variability existed among crown levels in black spruce (Figure 6c) which had initial highs of between 2.6

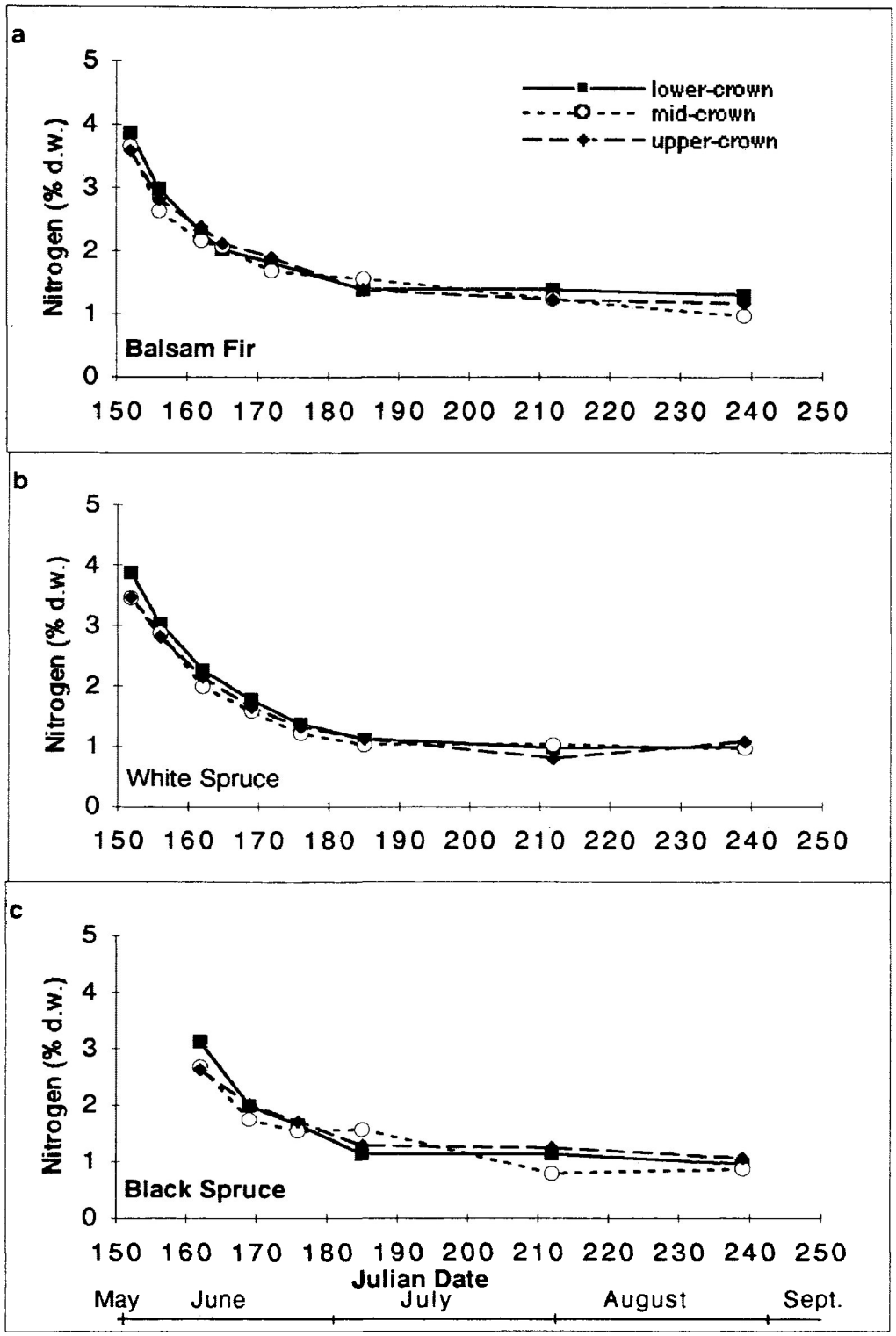


Figure 6. Mean foliar nitrogen content (% dry weight) from June to August,1990 for a) balsam fir, b) white spruce, and c) black spruce sampled near Kakabeka Falls, Ontario. First samples for each species were collected when vegetative buds were swollen. Mean values for lower- (n=4), mid- (n=2), and upper-crown (n=4) positions.

and 3.1 percent N on June 11 (JD 162) and lows of between 0.9 and 1.1 percent on August 27 (JD 239).

For all species and crown levels, with one exception, N concentrations were three to four times higher in the buds than in hardened foliage of late-August. The exception, upper-crown of black spruce, had N levels 2.5 times higher in the spring than in late-summer.

Foliar Indigestible Fibre

Foliar IF exhibited the general trend of low levels in swollen buds, a rapid increase to late-June or early-July which was followed by either a tapering off or slight increase to late-August (Figure 7 and Appendix VI). White spruce IF, although initially lower than in balsam fir, increased at a greater rate and stabilized at a higher level. In comparison, black spruce values increased slowly at first and exhibited the lowest concentrations of foliar IF during the first 15-20 days of growth but increased to a value similar to that of white spruce. There were few differences in levels of foliar IF among crown positions (Figure 8).

On June 1 (JD 152) IF levels in balsam fir (Figure 8a) began at about 30 percent, increased steadily to roughly 42 percent by June 21 (JD 172), gradually reached highs of about 45 percent by July 21 (JD 212) and decreased to close to 39 percent by August 27 (JD 239). Fibre values for white spruce (Figure 8b), started out at about 26 percent on June 1 (JD 152) and increased quickly to roughly 49 percent on June 25 (JD 176). A further but more gradual increase led to seasonal highs of close to 52 percent on July 4 (JD 185) followed by a tapering off to about 49 percent on August 27 (JD 239). Values for black spruce started near 27 percent on June 11 (JD 162) and increased gradually to June 25 (JD 176) after which they jumped to about 42

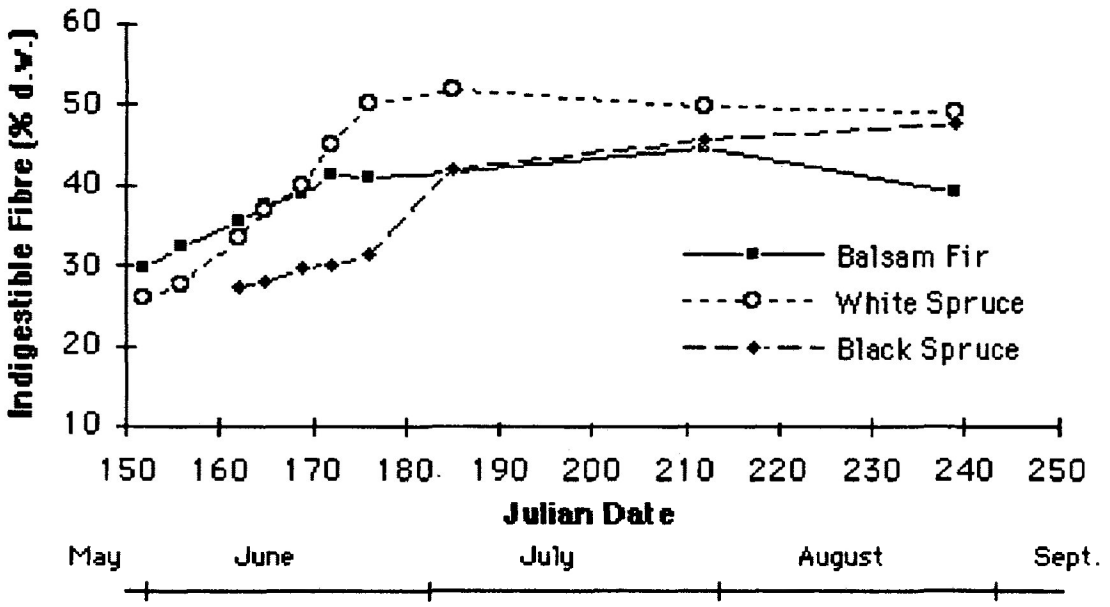


Figure 7. Mean seasonal values of indigestible fibre (% dry weight) in current-year foliage of balsam fir, white spruce, and black spruce.

percent on July 4 (JD 185) (Figure 8c). Percent IF continued to increase gradually to near 48 percent on August 27 (JD 239).

Comparing species for differences between the lowest and highest levels of IF, showed that white spruce had the greatest increase. The highest average level of fibre in white spruce was about 2.0 times greater than the lowest value. This difference for black spruce was 1.7 times and for balsam fir was 1.5 times.

IF:N

IF:N increased through the growing season for all three species, although patterns varied, as foliar N decreased and IF increased (Figure 9a). White spruce increased quickly to 50:1 during June and then levelled off during July and August. Black spruce increased steadily during June, July and August, finally reaching the same 50:1 level as white spruce. Balsam fir increased slowly during June, July and August and attained a final IF:N of 35:1.

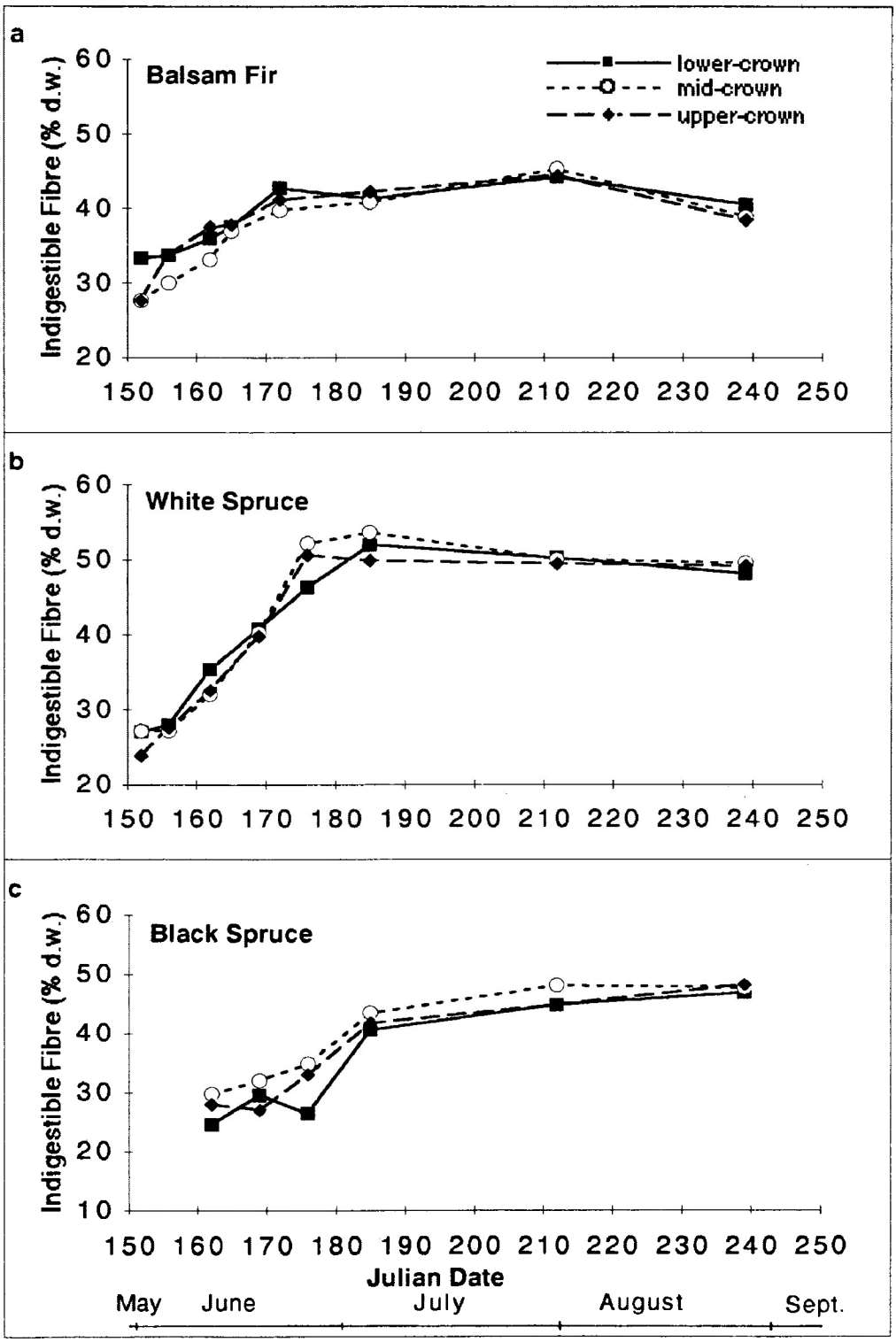


Figure 8. Mean foliar indigestible fibre content (% dry weight) from June to August, 1990 a) for balsam fir, b) white spruce, and c) black spruce sampled near Kakabeka Falls, Ontario. First samples for each species were collected when vegetative buds were swollen. Mean values for lower- (n=4), mid- (n=2) and upper-crown (n=4) positions.

Water :N

Water:N increased rapidly for all three species until early July and then changed little through the growing season, expect for black spruce for which a rapid increase in water:N occurred in August (Figure 9b). White spruce maintained the highest water:N ratio through most of the season, with a peak near 64:1, while balsam fir sustained the lowest, with a peak near 45:1. Black spruce was in between and followed a pattern of change similar to white spruce. These differences reflect the individual rates of change for foliar N and water.

DISCUSSION

Food quality variability within a crown and among crowns of trees of the same species may be a resistance mechanism which offers protection against herbivorous attack (Whitham 1983). A spatial and temporal mosaic of food quality within a tree canopy theoretically confers protection against insect feeding by: 1) increasing the risk of predation due to searching by insects for high quality food and 2) reducing insect fitness due to increased metabolic costs from searching movement and from the physiological "costs" associated with handling poor-quality food (Schultz 1983). Both factors should contribute to a reduction in insect populations and thereby restrict primary consumption (Schultz 1983).

My study is unique in that I investigated three components of food quality, namely, water, N, and IF from the foliage of the same trees, from three crown positions at frequent intervals through the growing season. To my knowledge, this is the first study of its kind. My results showed that food quality, as assessed

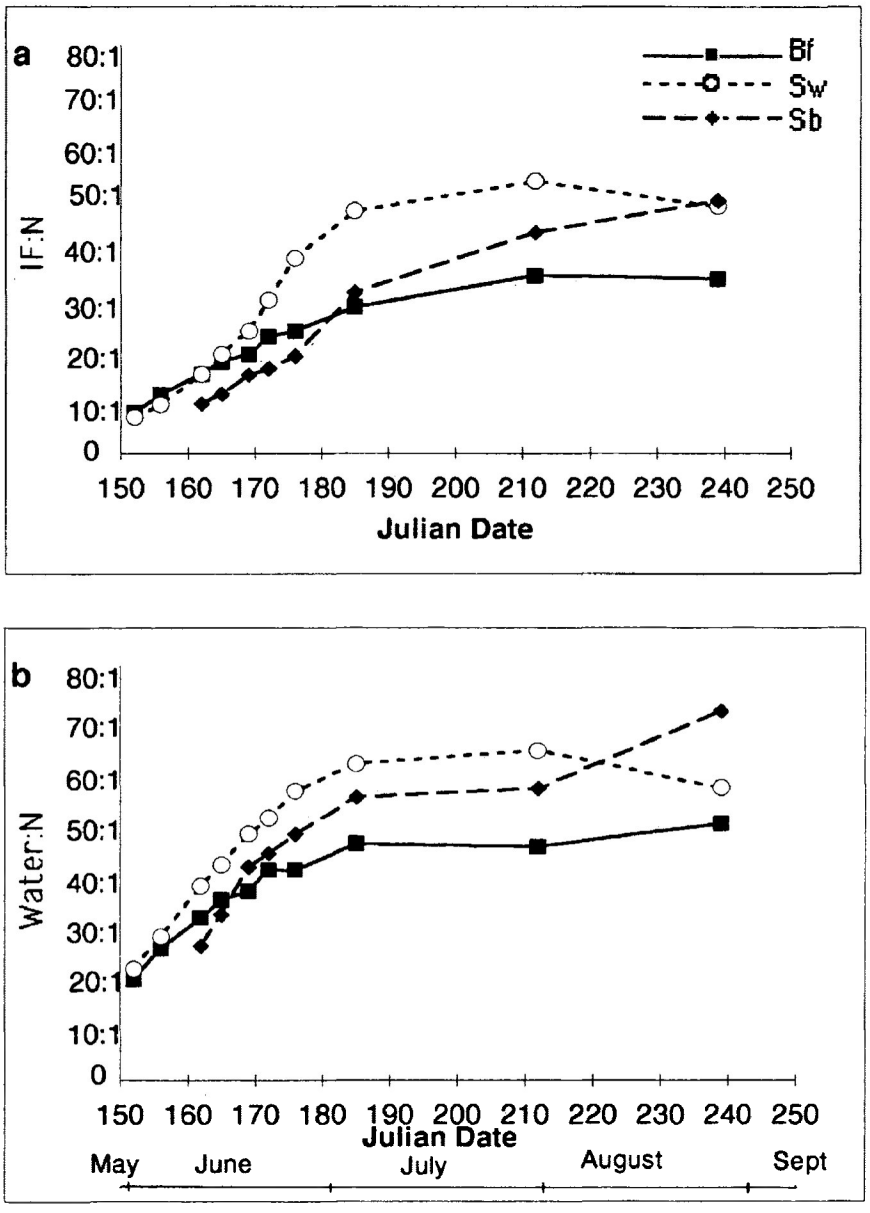


Figure 9. Change in the a)indigestible fibre (IF) to nitrogen (N) ratio and b) water to N ratio as foliage matures through the 1990 growing season for balsam fir (Bf), white spruce (Sw), and black spruce (Sb).

by water, N and IF of current-year needles, varied little within the crowns of balsam fir, white spruce, and black spruce. This spatial homogeneity of the preferred food source of spruce budworm is advantageous to the insect but not to the host tree. High quality, current-year needles are predictable to budworm and therefore highly susceptible to defoliation. Defoliation negatively affects tree growth and reduces wood production (MacLean 1985). However, as tissues mature food quality declines thus presenting a temporal heterogeneity in the food source. This could protect the host trees by presenting a relatively short, approximately six-week time period during which foliage is acceptable for insect consumption. By about late-June or early-July, spruce budworm pupate (Royama 1984) and thereafter host trees are free from attack by this insect for the rest of the growing season.

Variability of food quality may act as a defence during endemic population periods when host trees sustain defoliation primarily to current-year foliage, but older foliage is left intact to carry on with the tree's metabolic processes. During epidemic population periods, however, this defence is broken down because both current-year and older foliage is sometimes consumed (Mattson *et al.* 1988). Tree mortality usually starts to occur with balsam fir after five years of severe defoliation and with white spruce, after seven or eight years (MacLean 1985).

In spring, when trees break dormancy, physiological activity is high in all meristematic regions including shoot tips. On the stem and branch tips, new cells form (Zimmerman and Brown 1974) and cells which formed the year before and overwintered in terminal and lateral buds begin to elongate (Wilson 1970). Actively growing regions such as stem tips are characterized by high levels of water and N because of the integral, physiological roles that these components play in the growth process (Kramer and Kozlowski 1960; Wilson

1970). For the trees in this study, water formed between 70 to 80 percent of the fresh weight of bud tissue and N formed 2.5 to 4.0 percent of the dry weight of bud tissue.

As tissues mature through the growing season, the apparent demand for water and N declines (Kramer and Kozlowski 1960) as may be suggested here by declining levels of water and N in balsam fir, white spruce, and black spruce foliage. The general pattern of declining relative levels of foliar water and N during the growing season for current-year leaves is well established and has been reported by numerous investigators. For example, Kozlowski and Clausen (1965) reported this pattern for foliar water (percent d.w.) for 25- to 30-year-old conifers and deciduous species; Pharis (1967), for several species of two-year old conifer seedlings; Hough and Pimental (1978), Schultz *et al.* (1982) and Hunter and Lechowicz (1992), for several hardwood trees; and Prévost and Laing (1986), for 35-year-old black spruce. For foliar N (percent d.w.), a declining seasonal pattern was described, for example, by Feeny (1970) for oak; Shaw and Little (1972) for balsam fir; and Hough and Pimental (1978), Schultz *et al.* (1982) and Hunter and Lechowicz (1992), for several hardwood trees.

Coinciding with declining water and N levels were increasing IF levels in the foliage of balsam fir, white spruce, and black spruce. Levels of IF increase in plants as cell walls thicken through the accretion of cellulose, hemicellulose, and lignin. Cell wall components form from materials within the cell protoplasm (Northcote 1989) and serve a fundamental, primary function of providing strength to plant structure (Raven *et al.* 1986). The pattern of increasing IF levels in current-year foliage through a growing season as observed in this study has been described by Prévost and Laing (1986) for black spruce, Baltensweiler and Fischlin (1988) for *Larix* sp., and Bauce and Hardy (1988)

for balsam fir. Kozlowski and Clausen (1965) reported, for several hardwood and softwood species, a seasonal pattern of increasing foliar dry-weight which the authors attributed primarily to progressive cell wall thickening.

The high quality of early season foliage has been suggested as a reason that spruce budworm is an early season defoliator (Slansky and Scriber 1985). It may also be a reason that spruce budworm and the other species in the genus *Choristoneura* overwinter in the second instar. At the beginning of the growing season, food quality is optimally suited for larval consumption being high in water and N and low in IF. Sanders (in litt., 15 Dec 1994) stated that "[emerging] in the spring in the second instar gives *Choristoneura* species a flying start which may be viewed as an adaptation for exploiting an ephemeral food supply."

In the early growing season, the relative growth rate (RGR) of spruce budworm would be predicted as high because of the high foliar water and N levels of foliage (Scriber and Slansky 1981). RGR is defined as biomass accumulated per unit body weight per day and is usually expressed as mg/mg/day (Scriber and Slansky 1981). Scriber and Slansky (1981) looked at the particular influence of food quality on larval RGR by using foliar water and N as indices of food quality. They reviewed data on performance values from 265 experiments which had similarly aged larvae of 25 species of Lepidoptera and four species of Hymenoptera (sawflies) raised under similar photoperiod, temperature, and humidity regimes. Optimum performance values were plotted and the product of their efforts was isobars of varying RGR (Figure 10).

Using Figure 10, I plotted foliar N and water levels determined in this experiment to predict the RGR of spruce budworm when feeding on foliage of its host species. Feeding on early season, white spruce foliage, spruce budworm is predicted to show a superior RGR of 0.8 mg/mg/day. This compares to larvae

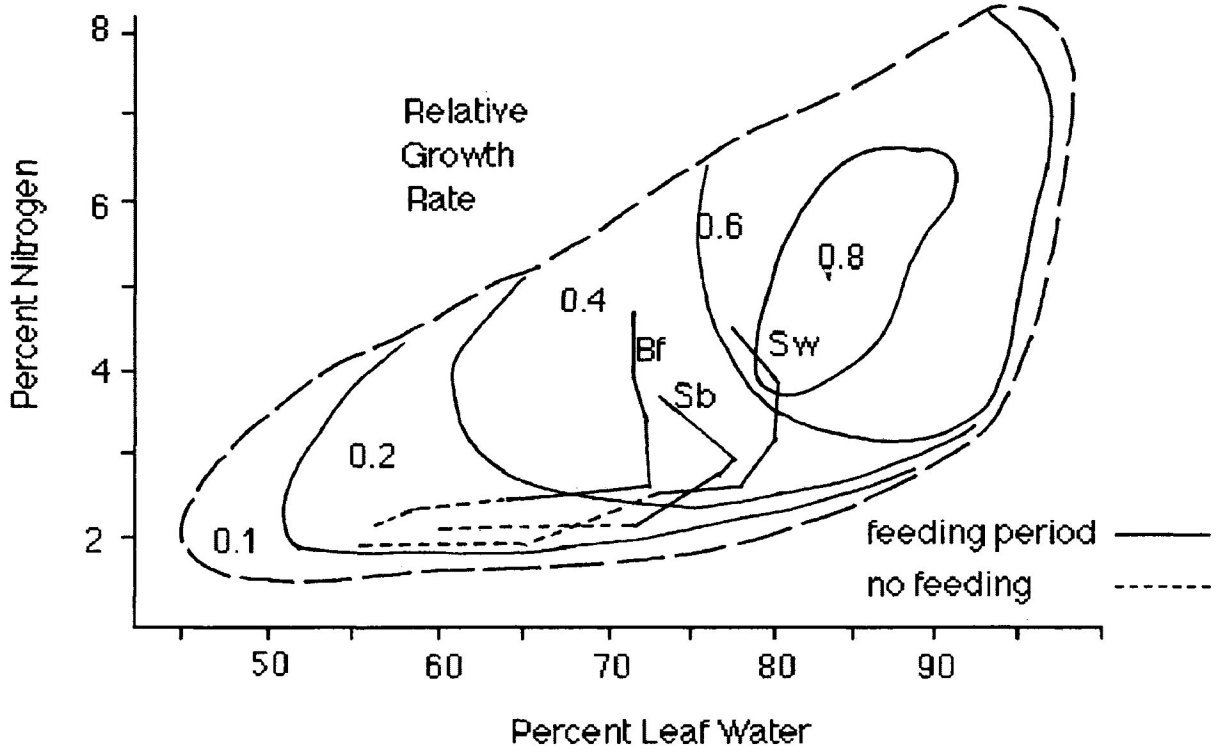


Figure 10. Predicted relative growth rate (mg/mg/day) of spruce budworm larvae feeding on foliage of balsam fir (Bf), white spruce (Sw) and black spruce (Sb) with seasonal foliar N and water levels as determined in this study (adapted from Scriber and Slansky 1981).

feeding on balsam fir and black spruce for which the RGR would be 0.4 mg/mg/day. The differences in RGR's are attributable to higher water levels in white spruce foliage compared to balsam fir and to both higher water and N than in black spruce.

As food quality (as indexed by foliar water and N) declines, RGR during the period of spruce budworm feeding is predicted to decline for white spruce; however, on balsam fir and black spruce RGR remains approximately the same. This is interesting considering that, of the three species, white spruce foliage experiences the most rapid increase in and highest mid-June to end-of-season

levels of IF. Slansky and Scriber (1985) offer the suggestion that the differences in food quality (as indexed by foliar water and N) may be due, in part, to changes within a food source such as occurs with the seasonal aging of leaves. Certainly other chemical constituents besides N and water affect food quality for insects. Perhaps IF, which constitutes such a large component of foliar composition, plays an important role in insect performance.

Clancy (1991a) has challenged the predictive power of foliar water and N arguing that these components are known to be strongly correlated with other tissue characteristics (Mattson and Scriber 1987). It may be that the budworm are responding to changing IF levels or to a combination of these components. Perhaps the changing ratios of IF to N should be considered as an index for food quality instead of N and water.

Seasonal values for foliar water varied among tree species; white spruce had slightly higher levels than black spruce throughout the growing season while balsam fir had the lowest. Koller and Leonard (1981) also found that, in understory trees, balsam fir contained lower levels of foliar water than white spruce. Seasonal highs of about 80 percent and lows of 56 percent for white spruce in this study were similar to values of 84 percent and 59 percent, respectively, reported by Thomas (1987). For black spruce, water levels were similar, although slightly higher, than those reported by Prévost and Laing (1986) who observed seasonal highs and lows of about 76 percent and 52 percent, respectively, while values for this study were 79 percent and 59 percent.

Foliar N varied among the three species with balsam fir and white spruce having similar late-May and early-June values; however, by mid-June and through to late-August, levels for white spruce had declined and were the lowest of the three species. These results agree with findings that showed that

white spruce had lower percent N than balsam fir (Mattson *et al.* 1983) and black spruce (Thomas 1989) by mid-June. Early season values for foliar N in balsam fir were about 3.7 percent which was mid-range of published values of 2.5 percent (Shaw and Little 1972), 4.4 percent (Shaw *et al.* 1978) and 5.5 percent (Mattson *et al.* 1983). Late season values of about 1.2 percent were comparable to published values of 0.88 percent (Shaw and Little 1972), 0.9 percent (Shaw *et al.* 1978), and 1.5 percent (Mattson *et al.* 1983) for balsam fir. For white spruce, the mean early season N value of 3.6 percent was similar to 3.2 percent reported by Thomas (1987) and lower than values of 5.5-8.0 percent reported by Mattson *et al.* (1983); late season values were similarly around 1.0 percent. For black spruce, early and late season values of 2.8 percent and 0.7 percent N, respectively, were slightly lower than values of 3.6 percent and 1.2 percent N reported by Prévost and Laing (1986). Differences in foliar N levels found in my study compared to other studies may be due to differences in site quality since levels of foliar N in conifers is positively affected by site quality (Watt and Heinselman 1965; Alban and Watt 1981; Timmer and Morrow 1984; Morrison and Foster 1990).

Rates and levels of IF accumulation in current-year needles varied by species. For balsam fir, the steady but gradual increase in percent IF from bud burst to about mid-July was similar to that reported by Bauce and Hardy (1988). Values of IF were higher in the trees in this investigation, however, with levels in the swollen buds of about 30 percent, seasonal highs of about 45 percent and end of season values of about 40 percent. Bauce and Hardy (1988) reported IF levels at bud burst of about 10 percent, seasonal highs of about 30 percent and end of season values of about 30 percent. Observed differences may be due in part to different genetic makeups or to different responses to environmental factors (Northcote 1989). It could be that the trees in my investigation had

higher IF levels as a response to prior attack by spruce budworm. These trees were on private property and had never been protected by aerial spraying whereas the trees in the study by Bauce and Hardy (1988) had been protected from defoliation for the previous 10 years.

For black spruce, the pattern of IF accumulation was similar to that described by Prévost and Laing (1986); in both investigations there was a slow but steady increase until late-June after which a rapid increase or "jump" occurred, followed by gradual increases to late-August. The range of seasonal values of 22 to 52 percent reported Prévost and Laing (1986) was similar to the range of 27 to 47 percent determined in this study.

The differences in interspecies susceptibility to spruce budworm defoliation probably has to do more with tree phenology and growth rates than it does with food quality. Black spruce is the least susceptible to defoliation and mortality because the life cycle of spruce budworm is not as closely synchronized with black spruce phenology as it is with white spruce and balsam fir phenology. Early-instar larvae commonly disperse or die while waiting for buds to flush (Morris 1963). White spruce suffers less defoliation than balsam fir primarily because of the faster growth and heavier shoots of white spruce (Lavallée and Hardy 1988; Régnière and You 1991). Although individual spruce budworm larvae consume almost identical amounts of white spruce and balsam fir foliage (Koller and Leonard 1981; Régnière and You 1991), balsam fir shoots elongate more slowly than those of white spruce (Greenbank 1963) which allows defoliation to outpace shoot expansion (Koller and Leonard 1981).

Intratree variation of water, N, and IF contents of current-year needles was minimal for the three tree species, as evidenced by the similarity of mean values among lower-, mid-, and upper-crown positions. Although these foliar

components do vary within a plant depending on season, tissue type (e.g. flowers, fruits, seeds, leaves, roots and cambium) and age (Kramer and Kozlowski 1960; Scriber and Slansky 1981; Mattson 1980), it appears that they vary little in current-year needles among crown positions with similar aspects. This finding suggests that it is possible to sample from any crown position with the same aspect to determine food quality of current-year needles as assessed by N:water, following recommendations of Scriber and Slansky (1981).

In contrast to these findings, Feeny (1970) determined that both crown position and aspect influenced levels of foliar moisture in pedunculate oak; upper crown, south-facing leaves had lower foliar moisture than lower-crown, north-facing leaves. It is unclear, though, if the effect was due to aspect or crown position, or both, because leaves of upper and lower crown positions with the same aspect were not both analyzed.

Food quality of current-year foliage does not appear to be a factor in determining budworm distribution within the tree crown. If it were, then one would expect a relatively equal distribution throughout the crown. Instead, Régnière *et al.* (1989) determined that the upper crown of balsam fir and white spruce trees contained the highest concentration of active second-instar to sixth-instar larvae, pupae and eggs. Overwintering, second-instar larvae have been found to be concentrated at mid-crown (Miller 1958; Moody and Otvos 1980; Régnière *et al.* 1989).

As an explanation of this distribution, insect behavioural and food availability factors have been suggested. After egg-hatch, first-instar larvae tend to disperse within crowns of host trees toward the inside of the crowns to escape the relatively high temperatures typical of the period of egg hatch and in search of hibernation sites (Régnière and Fletcher 1983; Régnière *et al.* 1989). After spring emergence, the active second instars gradually shift to the upper

crown and spend the remainder of their feeding stages highly concentrated in that location. Régnière *et al.* (1989) reported that this shift in vertical distribution of larvae from overwintering to feeding stages reflected the greater distribution of food resources as expressed by bud density. The number of buds per kilogram of foliage was determined to be greatest in upper crown positions of balsam fir and white spruce (Régnière *et al.* 1989). Eggs were concentrated in the upper crown, probably as a result of oviposition preference which appears to depend on physical and chemical stimuli (Wilson and Bean 1963; Städler 1974) and not food quality for emerging larvae (Renwick and Radke 1982).

Although food quality of current-year needles varies little among crown positions, few studies have actually investigated the effect of crown position on Lepidoptera performance. Watt (1992) discovered that crown height of lodgepole pine impacted upon the performance of pine beauty moth (*Panolis flammea* Den. & Schiff.). Growth and survival were reduced and rate of development was extended for larvae feeding on lower crown branches in comparison to those feeding at the top of the crown. This performance reduction may have been due to a cooler habitat in the lower crown or perhaps to some other factor not related to food quality.

Among tree species, food quality variability is a factor which makes a species more or less acceptable as a host. It is known that, of the three host species, balsam fir is the most vulnerable to mortality due to severe defoliation, followed by white spruce and then black spruce which is the least vulnerable (MacLean 1980). My investigations, however, with food quality (as assessed by water, N, and IF) showed that although differences in food quality did exist among species, these difference were not great.

EXPERIMENT 2

EARLY- AND LATE-SEASON FOLIAGE QUALITY EFFECTS ON SPRUCE BUDWORM PERFORMANCE

PURPOSE

The purpose of this experiment was to measure leaf toughness on early- and late-season foliage of balsam fir, white spruce, and black spruce to determine the seasonal variation of this component of food quality. In addition, spruce budworm were reared on early- and late-season foliage of these three host species to confirm that performance is superior on foliage with low N:IF (early-season foliage) than on foliage with high IF:N (late-season foliage).

METHODS

Foliage Collection

Foliage was collected from 40-60 year-old balsam fir, white spruce, and black spruce trees located on an upland site in Marks Township, near Kakabeka Falls, Ontario. Selected trees were open-grown and had minimal feeding damage. Current-year, mid-crown shoots were collected for rearing spruce budworm and for penetrometer and foliar analyses.

Early-season foliage was collected in June, 1990, for the penetrometer/foliar analyses and in June, 1992 for the budworm rearing trials. In both years, foliage collections occurred when the growing shoots were

elongating. This stage of phenological development corresponds to stage 5 of Turgeon's (1986) developmental index for white spruce. In 1990, balsam fir was collected on June 14 (JD 165), white spruce on June 18 (JD 169), and black spruce on June 28 (JD 179). In 1992, balsam fir was collected on June 10 (JD 161), white spruce on June 16 (JD 167), and black spruce on June 25 (JD 176).

Late-season foliage for both the penetrometer/foliar analyses and rearing trials was collected on September 3 (JD 246), 1990, after the needles had hardened. However, owing to experimental error, it was necessary to recollect white spruce on February 5 (JD 36), 1992. Needles were stored at -20°C in polyethylene bags until required for use at which time they were removed from the freezer and allowed to thaw for about two hours.

Foliar Characteristics

Leaf Toughness

A penetrometer (Appendix VII) was used to measure relative toughness of early- and late-season foliage of balsam fir, white spruce and black spruce. Current-year needles from different trees within species were randomly selected for measurement. Needles were positioned on the penetrometer so that the middle of the needle was penetrated. The penetrometer unit was assembled and fine-grained sand was poured into a plastic container resting on the punch pad. When the punch pad lowered, indicating that the needle had been penetrated by the punch, the pouring of sand was discontinued. The sand was weighed and the weight provided a relative measure of leaf toughness for each species and season of leaf collection.

Foliar Indigestible Fibre, Nitrogen and Water

Levels of IF were determined on the collected material according to the method of Goering and Van Soest (1970). Foliar N and water levels were not determined directly for the collected material. Instead, for approximately the same degree-days of foliage collection, foliar N and water levels were assumed to be similar to those determined for the foliage of experiment 1.

Insect Rearing

Rearing trials were conducted with spruce budworm on early- and late-season foliage of white spruce, black spruce and balsam fir. Second-instar spruce budworm larvae were obtained from the Forest Pest Management Institute, Sault Ste. Marie, Ontario. Foliage was placed in four litre rearing containers - one container per tree species per season of foliage collection.

Early-season foliage was collected and stored in polyethylene bags at 4°C until required for use. Rearing experiments were initiated within two to six days of the early-season branch collections. About 80 second-instar larvae were placed in each rearing container with early-season foliage.

Late-season foliage was removed from the freezer, allowed to thaw, and placed in rearing containers each with 30 second-instar larvae. Containers were kept at 21°C with 16:8 L:D photoperiod. Fresh or thawed foliage was added daily and old foliage and frass were periodically removed until the budworm had pupated.

At pupation, development time from second instar was recorded and pupae were weighed, sexed, and transferred to volume emergence cages. As moths emerged, the number of days spent in the pupal stage was recorded and moths were placed in mating jars, each with approximately five males and five females. Pieces of wax paper, folded accordian-style, for oviposition sites and

vials of sugar water were placed in the jars and changed about every second day. The wax paper holding egg masses was cut and placed in five centimetre diameter, closed petri dishes. These were placed on a wire screen above water in closed rearing containers. Eggs were counted after first-instar larvae emerged to provide a total of the number of eggs laid per female and percent hatch.

Second-instar larvae reared on the standard McMorran diet (Appendix VIII) were used as controls for the separate populations of budworm used in this trial.

Experimental Design

Foliar Characteristics

The experimental design was a two-way (3x2) factorial treatment with uneven replication in a completely randomized design structure. There were six treatment combinations made up of the following factors: 1) tree species - balsam fir, white spruce and black spruce and 2) season of foliage collection - early and late. The penetrometer response variable 'weight of sand' and IF levels (percent dry weight) were analyzed using the non-parametric, Mann-Whitney *U* test for two independent samples; the *U* statistic was transformed into a normally distributed *Z* statistic, as required for larger samples (Zar 1974). Foliar N levels, which were determined by the Lakehead University Instrumentation Laboratory, could not be tested for statistical differences due to the availability of only one N value per species per season of collection. Foliar water levels were analyzed using the non-parametric, Mann-Whitney *U* test for two independent samples.

Spruce Budworm Rearing Trials

The experimental design was a three-way (3x2x2) factorial treatment with one observation per treatment combination in a completely randomized design structure. There were 12 treatment combinations made up of the following factors: 1) tree species - balsam fir, white spruce, and black spruce; 2) season of foliage collection - early and late; and 3) sex of spruce budworm - female and male.

The data for the response variables 'number of days from second instar to pupa' and 'number of days in pupa' and 'pupal weight' were each analyzed using the non-parametric, Kruskal-Wallis one-way analysis of variance test (Zar 1974) for significant differences among tree species. Significant differences between the factors of season of foliage collection and sex of budworm were determined using the non-parametric, Mann-Whitney *U* test for two independent samples; the *U* statistic was transformed into a normally distributed *Z* statistic, as required for larger samples (Zar 1974). The data for the response variable 'number of eggs laid per female' were analyzed using a contingency table.

Unless stated otherwise, all tests of statistical significance were at the $P \leq 0.05$ level. All data analysis was performed using SPSS-X (SPSS 1988).

RESULTS

Foliar Characteristics

Leaf toughness as evaluated by weight of sand differed significantly between seasons of collection for each tree species and between each species within season, with the exception that early-season foliage of white spruce and black spruce did not differ significantly (Table 2 and Appendix IX).

Table 2. Foliar characteristics of early- and late-season, current-year needles of balsam fir, white spruce and black spruce[a].

	Toughness (g)		Indigestible Fibre (%dw)		Nitrogen (%dw)		Moisture (%fw)	
	Early	Late	Early	Late	Early	Late	Early	Late
Balsam Fir								
Mean	179.65a	487.54b	40.51f	43.88g	2.27	1.05	72.21k	56.87l
SD	118.15	149.60	1.68	2.05			1.86	3.03
CV(%)	65.77	30.68	4.14	4.68			2.57	5.33
n	38	30	11	10			7	7
White Spruce								
Mean	529.31c	946.90d	42.36h	49.34i	1.73	0.98	79.31m	56.74l
SD	207.45	178.39	0.75	1.05			1.15	1.17
CV(%)	39.19	18.84	1.77	2.13			1.45	2.06
n	31	30	10	11			7	6
Black Spruce								
Mean	570.18c	1170.30e	41.75fh	49.67i	1.59	0.99	76.38n	68.71o
SD	317.46	244.42	0.94	0.60			2.54	2.40
CV(%)	55.68	20.89	2.25	1.20			3.32	3.49
n	30	30	12	10			7	7

[a] The mean, standard deviation (SD), coefficient of variation (CV) and number of observations (n) are presented for toughness (i.e. weight of sand) and levels of foliar IF, N and moisture. For each variable, means within a column or row followed by different symbols are significantly different as determined by Mann-Whitney *U* tests.

Relative leaf toughness, on average, was least for balsam fir, followed by white spruce, and then black spruce. Although toughness measurements were highly variable, early-season foliage was significantly "softer" than later season foliage, for all tree species. Early-season needles of balsam fir required about one-third the force required for leaf penetration compared to late-season needles; early-season needles of white and black spruces required about half the force. In addition, leaves collected from these three trees in early season

were at least twice as variable in toughness as those collected in late season, as indicated by the coefficient of variation (standard deviation/mean).

IF levels were significantly lower in early-season foliage for all three tree species (Table 2 and Appendix IX). Within early-season foliage, white spruce had significantly higher IF levels than balsam fir, but black spruce did not differ significantly from either white spruce nor balsam fir. Within late- season foliage, the IF levels of the two spruces did not differ significantly but both had significantly greater IF levels than did balsam fir. The difference in percent IF from early- to late-growing season was highest for white and black spruce and lowest for balsam fir.

N levels, which were extracted from experiment 1 data, were higher in the early- than in the late-season foliage for all three tree species (Table 2). Early-season foliage of balsam fir had the highest level of N while black spruce had the lowest. Late-season N values were about one percent for all three species. The difference in percent N from early- to late- season was highest for balsam fir, followed by white spruce and then black spruce.

Moisture levels, which were extracted from experiment 1 data, were significantly higher in early-season than in late-season foliage for all three tree species (Table 2 and Appendix IX). Within early-season foliage, each species differed significantly in foliar moisture levels; white spruce had the highest level while balsam fir had the lowest. Within late- season foliage, foliar moisture levels were significantly higher for black spruce than for white spruce and balsam fir which did not differ significantly. The difference in percent moisture content from early- to late-season was highest for white spruce, followed by balsam fir and then black spruce.

Survival

Survival rates for budworm reared on early-season foliage could not be calculated because an undetermined number of budworm were placed on that foliage. On late-season foliage, however, combined survival rates for male and female budworm from the second-instar pupa and pupa to moth stages were 80.0 percent for balsam fir, 43.3 percent for white spruce, and zero percent for black spruce. Although no budworm survived on the diet of late-season foliage of black spruce, it was considered to be a valid result considering that the trial was attempted twice with similar results.

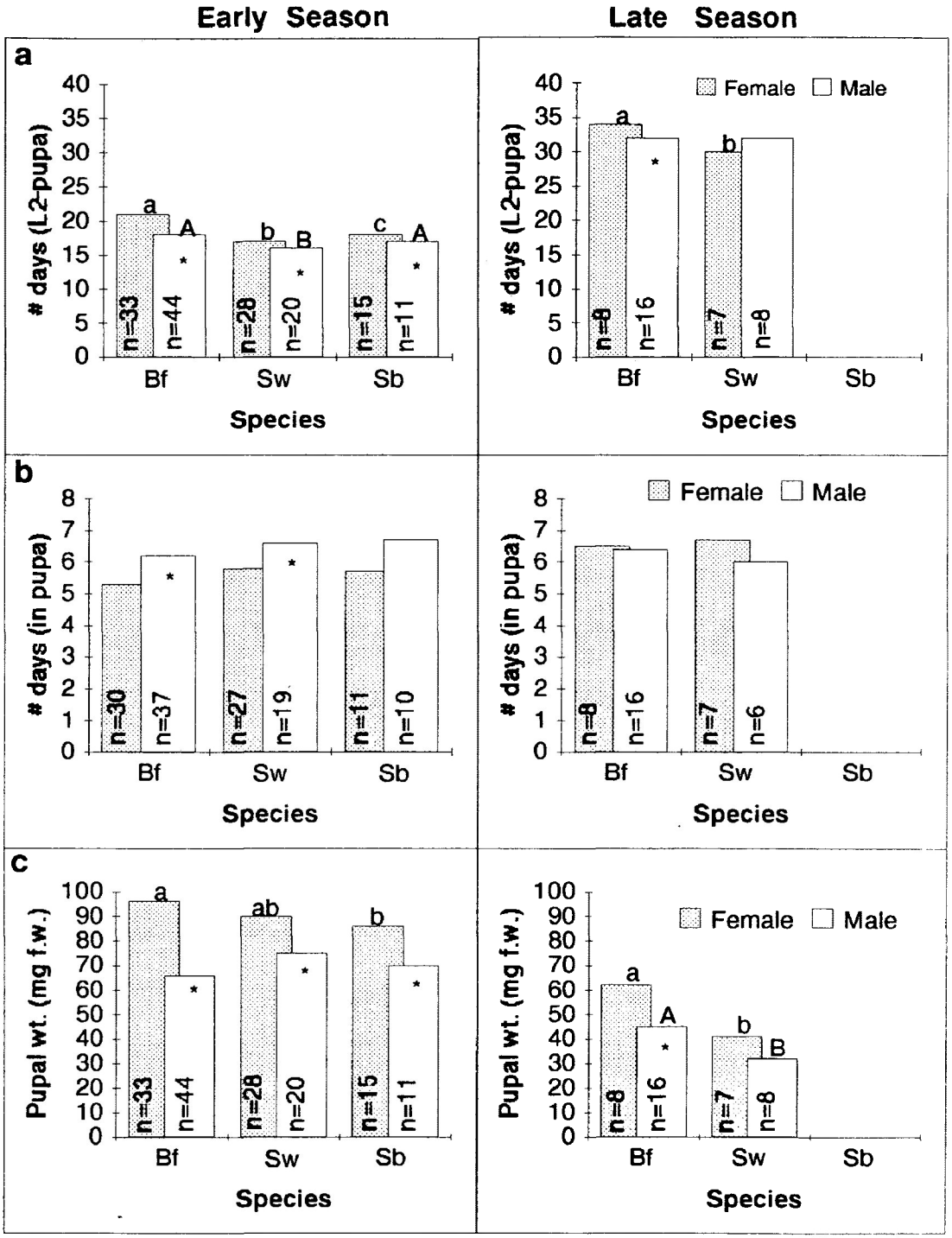
Development from Second Instar to Pupa

Early-Season Foliage

Female budworm took significantly longer than male budworm to develop from second instar to pupa when reared on early-season foliage of balsam fir, white spruce, and black spruce (Figure 11a and Appendices X and XI). Among tree species, females differed significantly in the number of days taken to develop from second instar to pupa. The longest development time occurred on the foliage of balsam fir, followed by black spruce and then white spruce. Males took significantly longer to develop on foliage of balsam fir and black spruce than on white spruce.

Late-Season Foliage

Females took significantly longer than males to develop on balsam fir but not on white spruce foliage (Figure 11a and Appendices X and XI). Neither sex survived to the pupal stage on late-season foliage of black spruce. Among tree species, females took significantly longer to develop on balsam fir foliage compared to those on white spruce while males did not differ significantly.



* - indicates significant differences between sexes
a, b, ab - indicate significant differences among diets for females
A, B, AB - indicate significant differences among diets for males

Figure 11. The a) number of development days from second instar (L2) to pupa, b) number of days in pupal stage, and c) pupal weights of male and female spruce budworm fed on balsam fir (Bf), white spruce (Sw), and black spruce (Sb) foliage, categorized by season (early and late) of collection.

Early- Versus Late-Season Foliage

Female and male spruce budworm took significantly longer to develop on late-season than on early-season foliage of balsam fir and white spruce (Figure 11a and Appendix XI). On late-season balsam fir, females and males took, respectively, about 1.6 and 1.8 times longer to develop than on early-season balsam fir and on late-season white spruce, about 1.8 and 2.0 times longer.

Number of Days in Pupal Stage

Early-Season Foliage

Male spruce budworm took significantly longer than female budworm to pass through the pupal stage when reared on early-season foliage of balsam fir and white spruce but not black spruce (Figure 11b and Appendices X and XI). Among tree species, neither sex differed significantly in the number of days required to pass through the pupal stage.

Late-Season Foliage

Females and males did not differ significantly in development days through the pupal stage on either balsam fir or white spruce foliage (Figure 11b and Appendices X and XI). Among tree species, neither females nor males differed significantly in number of days required to develop through the pupal stage.

Early- Versus Late-Season Foliage

Development times for females were significantly greater on late-season foliage than on early-season foliage (Figure 11b and Appendix XI). Females took about 1.2 times longer to develop through the pupal stage on late-season foliage of both balsam fir and white spruce. Males did not differ significantly on early- and late-season foliage of balsam fir and white spruce. In addition, there

was a reversal of trends on early- and late-season foliage. On early-season foliage, females developed more slowly than did males but on late-season foliage, males developed more slowly than did females.

Pupal Weights

Early-Season Foliage

Female budworm were significantly heavier than male budworm on each of the tree species (Figure 11c and Appendices X and XI). Among tree species, females on balsam fir were significantly heavier than their counterparts on black spruce; those on white spruce did not differ from either balsam fir or black spruce. Males did not differ significantly in weight among host tree species.

Late-Season Foliage

Female pupal weights were significantly heavier than male weights for balsam fir but not for white spruce foliage (Figure 11c and Appendices X and XI). Among tree species, both female and male budworm were significantly heavier when reared on balsam fir foliage than when reared on white spruce.

Early- Versus Late-Season Foliage

Female and male pupal weights were significantly lighter for budworm reared on late-season foliage compared to those reared on early-season foliage (Figure 11c and Appendix XI). On balsam fir, female and male pupal weights were, respectively, about 36 and 33 percent lighter and on white spruce, about 55 and 57 percent lighter.

Fecundity

Females reared on early-season foliage displayed significantly higher [Contingency table $\chi 2 = 69.92$ $\alpha = 0.00$] fecundity than those reared on late-season foliage (Table 3). About three times as many eggs were laid by females

reared on early-season foliage compared to those reared on late-season foliage of balsam fir and white spruce. There was no survival of females reared on the late-season foliage of black spruce. Females reared on early balsam fir laid the highest number of eggs with an average of 373 while those on early white and black spruces laid about 215 eggs. Additionally, females reared on late balsam fir laid more eggs than those reared on late white spruce. Percent egg hatch was similar for budworm reared on both seasons of balsam fir and white spruce; black spruce foliage-reared females yielded slightly lower percent hatch.

Table 3. Mean number of eggs laid per female and percent egg hatch on early- and late-season foliage diets of balsam fir, white spruce, and black spruce.

Season	Balsam fir			White spruce			Black spruce		
	Eggs per female	% hatch	n[a]	Eggs per female	% hatch	n[a]	Eggs per female	% hatch	n[a]
Early	373	87.2	10	211	83.4	10	218	77.0	10
Late	113	83.2	5	81	83.0	8	-	-	0

[a] Number of females.

DISCUSSION

Spruce budworm reared on current-year needles showed superior performance on early-season compared to late-season foliage. In this study, budworm reared on early-season foliage developed more rapidly, attained greater pupal weights and had higher fecundity than those reared on late-season foliage of balsam fir, white spruce, and black spruce. On early-season foliage of balsam fir, female and male budworm respectively developed about 38 and 44 percent faster and attained pupal weights which were about 56 and

48 percent heavier than their counterparts on the late-season foliage diet. About 3.3 times as many eggs were laid by females reared on early- compared to late-season foliage. On white spruce early-season foliage, female and male budworm respectively developed about 43 and 50 percent faster and attained pupal weights which were about 120 and 131 percent heavier than budworm reared on the late-season foliage diet. About 2.6 times as many eggs were laid by females reared on early- compared to late-season foliage. Comparisons of performance on black spruce foliage are not possible because no budworm survived to the pupal stage on the late-season foliage. These findings are corroborated by those of Thomas (1983, 1987, 1989) whose research has suggested that the rapid deterioration of early-season foliage is the causal agent for reduced performance by spruce budworm.

The characteristics which render early-season foliage more suitable than late-season foliage to spruce budworm growth, development and reproduction involve differences in the nutritional, allelochemical and physical attribute components of food quality. In comparison to late-season foliage, early-season foliage of balsam fir, white spruce, and black spruce had higher levels of the nutritional components of water and N and lower levels of IF, the allelochemical component. Leaf toughness, the physical attribute of food quality, was lower in early-season than in late-season foliage for all three host species.

As demonstrated in my and other studies (Feeny 1970; Hough and Pimental 1978; Schultz *et al.* 1982; Hunter and Lechowicz 1992), leaf toughness increases over a growing season with the increasing accumulation of cell wall materials, in particular, lignins (Raven *et al.* 1986; Swain 1979). It is, however, strongly associated with decreasing levels of other foliar components such as water and N. For this reason, it is difficult to assess the effects of leaf

toughness alone on herbivore feeding and performance without considering the effects of changes in other components of food quality.

In my study, spruce budworm growth and development was clearly superior when completed on the softer, more nutritious leaves of early season than on the tougher, less nutritious leaves of late season. These findings are consistent with those of other investigations where leaf toughness negatively influenced lepidoptera pupal weights (Feeny 1970; Hough and Pimental 1978; Rausher 1981), survival and development times (Hough and Pimental 1978).

Leaf toughness may act as a defence by reducing the suitability of leaves as a food source in several ways. It provides a physical toughness to tissue which is difficult for some mandibulate insects to chew through (Rausher 1981). The mortality of all larvae, in this experiment, reared from early instars on the late-season black spruce foliage is similar to findings of Blais (1957) who reared third-instar budworm on one-year-old, black spruce and balsam fir foliage. Poor survival of third- and fourth-instar larvae was reported by Heron (1965) for spruce budworm forced to feed on one-year-old needles of white spruce. Heron observed that the physical deterrent presented by leaf toughness was overcome later in the development of larvae.

Physical toughness of late-season or old foliage may explain why no spruce budworm survived to the pupal stage on black spruce in this experiment. My results showed that black spruce had the toughest, late-season foliage of the three species. In contrast, balsam fir late-season foliage was the least tough and allowed the highest survival rate (80 percent) while white spruce had the second toughest foliage and survival was intermediate (43 percent).

Evidence for the inhibition of feeding due to leaf toughness has been shown for several taxa such as young grasshoppers (Williams 1954) and leaf

cutter ants (Nichols-Orians and Schultz 1990). Tanton (1962) ranked toughness of turnip leaves on an arbitrary scale and determined that there was a threshold level of toughness which matched the mandibulate power of mustard beetles; above that toughness level, leaf area eaten by the beetles decreased dramatically.

It is probable that early-instar spruce budworm feeding is also impaired by increasing leaf toughness. Field investigations by Morris (1963) revealed that only large larvae were able to complete their development on older foliage; presumably their stronger mandibulate muscles and larger mandibles allowed them to chew through tougher needles that smaller larvae could not penetrate. This suggests that young spruce budworm with weaker mandibulate muscles and smaller mandibles are not equipped to chew successfully through tough, older needles.

Another way in which toughness reduces the suitability of leaves as a food source is by reducing nutrient availability to larvae. Compared to soft, young foliage, tough, mature foliage contains lower levels of the nutritional components of water and N. This decrease in nutrient concentrations may reflect a simple dilution due to increases in IF levels or may represent an actual decrease in intracellular concentrations as well (Rausher 1981). Leaf acceptability and insect performance has been shown to be reduced for insects reared on mature host leaves but it is difficult to say whether toughness or low nutrient status, or either, plays the more important role.

Feeny (1970) believed that the seasonal decline in N was the most important ultimate factor but that leaf toughness was probably the chief proximate factor preventing winter moth larvae from feeding normally on mature oak leaves. Coley (1983) reported that of the several chemical, physical and

nutritional leaf characteristics that she correlated to rates of herbivory, leaf toughness showed the highest negative correlation, followed by fibre content and nutritive value. On the other hand, Hunter and Lechowicz (1992) found that, although leaf toughness was strongly correlated to acceptability to gypsy moth, it explained only about 10 percent of the variation in acceptability of host species; in fact, leaf water, N, and toughness each accounted for no more than 10 percent of this variation. They concluded that characteristics that were not measured were "undoubtedly important in gypsy moth host choice."

It is very likely that the three components of food quality - nutritional, allelochemical and physical attributes - act together to determine the quality of leaves as a food source for insects. For spruce budworm, foliage is available year round due to the long leaf longevity of its evergreen hosts. Evergreen species probably evolved this characteristic as a response to growing on sites with limited resources (Bryant *et al.* 1983) and have developed defences to protect the leaves, which are expensive to replace, from removal by herbivores.

From the perspective of the tree, varying tissue food quality allows for a level of defoliation by spruce budworm which is limited to the beginning of the growing season and which can be tolerated and compensated for. Tolerance is considered (Mattson *et al.* 1988b; Mattson *et al.* 1991) to be the first line of defence evolved by plants against folivory. White and black spruce tolerate a level of defoliation of current-year needles by having well defended old foliage which which provides reserve photosynthetic capacity and is seldom depleted even during epidemics (Mattson 1985). Balsam fir tolerates a certain level of attack by compensating for injury through the production of epicormic shoots, combined with the increased retention of older age-class needles, and the development of new foliage at the expense of volume growth (Piene 1989).

Obviously tolerance to severe levels of defoliation cannot last forever and mortality occurs when tree energy reserves are depleted (Mattson *et al.* 1991).

Another line of defence that trees may use to defend against insect attack is a delayed inducible resistance (DIR) which changes the quality of leaf tissue in the year following defoliation thereby negatively affecting future generations (Haukioja and Neuvonen 1987). A DIR depends on herbivore density and may act as a regulator of herbivore populations (Haukioja and Neuvonen 1985). Such a defence is thought to exist for two deciduous tree-herbivore systems: *Larix decidua/Zeirpahera diniana*, and *Betula pubescens tortuosa/Epirrita autumnata* (Haukioja 1990). Although there is little substantive evidence for their existence in evergreen conifers (Mattson *et al.* 1991; Neuvonen and Niemelä 1991), their possibility has been suggested for white spruce (Beaudette 1986) and balsam fir (Bauce and Hardy 1988). For these two host species of spruce budworm, increased levels of IF have been noted in defoliated over nondefoliated leaves (Beaudette 1986; Bauce and Hardy 1988). Moreover, feeding tests with spruce budworm showed reduced performance when reared on leaves with the higher levels of IF (Bauce and Hardy 1988). The reduced performance of budworm observed by Bauce and Hardy (1988) may have been due to other foliar characteristics rather than toughness.

EXPERIMENT 3

DETERMINATION OF A THRESHOLD NITROGEN LEVEL FOR EVALUATING SPRUCE BUDWORM PERFORMANCE

PURPOSE

The purpose of this investigation was to vary casein levels in the standard McMorran diet to determine a lower limit below which budworm performance would be poor. Casein, a complete milk protein, (West *et al.* 1966) was taken as the source of assimilable N in the diet due to its highly purified, vitamin-free preparation (Dadd 1985). The lower level of casein was then included in the cellulose:casein N diets of experiment 4.

METHODS

Diet Preparation

Components in the standard McMorran diet were first analyzed for sources of N and IF according to methods described in experiment 1.

Treatment diets were prepared by adding casein at four levels. Each level was a percentage of the dry weight of casein in the standard diet. Diets 1, 2, 3, and 4 contained 12, 18, 25, and 100 percent casein in the standard diet, respectively. Diets were poured into labelled, 5 cm diameter petri plates and stored fresh in the refrigerator for one day prior to use.

Insect Rearing

Spruce budworm were placed as second instars on the diet plates with one budworm per plate and 40 plates per diet. Budworm were kept at 21°C with 16:8 L:D photoperiod and reared to pupa. At each first day of the fifth and sixth instars, fresh weights were measured and the number of days recorded for development from second to fifth instar and in fifth and sixth instars. Earlier instars are very fragile and were not weighed because of the risk of mortality associated with handling. Pupae, moths and eggs were treated according to the methods of experiment 2.

Experimental Design and Data Analysis

The design of the experiment was a two-way factorial with factors diet and sex having four and two levels, respectively. There was uneven replication of female and male budworm per diet due to the impossibility of sex determination at the second instar and the design structure was completely randomized.

Data were first tested for homogeneity of variance using the Bartlett test (Steel and Torrie 1980). Where homogeneous variances existed, a one-way analysis of variance was used to determined significant differences among diets for a particular response variable within each level of sex, i.e. differences among female, sixth-instar weight on diets 1, 2, 3, and 4 and differences among male, sixth-instar weight on diets 1, 2, 3, and 4. The Least Significance Difference (LSD) test was used for multiple comparison among means. Differences between two means were tested with the two-sample *t* test.

Non-parametric statistics were used where homogeneous variances did not exist and where re-expression was not helpful in transforming data to achieve a normal distribution. This situation occurred when the ratio of largest datum value to smallest datum value was less than two (Emerson and Stoto

1983). Significant differences between two independent samples were determined with the Mann-Whitney *U* test; where required for larger samples, the *U* statistic was transformed into a normally distributed *Z* statistic (Zar 1974).

Unless stated otherwise, all tests of statistical significance were at the $P \leq 0.05$ level. All data analysis was performed using SPSS-X (SPSS 1988).

RESULTS

Diet Components

Components of the standard McMorran diet which contained the highest N levels were aureomycin, casein and wheat germ (Table 4). Alphacel contained the highest level of IF, followed by wheat germ (Table 4).

Table 4. N and IF levels (% dry weight) in selected dietary components of the standard McMorran diet.

Diet component	%N[a]	%IF
Aureomycin	33.70	-
Casein	14.60	-
Wheat germ	5.69	28.68
Vanderzant vitamin	0.52	-
Agar	0.31	0.38
Wesson salt	0.01	-
Alphacel	-	99.07

[a] Determined by Lakehead University Instrumentation Laboratory.

Nitrogen concentration contributions were determined for individual dietary components in each of diets 1, 2, 3, and 4 (Table 5). Diets with 12 (diet 1), 18 (diet 2), 25 (diet 3) and 100 (diet 4) percent casein in the standard diet were calculated to contain 3.29, 3.47, 3.66, and 5.42 percent N. These values are slightly less than those determined by Lakehead University Instrumentation

Laboratory which were 3.50, 3.59, 4.07, and 5.48 percent N for diets 1, 2, 3, and 4, respectively. Total percent IF was calculated to be 10.36 for diet 1, 10.20 for diet 2, 10.02 for diet 3, and 8.40 for diet 4 (Table 6).

Table 5. Percent N levels of components of the standard McMorran diet as a percentage of the dry weight of diets 1, 2, 3, and 4 and total percent N.

	Diets			
	1	2	3	4
% Casein	12	18	25	100
	% N in diets			
Aureomycin	1.42	1.40	1.38	1.15
Casein	0.47	0.69	0.94	3.14
Wheat germ	1.30	1.28	1.25	1.05
Vanderzant vitamin	0.04	0.04	0.03	0.03
Agar	0.06	0.06	0.06	0.05
Wesson salt	0.00	0.00	0.00	0.00
Total calculated % N in diet	3.29	3.47	3.66	5.42
Total % N in diet [a]	3.50	3.59	4.07	5.48

[a] Determined by Lakehead University Instrumentation Laboratory.

Table 6. Percent IF of components of the standard McMorran diet as a percentage of the dry weight of diets 1, 2, 3, and 4 and total percent IF and IF:N for these same diets.

	Diets			
	1	2	3	4
% Casein	12	18	25	100
	% IF (dry weight) in diets			
Alphacel	3.76	3.70	3.63	3.04
Wheat germ	6.53	6.43	6.32	5.30
Agar	0.07	0.07	0.07	0.06
Total calculated % IF in diets	10.36	10.20	10.02	8.40
IF:N calculated	3.2:1	2.9:1	2.7:1	1.2:1

Survival

Survival from second instar to moth for both female and male spruce budworm was 100 percent on diet 4, 90 percent on diet 3, 75 percent on diet 2, and 35 percent on diet 1 (Figure 12).

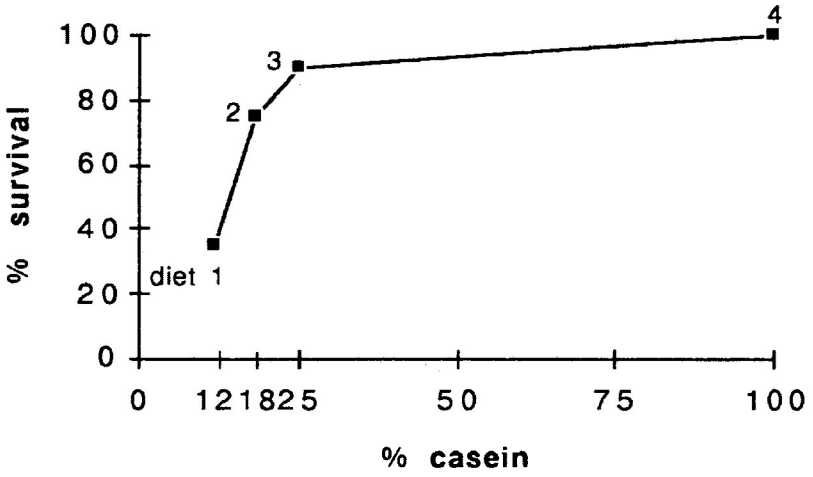


Figure 12. Combined percent survival of female and male spruce budworm from second instar to adult moth on diets with 12, 18, 25, and 100 percent casein in standard McMorran diet.

Development

Female and male spruce budworm did not differ significantly in the number of days spent in fifth instar on diets 1, 2, 3, or 4 (Figure 13a and Appendices XII and XIII). Among diets, the number of days spent in fifth instar by females on diet 2 was significantly longer than that taken by females on diets 1 and 3, which did not differ. Females on diet 4 took the least amount of time, significant at $P \leq 0.05$. Males on diets 1 and 2 did not differ and took significantly longer to pass through fifth instar than males on diets 3 and 4, which themselves did not differ.

Female budworm spent a significantly greater number of days in sixth instar than male budworm on diets 2 and 3 only (Figure 13b). Among diets,

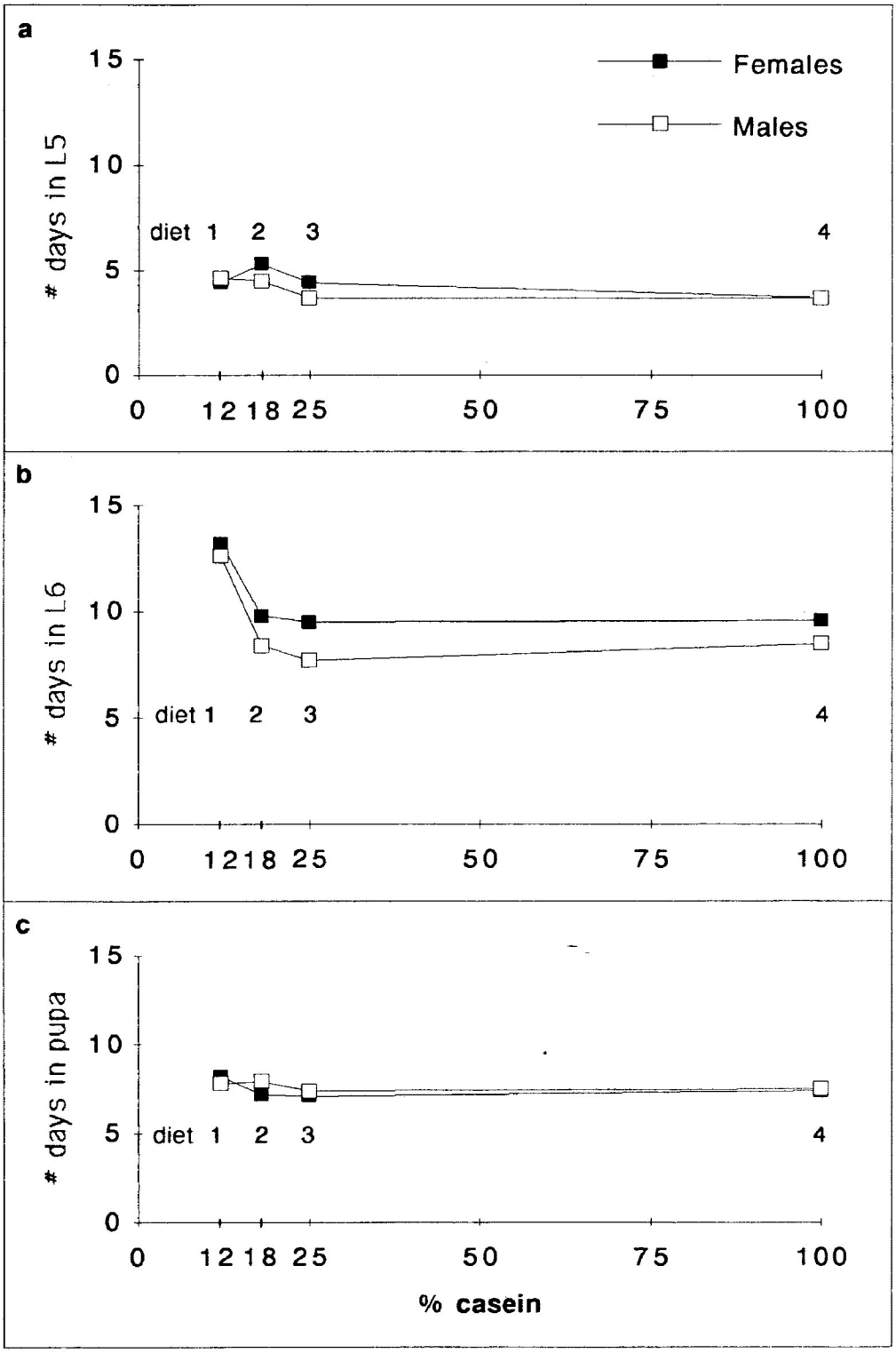


Figure 13. Number of days spent in a) fifth instar (L5), b) sixth instar (L6), and c) pupa by female and male spruce budworm which were reared from secondinstar to pupa on artificial diets with 12, 18, 25, and 100% casein in standard McMorran diet.

both sexes on diet 1 spent a significantly greater number of days in this instar than their counterparts on the other three diets.

Female and male budworm did not differ significantly in the number of days spent in the pupal stage on diets 1, 3, or 4 (Figure 13c). On diet 2, however, males spent a significantly greater number of days in this instar compared to females . Among diets, there were no significant differences in number of pupal development days.

Weights

Female spruce budworm were significantly heavier than male at fifth instar on diet 4 only (Figure 14c and Appendices XII and XIV). Among diets, females on diet 4 and males on diets 3 and 4 were significantly heavier than their counterparts on the other diets .

Weight at sixth instar was significantly heavier for females than males on diets 2, 3, and 4 but not on diet 1 (Figure 14b). Among diets, female budworm on diet 4 were significantly heavier than those on diet 1 but not heavier than females on diets 2 and 3. Males on diet 4 were significantly heavier than males on diet 2 but not significantly different than those on diets 1 and 3.

Female budworm yielded significantly heavier pupae than did male budworm on all diets (Figure 14c). Among diets, female pupae on diet 4 weighed the most and were significantly heavier than those on diets 1, 2, and 3. In turn, females on diet 3 were significantly heavier from all other diets; females on diets 1 and 2 did not differ. Male budworm on diets 3 and 4 did not differ and were significantly heavier than those on diets 1 and 2, which also did not differ.

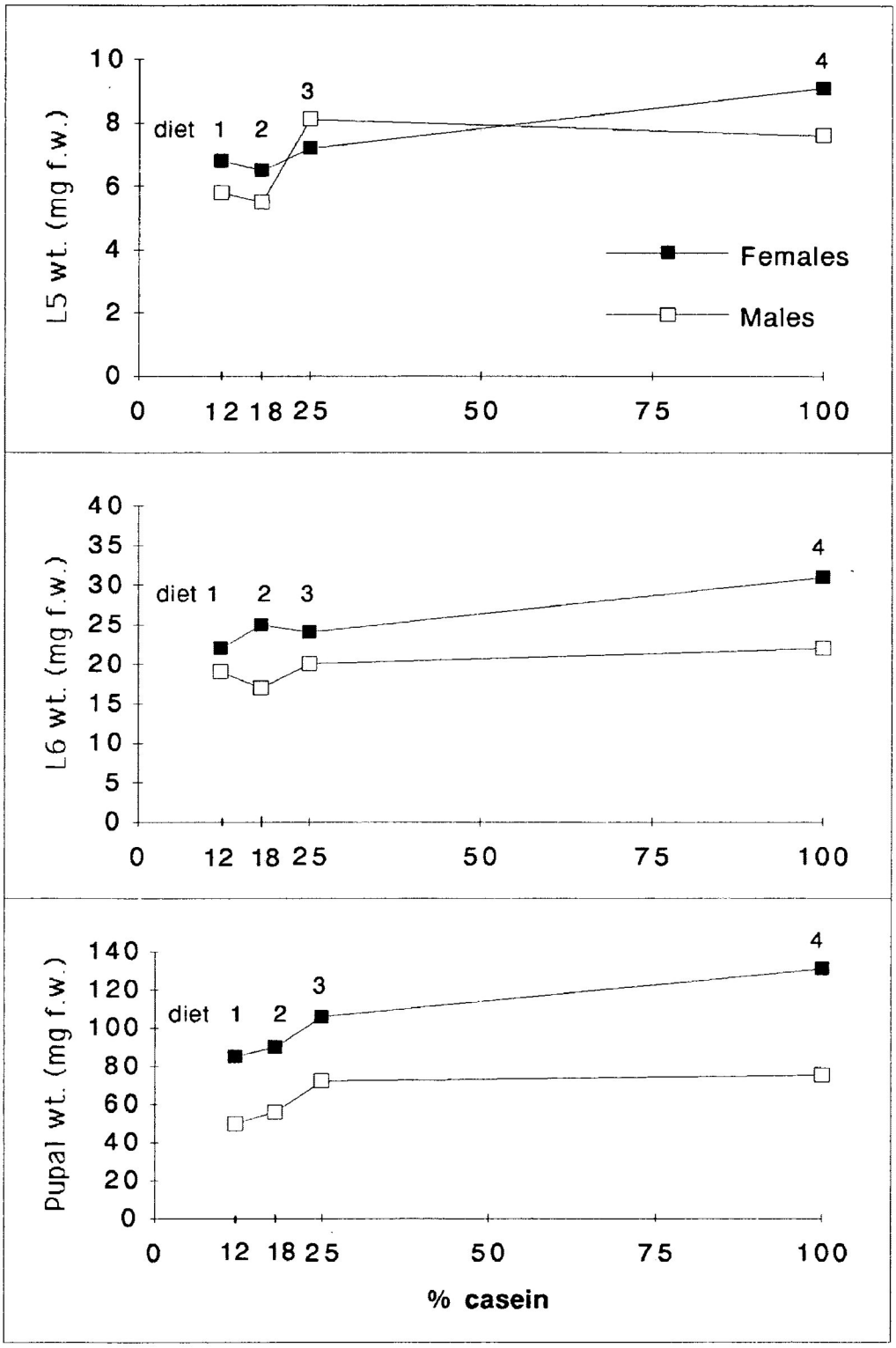


Figure 14. Weight on first day of a) fifth instar (L5), b) sixth instar (L6), and c) pupa of female and male spruce budworm which were reared from second instar to pupa on artificial diets with 12, 18, 25, and 100% casein in standard McMorran diet.

Fecundity

The average number of eggs laid per female increased with increasing casein from 12 to 25 percent in the diet and was similar for 25 and 100 percent casein (Figure 15). The greatest number of eggs, 247, were laid by females reared on diet 3, followed closely by 234 eggs on diet 4, 178 eggs on diet 2, and 54 eggs on diet 1.

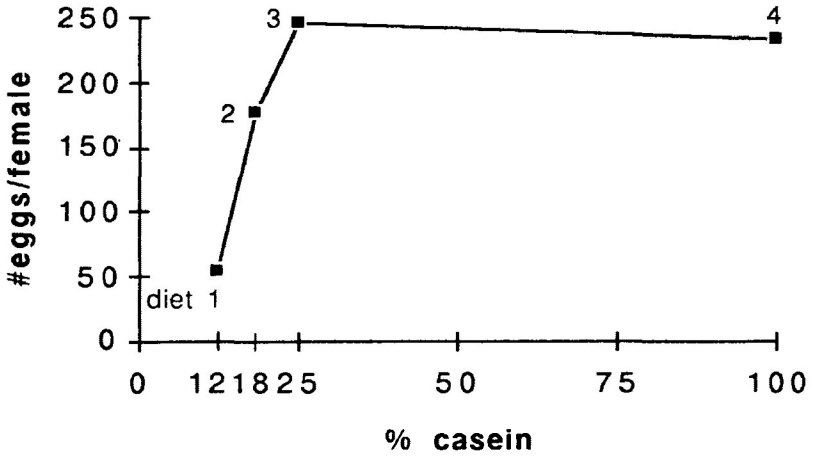


Figure 15. Average number of eggs laid per female on diets with 12, 18, 25, and 100% casein in standard McMorran diet .

A comparison of pupal weight and fecundity showed that although females on the 12 and 18 percent diets had similar weights there was a three-fold increase in the number of eggs laid by females on the 18 percent casein diet (Figure 16). As well, significantly lighter females on the 25 percent casein diet laid slightly more eggs than their heavier counterparts on the 100 percent casein diet.

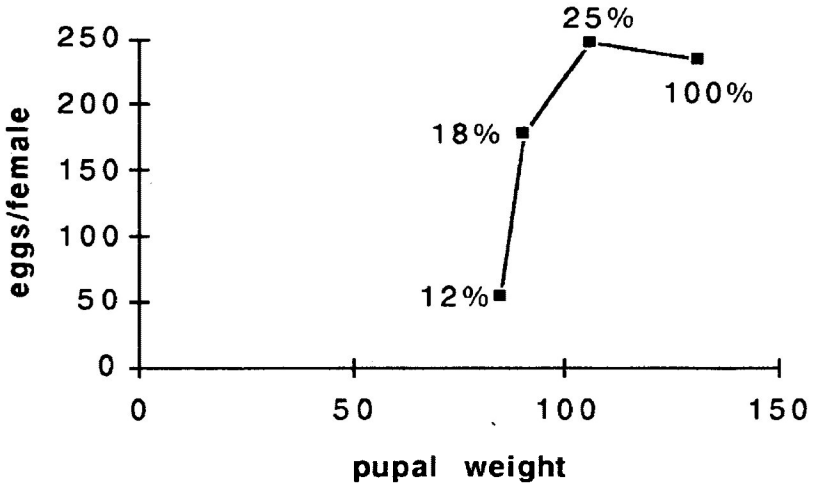


Figure 16. Relation between female pupal weight and fecundity on diets with 12, 18, 25, and 100% casein in standard McMorran diet .

DISCUSSION

Nitrogen is an important dietary component which has been regarded as a limiting factor to insect growth (Mattson 1980), although, recently its putative importance has been questioned in favour of a proper balance of many different nutrients as the most important factor in determining the performance of insect herbivores (Clancy 1992). As shown in this and other studies (e.g. Harvey 1974; Mattson 1980; Scriber and Slansky 1981; Bauer and Nordin 1988; Clancy 1992), increasing levels of dietary nitrogen frequently leads to increasing insect performance.

In this study, nitrogen levels were varied in the standard diet (McMorran 1965) to determine a minimum level below which spruce budworm performance would be relatively poor. This level was then to be used as the lowest concentration of N in experiment 4 in which dietary ratios of cellulose:casein N were manipulated. Spruce budworm performance was determined by measuring survival to adult moth, development times and weights of fifth and sixth instars and pupa, and fecundity as defined by number of eggs laid. Fifth

and sixth instars are important life stages because it is during these periods that the majority of food is consumed (Miller 1977; Retnakaran 1983). Extended development times in these instars can leave insects more vulnerable to mortality by increasing the time that larvae are exposed to natural enemies (Feeny 1976; Price *et al.* 1980). Weight is an important indicator of fitness because of the direct relationship of pupal weight to fecundity (Miller 1957; Miller 1963), although, Leather (1988) has refuted the reliability of this relationship.

Spruce budworm performance in terms of development and growth was best on the diet with 100 percent casein in the standard McMorran diet and worst on the diet with 12 percent casein in the standard McMorran diet. Intermediate results occurred on the diets with 18 and 25 percent casein in the standard McMorran diet.

In fifth instar, females on the 100 percent casein diet had the shortest development times and greatest weight. Comparing the 100 with the 12 percent casein diet, both females and males on the 12 percent casein diet took between one-half to one day longer to pass through this instar (Figure 13) and were about one milligram lighter (Figure 14).

By sixth instar, nutritional differences in the diets became more apparent as both female and male budworm on the 12 percent casein diet took about four days longer to pass through this instar and females were significantly lighter by about 8 mg than those reared on the 100 percent casein diet. This extended development probably represents the increased time spent feeding by larvae in order to compensate for a low quality diet. Compensation is a behaviour exhibited by a number of insects which is ecologically relevant because of the heterogeneity of food quality that insects must adapt to in nature (Scriber and Slansky 1981). It can, however, leave insects more vulnerable to mortality.

Koller (1987) noted that spruce budworm larvae regulated their consumption in response to levels of foliar N - lower levels of foliar N led to increased consumption.

At the pupal stage, differences in weights clearly showed the effect of increasing dietary N. Females reared on 100 percent casein were about 1.5 times heavier than those reared on 12 percent casein while, in comparison, males showed a weak although significant response to increasing N. These sex differences illustrate the higher requirement for dietary N that female budworm appear to have compared to male budworm. Mattson *et al.* (1983) reported similar findings for budworm reared on artificial diets with N levels increasing from two to four percent as well as on intact foliar diets which received N fertilization treatments ranging from 0.47 to 2.05 percent. That study revealed that female larvae responded to increasing N levels with increasing weight gains whereas male weight gains showed little tendency to increase with foliar N levels above 1.5 percent. Mattson *et al.* (1983) suggested that the difference between males and females may be due to the female requirement for additional protein intake for oogenesis.

Pupal weight has been shown to be directly related to adult fecundity (Miller 1957) but in this study a direct relationship was not clearly evident. Female pupal weights on diets with 12 and 18 percent casein did not vary significantly but females on the 18 percent casein diet laid about three times as many eggs as those on the 12 percent casein diet. It appears that for females on the 12 percent casein diet a certain minimum weight of about 80 mg was attained before pupation occurred but, even at that, fecundity was drastically reduced (Figure 16). Over its developmental period, a larva must grow large enough to pupate and accumulate enough energy reserves to fuel its metabolism during the non-feeding pupal stage in order to optimize potential

fitness (Dadd 1985). It appears that budworm on the 12 percent casein diet struggled to reach a minimum weight by extending the sixth instar feeding period, but this compensation could not outweigh the effects of a N-poor diet.

Another example of an unclear weight/fecundity relationship is the fact that females on the 25 percent casein diet were significantly lighter than those on the 100 percent casein diet but laid slightly more eggs. It may be that the 100 percent casein diet (which is the standard McMorran diet), while allowing significant larval growth, is too N-rich or out of nutrient balance to allow for optimal egg production. Another possibility for these inconsistencies may be that the weight/fecundity relationship is not completely reliable, as suggested by Leather (1988). In his paper, Leather (1988) indicated studies in which lepidopteran pupae of similar sizes had significant differences in fecundities, for example, for moths of *Epiphyas postvittana* which had arisen from pupae of similar weights but were derived from different larval food hosts (Danthanarayana 1975) and also for same sized pupae of *Pieris rapae* which had different genetic make ups and produced different weight/fecundity relationships (Gilbert 1986). Leather (1988) also pointed out that pupal weight varies according to season and environmental conditions (Miller 1957) and does not consider it to be a satisfactory index. In addition, the number of eggs contained within an individual may have little bearing on the number of eggs actually laid (Leather *et al.* 1985). Although adult weight is closely related with egg load (Klomp 1958), the question arises of how may insects actually achieve their full reproductive potential. Leather (1988) indicated that, even in the laboratory, less than 15 percent of individuals of the Lepidoptera *Panolis flammea* lay 70 percent of their full egg load and in the field it is estimated that the maximum fecundity is only 28 percent of its full reproductive potential (Leather *et al.* 1985). Leather (1988) asserts that longevity and the factors that

influence it, such as temperature and adult food supply, appear to be the single most important factor influencing fecundity within the Lepidoptera. Although the weight/fecundity relationship is present, it is often masked by other factors and should thus be treated with caution (Leather 1988).

In contrast to pupal weights which showed an increasing trend with increasing casein, the mean number of days in the pupal stage varied little between sexes (except on diet 2) and among diets with 18, 25 and 100 percent casein (Appendix XII). There was slightly more variability associated with the diet with 12 percent casein. In general it took an average of seven or eight days to pass through the pupal stage. It is possible that the number of days required to pass through the pupal stage is relatively constant and may not be dependent on food quality. Similar to these findings, Lavallée and Hardy (1988) found that the period of pupal development was relatively constant for spruce budworm which were reared on three different host foliages in the laboratory. On white and red spruces, females took 11.3 ± 1.0 days and on balsam fir, 10.9 ± 0.8 days to pass through the pupal stage.

Other studies have shown the importance of dietary N for insect growth. Mattson *et al.* (1983) reported that foliar analysis of white spruce and balsam fir revealed that N was the only variable consistently and positively related to spruce budworm growth, i.e. dry weight of fifth and sixth instar. Bauer and Nordin (1988) noted increased pupal weights and reduced development times for spruce budworm reared on artificial diets with 4.5 percent N compared to those on 2.5 percent N diets. The cinnabar moth, *Tyria jacobaeae* L., was observed to have greater survival, moth weight and fecundity in natural areas when feeding on host plants that had received N fertilization compared to moths feeding on unfertilized host plants (Myers and Post 1981). The large aspen tortrix, *Choristoneura conflictana* (Walker), was reported to possess heavier

pupal weights when reared on intact foliage of quaking aspen, *Populus tremuloides* Michx., which had received N-plus-P fertilization compared to larvae that had fed on foliage receiving P-fertilization only (Bryant *et al.* 1987). Bryant *et al.* (1987) also reared larvae from third to fifth instar on artificial diets containing a low N level (1.5 percent N, dry mass) and a high N level (3.6 percent N, dry mass) and noted significantly heavier fifth-instar weight on the high N diet.

Although spruce budworm performance in this study did generally decrease with decreasing casein in the diet, the quality of performances on the 18 and 25 percent casein diets was greater than anticipated. Casein, which was determined to contain 14.6 percent N, is thought to be the largest contributor of available N in the McMorran diet, followed by wheat germ which contains 5.69 percent available N (Table 4). The remainder of the dietary N is found primarily in aureomycin and Wesson vitamin and may be unavailable for insect growth being excreted or used for maintenance. Therefore, as a result of the cutback in the primary source of N, i.e. casein, I had expected a dramatic reduction before 12 percent casein in survival, days in sixth instar, weight of fifth and sixth instars, as well as pupal weight and egg production. Instead, there were no significant differences in number of days in sixth instar for males and females nor in sixth-instar weight for females among diets with 18, 25, and 100 percent casein. Survival and egg production were also generally high.

The reason for these results may lie in the N contribution of wheat germ to the diets. Wheat germ levels were constant among the four diets but contributed proportionally more utilizable N to the lower casein diets than to the upper. The combination of utilizable N from wheat germ plus casein in the diets appears to have provided an acceptable level of N for insect performance. For example, in the 12 percent casein diet which was analyzed to contain 3.50

percent total N, 1.77 percent was utilizable N contributed by wheat germ and casein (Table 5). In the 18, 25, and 100 percent casein diets, 1.97, 2.19, and 4.19 percent utilizable N, respectively, was contributed by the combination of wheat germ and casein. Except for the 4.19 percent N value which is high, these N values are comparable to the total foliar N values found in white spruce and balsam fir in mid- to late-June when spruce budworm are feeding in fifth and sixth instars (Appendix V). Considering that not all foliar N is available for insect growth, the values of utilizable N in the leaves are probably less than the values in the four treatment diets. It would appear then that N values in the low casein diets are adequate for spruce budworm growth and maintenance. Spruce budworm have adapted to varying food quality in their host species and appear to be capable of metabolizing food containing unnaturally high levels of N, as in the 100 percent casein diet, as well as food containing low levels of N.

In fact, the 18 and 25 percent casein diets may be fairly representative of food encountered in nature. Harvey (1974) reported that field-collected, female pupae generally weigh from 70 to 140 mg and average close to 100 mg (Miller 1957). In this study on the 18 percent casein diet, female pupal weights ranged from 30 to 135 mg and averaged 90 mg; on the 25 percent casein diet, they ranged from 62 to 137 mg and averaged 106 mg (Appendix XII). In comparison, females reared on the 100 percent casein diet were significantly heavier with pupal weights ranging from 69 to 170 mg and averaging 131 mg. These results are in general agreement with McMorran (1965) who reported pupal weights ranging from 93.5 to 154.0 mg and averaging 121.7 mg for spruce budworm reared on the standard diet.

The heavier weights on the standard McMorran diet probably reflect its N-rich condition for which there is no match in the foliar diets of host trees. The 100 percent casein diet, which is the standard McMorran diet, was analyzed to

contain 5.48 percent total N (Table 5) whereas balsam fir, white spruce, and black spruce mid-crown foliage contained 3.65, 3.46, and 2.69 percent total N at the beginning of the growing season when foliar N is at its highest level (Appendix V). The McMorran diet is, therefore, a "luxury" diet to which spruce budworm appear readily able to adapt.

Similarities exist, however, between spruce budworm performance on the lower casein diets of this experiment and on the early-season foliar diets related in experiment 2. Female pupal weights for budworm reared on the 12, 18, and 25 percent casein diets were generally comparable to those on black spruce, white spruce, and balsam fir, respectively. Female pupal weights on the 12 percent casein diet were from 65 to 102 mg and averaged 85 mg. On the foliage of black spruce, these weights ranged from 76 to 101 mg with an average of 86 mg (Appendix X). Female budworm reared on the 18 and 25 percent casein diets had average pupal weights of 90 and 106 mg, respectively, which were generally comparable to those found on white spruce that were between 54 to 134 and averaged 90 mg and on balsam fir which ranged from 50 to 123 mg and averaged 96 mg.

Although pupal weights for females reared on the artificial and foliar diets were similar, the similarities cannot be completely attributed to N levels. In the 12, 18 and 25 percent casein diets, total percent N was analyzed to be 3.5, 3.59, and 4.07, respectively (Table 5). In the black spruce, white spruce, and balsam fir foliar diets, total percent N was 1.59, 1.73, and 2.27, respectively (Table 2). Nitrogen values increased progressively in both artificial and foliar diets but values in the foliar diets were about one-half that found in the artificial diets and yet pupal weights were comparable. One possible reason for this result may be that the amount of available N in both types of diets was similar. Total N values for the artificial diets were high, relative to the foliar diets, but

available N may have differed little. Casein and wheat germ are considered to be the sources of available N in the artificial diet but it is uncertain what the actual amounts of available N are; it may be less than suspected. Another reason for this result of comparable pupal weights at seemingly very different N levels in foliar and artificial diets may lie in the controversy surrounding the singular importance of N in isolation from other diet components (Clancy 1992). Clancy believes that it is a proper balance of many different nutrients and not N alone that is the most important factor in determining the performance of insect herbivores. This finding may provide evidence for that idea.

Unlike early-season foliar diets, pupal weights for females reared on late-season diets were not comparable to those found on even the poorest N diet, i.e. 12 percent casein. On balsam fir, weights ranged from 50 to 75 mg with an average of 62 mg while on white spruce these values were between 33 and 53 mg with an average of 41 mg (Appendix X).

Male spruce budworm did not exhibit the similarities in performance that females did when reared on early-season foliage and artificial diets with varying casein. Male pupal weights increased with increasing N in the artificial diets but not with increasing N in the foliar diets. Unlike the females, there was little similarity between performance on the lower casein diets and the early-season foliar diets. Interestingly, weights on the 25 and 100 percent casein diets were similar to those on the white and black spruce foliar diets showing a range of average weights from 70.4 ± 6.7 mg to 74.8 ± 13.3 mg on the four diets. These similarities probably reflect the tendency for male spruce budworm to stabilize weight gain over a threshold N level (Mattson *et al.* 1983).

The number of days required to pass through the pupal stage was longer for spruce budworm reared on artificial diets than for those on both early- and late-season foliar diets. Females on artificial diets took about 7.5 days which

was about two days longer than those on early-season foliage and about one day longer than on late-season foliage. Males on artificial diets took an average of 7.6 days which was about one day longer than their counterparts on foliage diets collected in both seasons. These differences may be due, in part, to the inherent differences between artificial and foliar diets or to other factors such as budworm population differences or to timing of application of second-instar larvae to the artificial diet.

Five other rearing trials that I conducted of spruce budworm on the standard McMorran diet (100 percent casein) showed that the number of days spent as pupae did not vary between sexes but did vary significantly between two of the trials. Mean values for the number of days spent as pupae varied from 6.3 to 8.5 days. Because all attempts were made to standardize the rearing of the budworm on the diet, this variability may represent population differences or perhaps inadequacies in the mass rearing of the insect according to Grisdale (1972). Mulye and Gordon (1990) suggest that the rearing method can be improved by the use of antiseptic procedures for setting up and maintaining larval cultures and the maintaining of a relative humidity of 65 ±2 percent in a controlled environment condition. These authors assert that their method of rearing spruce budworm larvae results in higher survival, synchronous development and less variability in larval growth than the Grisdale (1972) method.

Contrasting fecundity for females reared on artificial compared to foliar diets again showed that there was no clear weight/fecundity relationship. The heaviest female pupal weights occurred on the 100 percent casein diets but the greatest average number of eggs (373) were laid by females reared on the balsam fir early-season, foliar diet. Although the 100 percent casein diet is a "luxury" diet which contained the highest N level of the diets, as mentioned, it

may not be a nutritionally optimal one for spruce budworm. On the artificial diets, the highest number of eggs (247) was laid on the 25 percent casein diet, but this number also does not compare with the fecundity on the balsam fir foliage. These findings are contrary to those of McMorran (1965) who reported higher fecundity for spruce budworm reared on the standard diet than on balsam fir buds.

Spruce budworm performance varied with the four levels of casein used in this experiment; it was greatest on the 25 and 100 percent casein diets and poorest on the 12 percent casein diet. Because performance would be poorest on a diet containing less than 12 percent casein, it was decided that these same four levels would be used in experiment 4.

EXPERIMENT 4

DETERMINATION OF THRESHOLD IF:N ABOVE WHICH SPRUCE BUDWORM PERFORMANCE DECLINES

PURPOSE

The purpose of this investigation was to rear spruce budworm on diets with varying cellulose:casein N to test the hypothesis that there is a threshold IF:N above which spruce budworm performance declines. Indigestible fibres interfere with insect feeding by providing mechanical toughness to food sources and by combining with and making unavailable nutrients and midgut enzymes. Integrated with a N-poor diet, an imbalance of IF:N probably has negative dietary effects on spruce budworm performance.

METHODS

Diet Preparation

Nine diets were prepared with varying cellulose:casein N; one standard McMorran diet and eight experimental (Table 7). Casein was added, as in experiment 3, at levels which were 12, 18, 25, and 100 percent casein in the standard diet. Powdered alphacel was then added to the base IF level (Appendix XV) to obtain cellulose:casein N ratios of 15:1, and 50:1; the standard diet McMorran diet has a ratio of 3:1. Diets were poured into labelled,

5 cm diameter petri dishes and stored fresh in a refrigerator for three or four days before use.

Table 7. Numbers assigned to each of the 9 diets.

Cellulose:casein N	Percent Casein in Standard Diet			
	100%	25%	18%	12%
Standard (3:1)	1			
15:1	2	4	6	8
50:1	3	5	7	9

Insect Rearing Trials

Second-instar spruce budworm larvae were reared to the end of the fourth instar on the standard diet in 5 cm diameter petri dishes, six larvae per dish. Twelve-hours-old, or less, fifth-instar larvae were individually and randomly assigned to one of the 9 experimental diets. There were 10 females and 10 males per diet for a total of 180 budworm. When these budworm had molted to the sixth instar, they were transferred to fresh experimental diets. The budworm were reared at 21^{o}C with 18:6 L:D photoperiod.

At each of the fifth and sixth instars and pupal molts, fresh weights and number of days spent in each instar were recorded. Total development time was also noted. Pupae, moths and eggs were treated according to the methods of experiment 2.

Experimental Design

The design of the experiment was a two-way factorial with factors diet and sex having 9 and two levels, respectively. There was even replication (10 male and 10 female replicates per diet) in a completely randomized design structure. Data were analyzed as stated in experiment 3.

RESULTS

Survival

Survival was very high on all diets (Figure 17). It was 100 percent for both female and male spruce budworm on the standard McMorran diet. On diets with 15:1 cellulose:casein N, female survival ranged from 70 percent on the 12 percent casein diet to 90 percent on the 100 percent casein diet. Male survival ranged from 70 to 100 percent on those same two diets, as well. On diets with 50:1 cellulose:casein N, the lowest survival rate occurred on the 12 percent casein diet upon which 60 percent of females survived. This rate for females increased to 100 percent survival on both 25 and 100 percent casein diets. Male survival ranged from 80 to 100 percent on the 12 and 25 percent casein diets, respectively.

Development

Number of Days in Fifth Instar

On all diets, female and male spruce budworm did not differ significantly in the number of days spent in fifth instar (Figure 18 and Appendices XVI and XVII). On diet 9, the difference in number of days between the two sexes were almost significant (P = 0.0524). Among diets, there were no significant differences.

Number of Days in Sixth Instar

On nearly all diets, female and male spruce budworm differed significantly in the number of days spent in sixth instar (Figure 19 and Appendices XVI and XVII). Diet 9 was the exception upon which females and males did not differ significantly. Among diets, there were no significant differences.

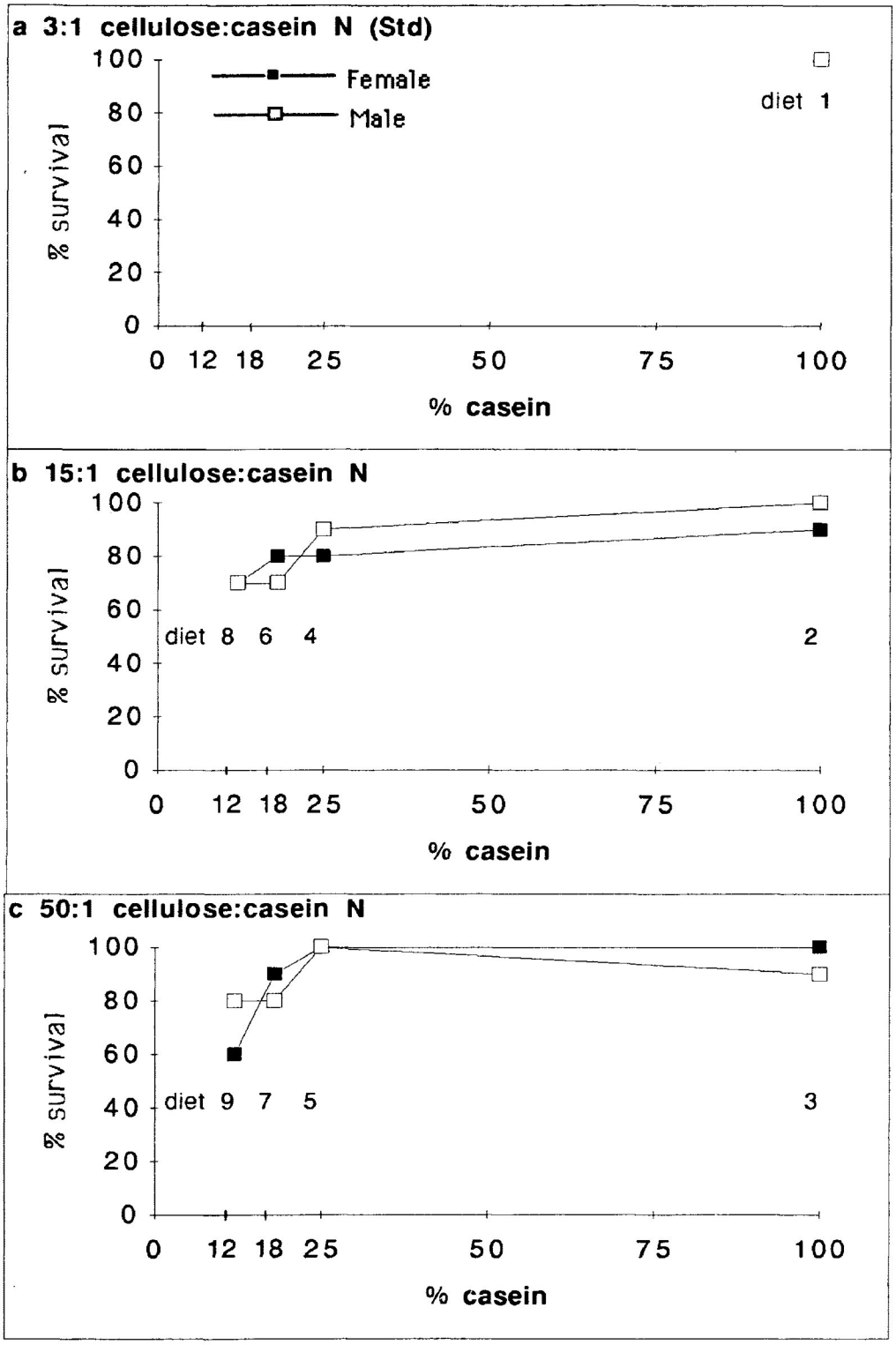


Figure 17. Percent survival of female and male spruce budworm from fifth instar to pupa on artificial diets with 12, 18, 25, and 100% casein in standard McMorran diet and cellulose:casein N levels of a) 3:1 (Std), b) 15:1, and c) 50:1.

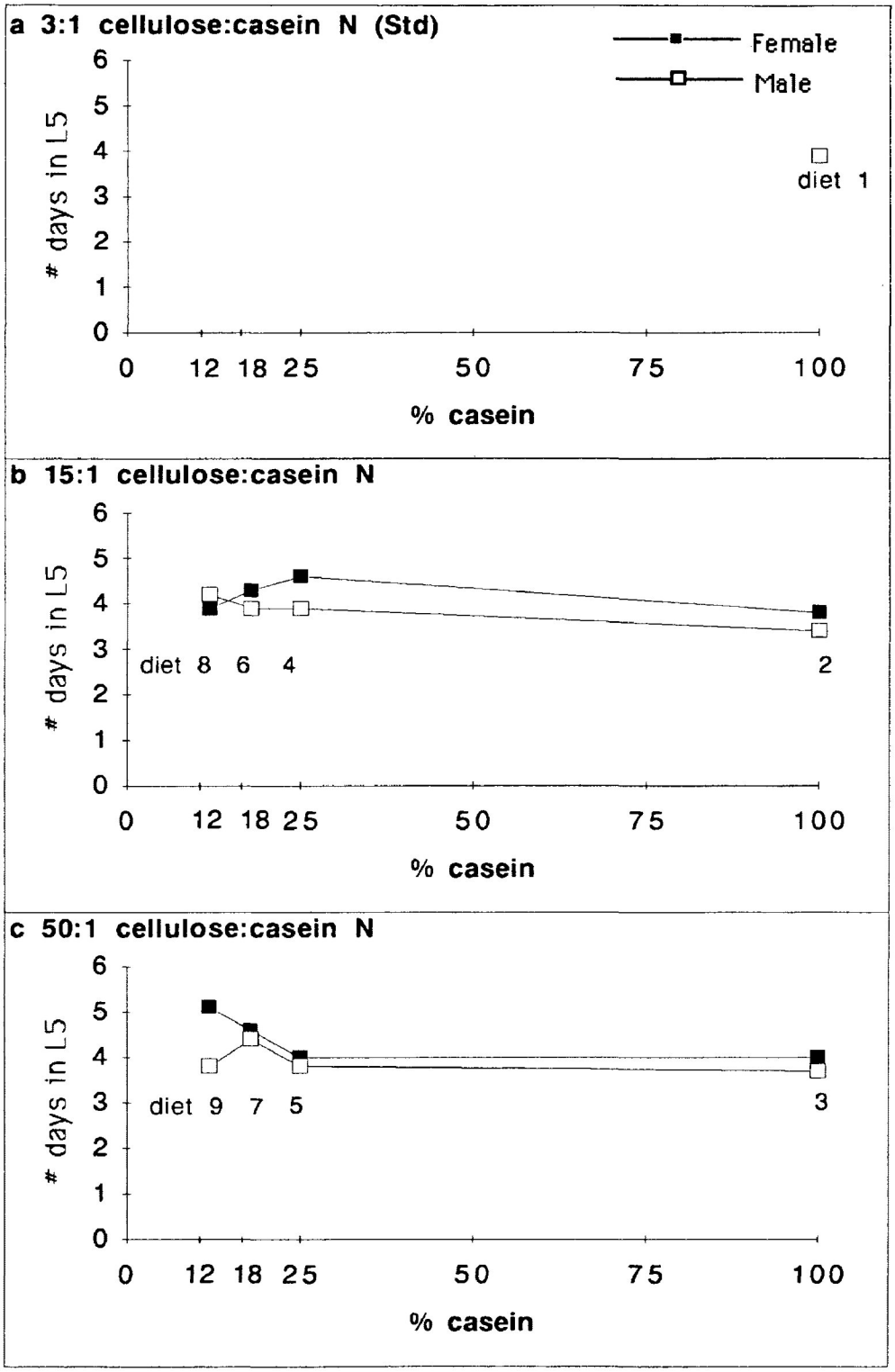


Figure 18. Mean number of days spent in fifth instar (L5) by female and male spruce budworm reared from L5 to pupa on artificial diets with 12, 18, 25, and 100% casein in standard McMorran diet and cellulose:casein N levels of a) 3:1 (Std), b) 15:1, and c) 50:1.

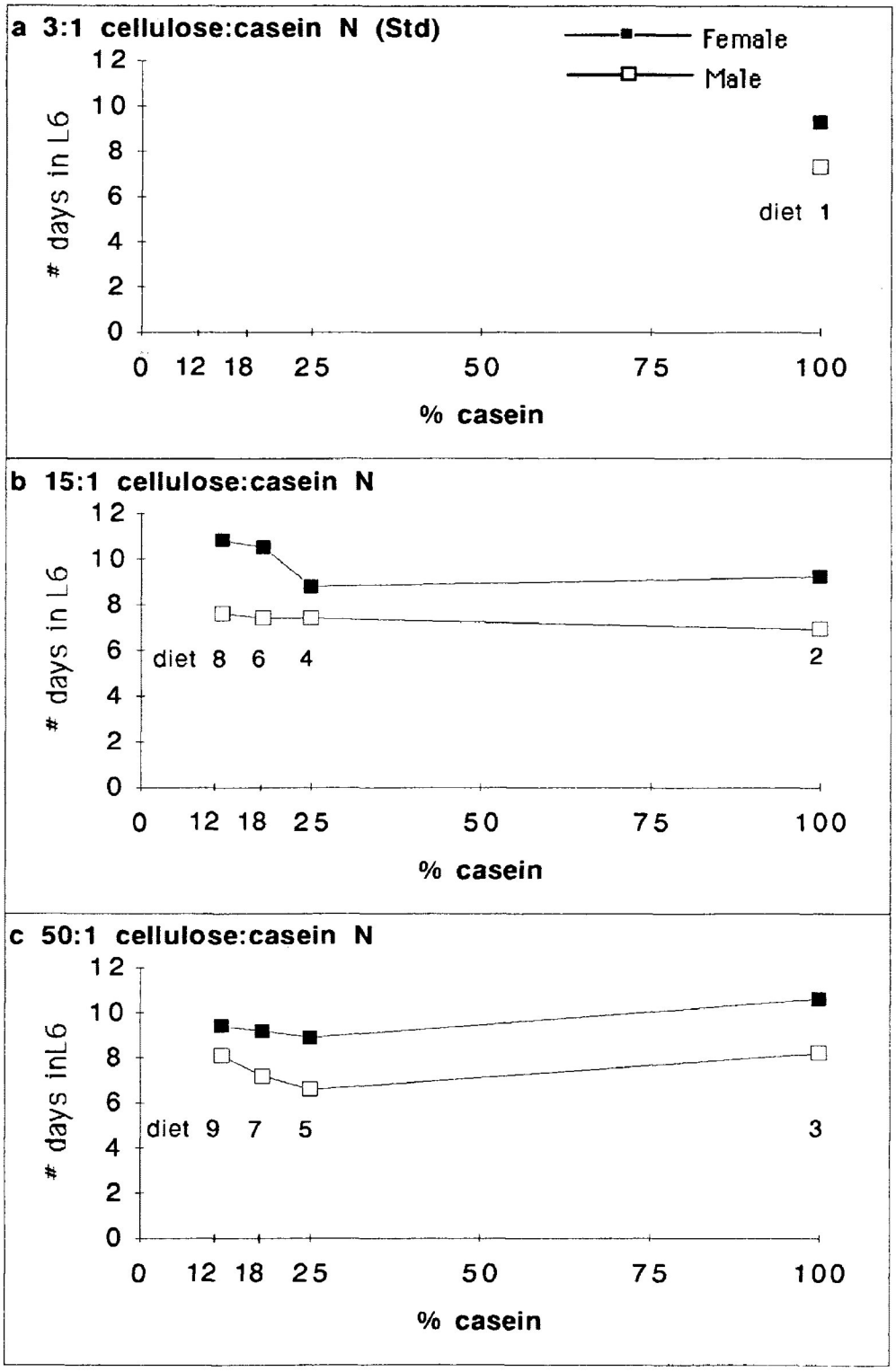


Figure 19. Mean number of days spent in sixth instar (L6) by female and male spruce budworm reared from fifth instar to pupa on artificial diets with 12, 18, 25, and 100% casein in standard McMorran diet and cellulose:casein N levels of a) 3:1 (Std), b) 15:1, and c) 50:1.

Number of Days in Pupa

On all but two diets, female and male spruce budworm did not differ significantly in the number of days spent in the pupal stage (Figure 20 and Appendices XVI and XVII). The significant differences were found on diets 6 and 7 with 18 percent casein and 15:1 and 50:1 cellulose:casein N, respectively. Among diets, there were no significant differences in number of pupal development days.

Weights

Weight at Fifth Instar

Female spruce budworm had significantly heavier first-day, fifth-instar weights than did male budworm on two diets only (Figure 21 and Appendices XVIII and XIX). These differences occurred on diets 2 and 6 with 15:1 cellulose:casein N and 100 and 18% casein, respectively. Among diets, there were no significant differences.

Weight at Sixth Instar

Female budworm were significantly heavier than males at sixth instar on all diets except 4 and 8 with 15:1 cellulose:casein N and 25 and 12% casein, respectively, and diet 5 with 50:1 cellulose:casein N and 25% casein (Figure 22 and Appendices XVIII and XIX). There were no significant sex differences on diets 5, 6 and 9.

Significant differences among diets occurred only for those with 12 percent casein, female budworm on diet 9 with 50:1 cellulose:casein N were significantly heavier than females on diet 8 with 15:1 cellulose:casein N (Figure 23a). Among diets with 15:1 cellulose:casein N, females on diet 2 with 100 percent casein were significantly heavier than females reared on the other three diets. There were no significant differences among males among any of the diets (Figure 23b).

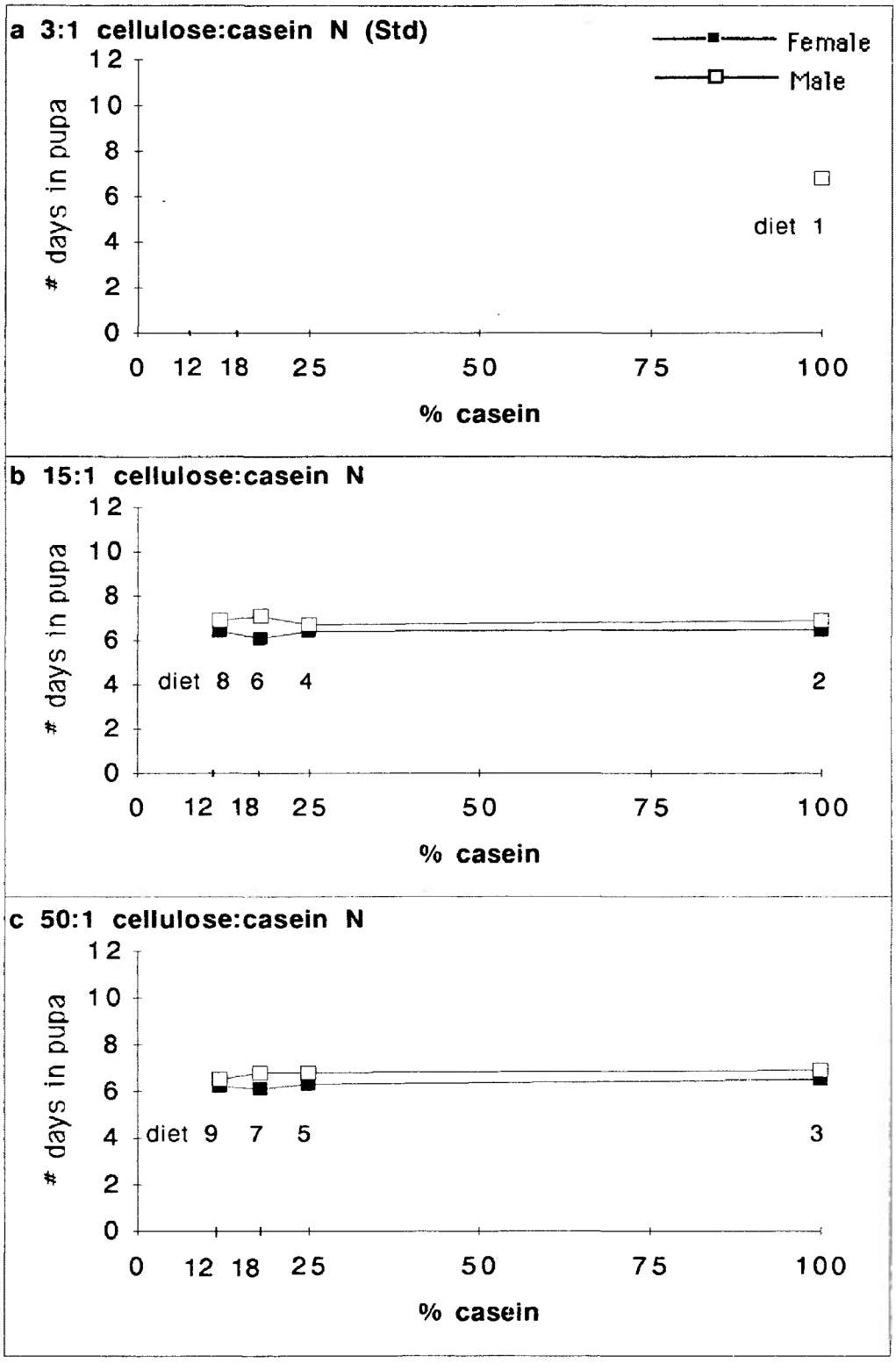


Figure 20. Mean number of days spent in pupa by female and and male spruce budworm reared from fifth instar to pupa on artificial diets with 12, 18, 25, and 100% casein in standard McMorran diet and cellulose:casein N levels of a) 3:1 (Std), b) 15:1, and c) 50:1.

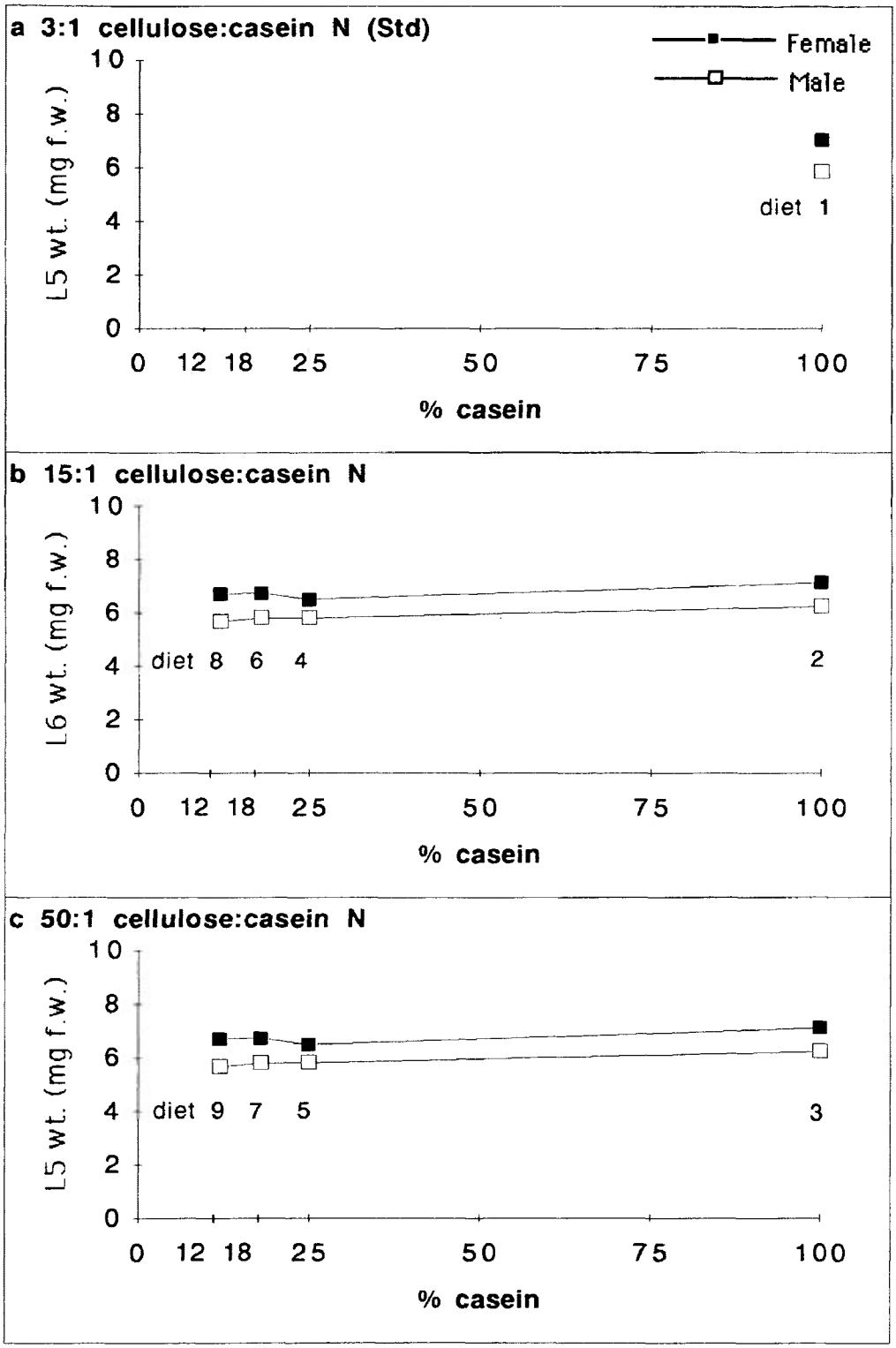


Figure 21. Mean weight on first-day of fifth instar (L5) female and male spruce budworm which were reared from L5 to on artificial diets with 12, 18, 25, and 100% casein in standard McMorran diet and cellulose:casein N levels of a) 3:1 (Std), b) 15:1, and c) 50:1.

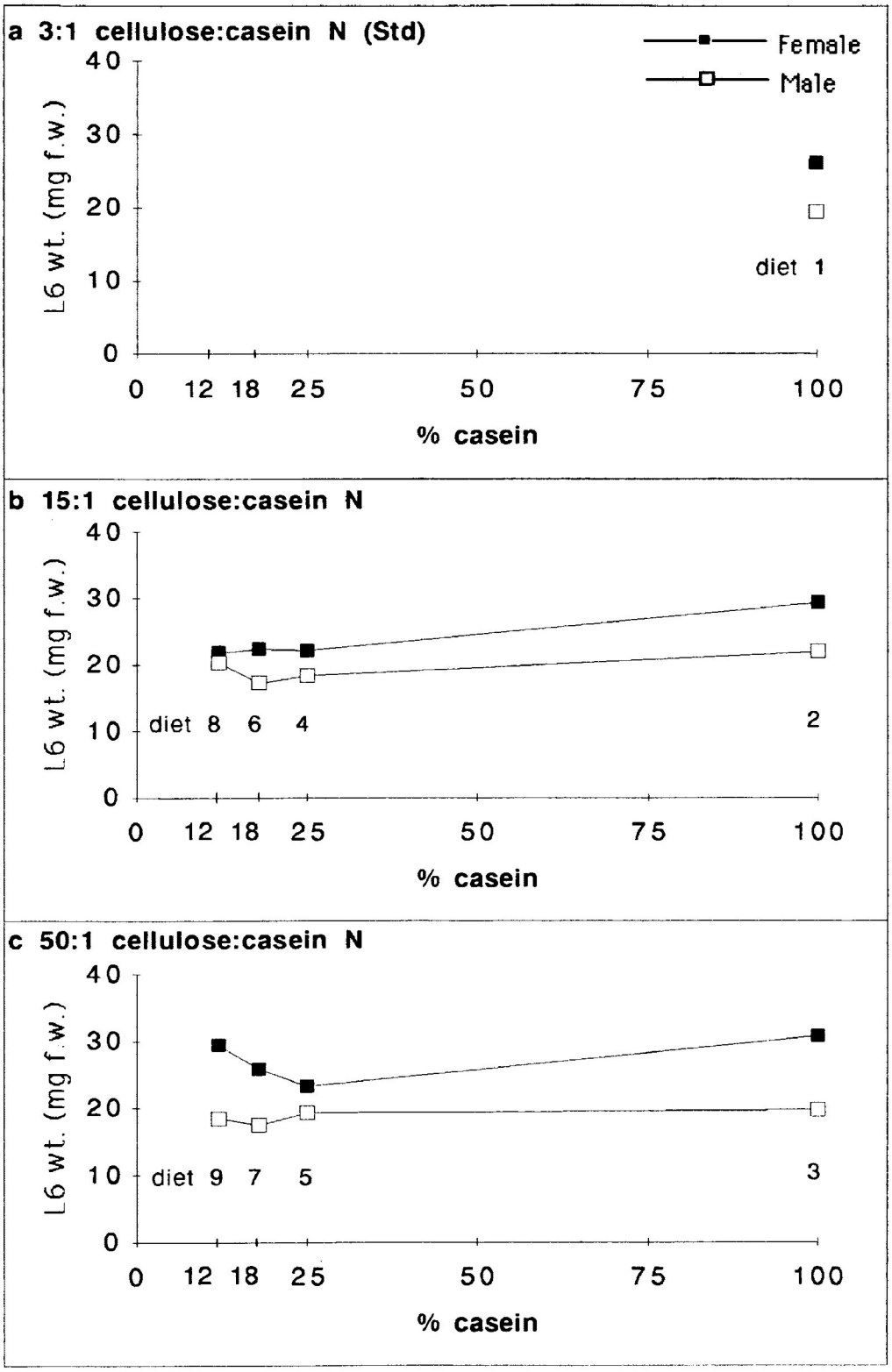
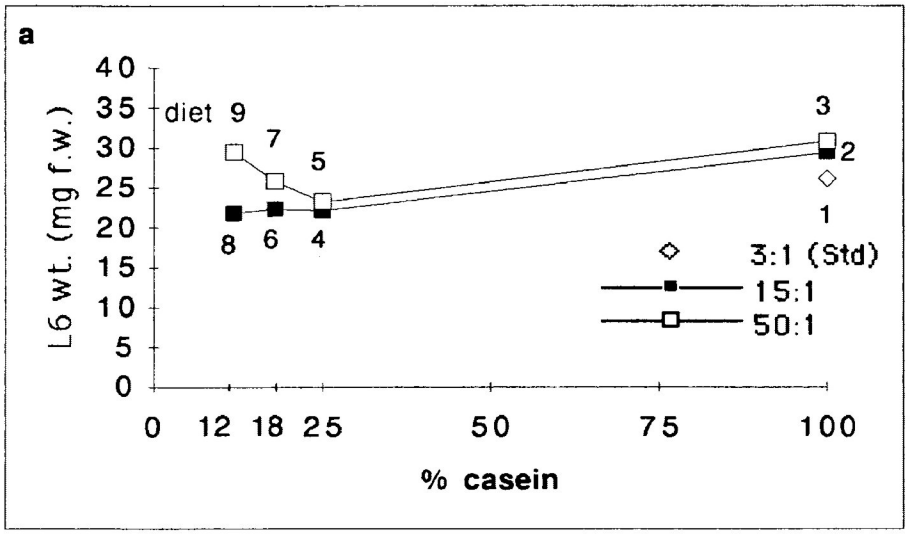


Figure 22. Mean weight on first-day of sixth instar (L6) female and male spruce budworm which were reared from fifth instar to pupa on artificial diets with 12, 18, 25, and 100% casein in standard McMorran diet and cellulose:casein N levels of a) 3:1 (Std), b) 15:1, and c) 50:1.



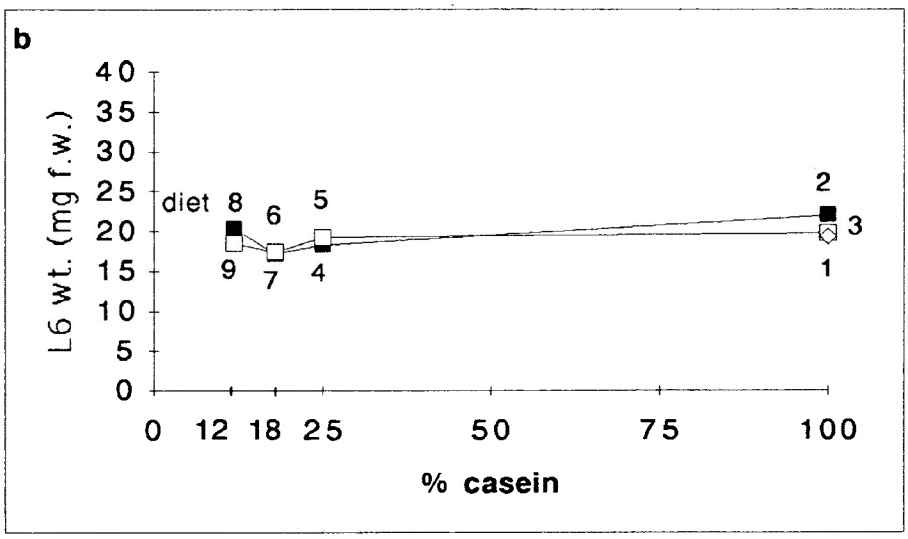


Figure 23. Mean weight on first-day of sixth instar (L6) for a) female and b) male spruce budworm which were reared from fifth instar to pupa on artificial diets with 12, 18, 25, and 100% casein in standard McMorran diet and varying cellulose:casein N.

Pupal Weight

Female budworm yielded significantly heavier pupae than male budworm on all diets (Figure 24 and Appendices XVIII and XX).

Among diets with 100 percent casein, females on diet 2 were significantly heavier than their counterparts on diets 1 and 3 but females on diets 1 and 3 did not themselves differ (Figure 25a). Males on diets 1 and 2 did not differ significantly from each other and neither did those on 1 and 3; however, males on diet 2 were significantly heavier than their counterparts on diet 3 (Figure 25b). There were no significant differences among diets with 25, 18 or 12 percent casein.

Among diets with 15:1, cellulose:casein N, both females and males on diet 2 were significantly heavier than their counterparts on the other three diets which themselves did not differ (Figures 25a and b). Among diets with 50:1, cellulose:casein N, males on diet 3 were significantly heavier than males on diets 9 and 7 but not on diet 5. Those on diets 9, 7 and 5 did not differ significantly. Females did not differ in weight among diets with 50:1 cellulose:casein N.

Fecundity

Female budworm reared on diets with 100% casein laid the greatest number of eggs on diet 2, followed by diet 3 and diet 1 (Figure 26). Females reared on diets with 18 and 25 percent casein, laid approximately the same numbers of eggs. On diets with 12 percent casein, females laid a greater number of eggs on diet 9 than on diet 8 .

Female budworm reared on diets with 15:1 (cellulose:casein N) generally laid increasing numbers of eggs on diets with increasing casein levels. The greatest number of eggs were laid on diet 2; similar amounts, on diets 4 and 6, and the least number, on diet 8. On diets with 50:1

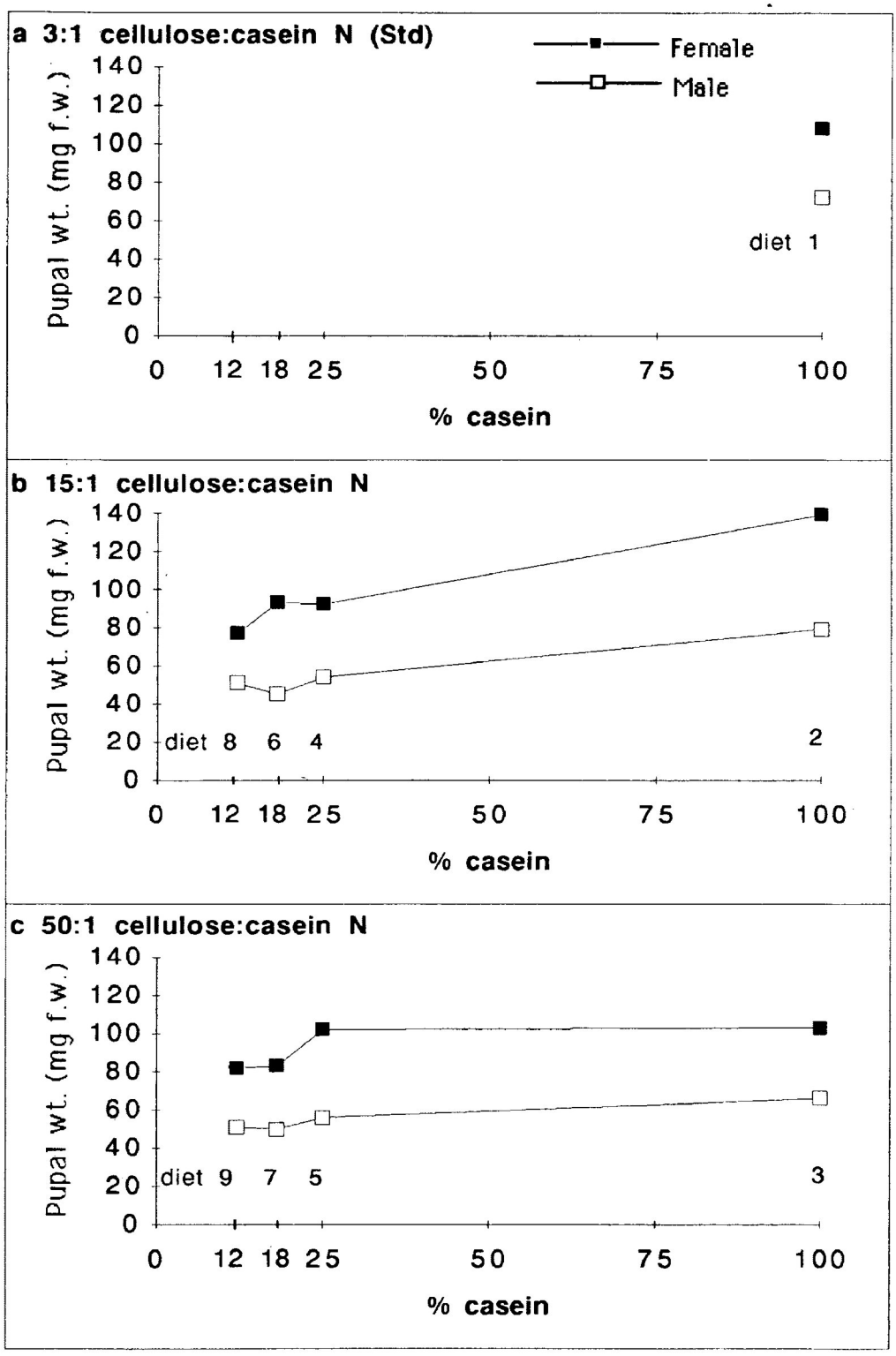
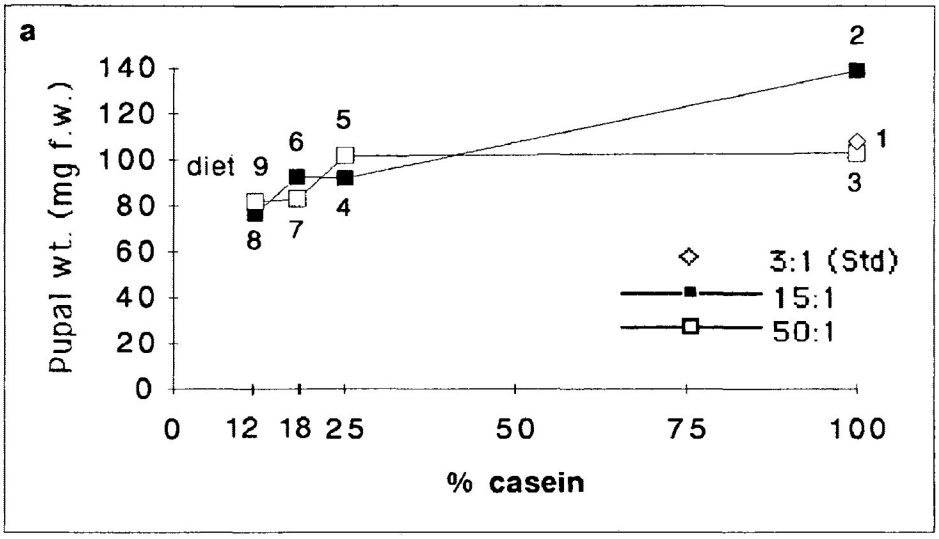


Figure 24. Mean first-day pupal weights of female and male spruce budworm which were reared from fifth instar to pupa on artificial diets with 12, 18, 25, and 100% casein in standard McMorran diet and cellulose:casein N levels of a) 3:1 (Std), b) 15:1, and c) 50:1.



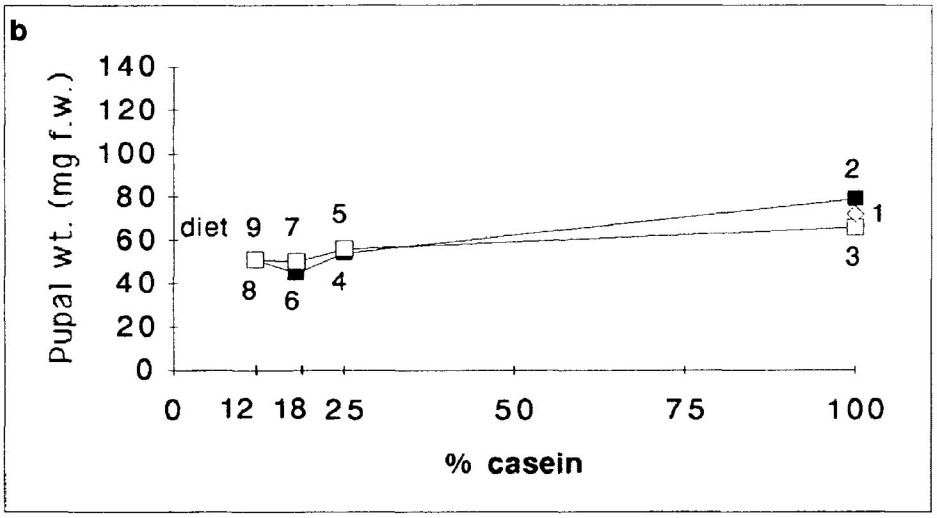


Figure 25. Mean first-day pupal weights for a) female and b) male spruce budworm which were reared from fifth instar to pupa on artificial diets with 12, 18, 25, and 100% casein in standard McMorran diet and varying levels of cellulose:casein N.

(cellulose:casein N), increasing numbers of eggs were laid by females reared on diets with increasing levels of casein. The greatest number of eggs were laid by females reared on diet 3, followed by diet 5, diet 7, and diet 9.

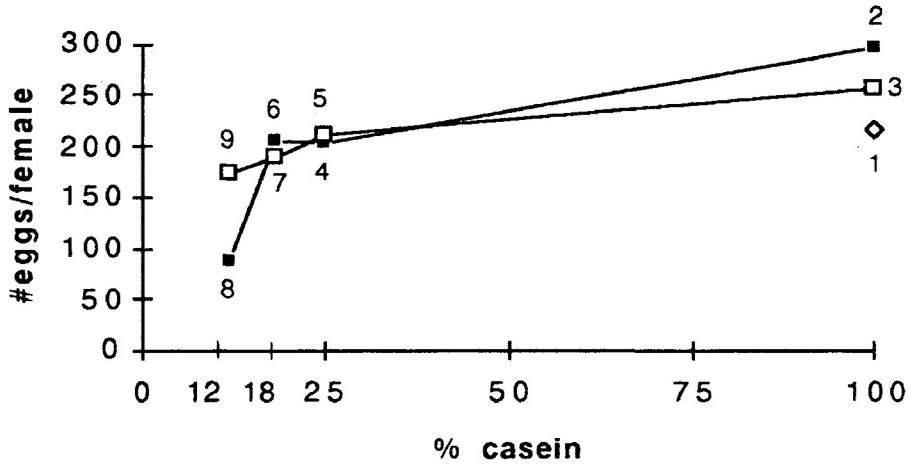


Figure 26. Mean number of eggs laid per female spruce budworm which were reared on diets with 12, 18, 25, and 100% casein in standard McMorran diet and varying cellulose:casein N.

DISCUSSION

Spruce budworm performance was minimally affected by treatment diets with varying cellulose:casein N. Increasing dietary N had no significant effects on development times and weight responses for sixth instar, and differences among pupa were significant only on diet 2 with 100 percent casein and 15:1 cellulose:casein N. In addition, survival was generally high for all levels of casein ranging from 70 to 100 percent. These results are in contrast to those of experiment 3 in which the same levels of casein were employed and for which significant effects occurred with development times for fifth and sixth instars and

weights of fifth and sixth instars and pupae. Survival ranged from 35 to 100 percent.

The probable reason for the lack of responses in this experiment and for the differences related in experiment 3 is that budworm were reared from second to the end-of-sixth instar on the treatment diets, whereas, in this investigation they were reared from second to the end-of-fourth instar on the standard diet and then transferred as fifth instars to the treatment diets. It appears that the nitrogen-rich, early diet optimized some performance factors later in the life cycle of those larvae transferred to the very casein N-poor diets. This was especially apparent for spruce budworm reared on the 12 percent casein diets. In this experiment, spruce budworm larvae reared on 12 percent casein in the standard diet and 15:1 and 50:1, cellulose:casein N, respectively, (diets 8 and 9) showed greater mean survival and fecundity and shorter mean development times than larvae reared on the 12 percent casein in the standard McMorran diet (diet 1) related in experiment 3.

Other investigations have reported similar findings. Harvey (1974) used transfer experiments and two artificial diets that mimicked early (high N, low sugar) and late (low N, high sugar) season foliage in order to study the nutritional needs of spruce budworm in relation to its host foliage. Harvey reported that budworm that were started (as second- and third-instar larvae) on the early diet and then transferred (as late third- and early fourth-instar larvae) to the late diet developed more quickly than those that were started on the late diet and then transferred to the early diet, although the final weights averaged out to be about the same. In another transfer experiment, Mattson *et al.* (1983) used two different diets to assess any enhancing effect on ultimate weight gain that would be incurred by feeding second- to fourth-instar spruce budworm larvae on a high N McMorran diet and then transferring them at fifth instar to a

low N foliage diet. Mattson *et al.* (1983) reported that budworm started (as second to fourth instars) on the artificial diet and transferred to balsam fir foliage diets were significantly larger than foliage to foliage reared insects. These authors observed that the early diet N ration seemed to "bump up" final adult weights.

Increasing dietary fibre through cellulose additions had minimal effect on spruce budworm performance as well. Powdered cellulose added to the artificial diets in varying levels was found to have little effect on spruce budworm survival, development, growth and fecundity, similar to reports of investigations with other insects (McGinnis and Kasting 1967; Peterson *et al.* 1988; Slansky and Wheeler 1991). In studies with grasshoppers (McGinnis and Kasting 1967) and southern armyworms,*Spodoptera eridanis* (Cramer), (Peterson *et al.* 1988) inclusion of powdered cellulose in artificial diets did not affect development. Rather, lowered digestibility resulted which was compensated for by increased consumption. Peterson *et al.* (1988) reported that southern armyworm showed no significant differences in nutritional indices due to varying levels of cellulose but dry weight of feces did differ significantly. Although not measured, I also observed that spruce budworm larvae reared on diets with 50:1 cellulose:casein N produced greater amounts of feces than those reared on 15:1, indicating increased consumption in the former.

Timmins *et al.* (1988), on the other hand, reported that even with compensatory feeding tobacco hornworm caterpillars suffered significantly reduced growth when reared on artificial diets with varying levels of cellulose. They reported that the increased rates of consumption were insufficient to compensate for the lowered levels of nutrients in the diets.

Two conclusions may be drawn from these investigations. First, that powdered cellulose probably acts as an inert diluent to nutrients in budworm

artificial diets that does not influence digestion (McGinnis and Kasting 1967; Peterson *et al.* 1988). Second, spruce budworm are well adapted to feeding on low quality diets which Koller (1987) also stated and reported that compensation by increased feeding was effective.

While powdered cellulose appears to have little effect on the availability or digestibility of nutrients in artificial diets, this conclusion cannot be extended absolutely to the natural system (Peterson *et al.* 1988; Slansky and Wheeler 1991). In this experiment, powdered cellulose was added to the artificial diets in ratios of 15:1 and 50:1 (cellulose:casein N) to mimic naturally occurring IF:N in spruce budworm host foliage in the early and late growing season. In no way, though, does cellulose powder resemble the tightly bound matrix of interlocking structures that characterize plant cell walls, especially at the end of the growing season when cell wall formation is complete (Northcote 1989). As fibre is accrued by cell walls, leaf toughness increases correspondingly making insect chewing difficult. This combined with the potential for fibres to join with nutrients and midgut enzymes and render them unavailable for insect digestion gives indigestible fibres the characteristics that make them protective for plants against insect feeding.

Several studies have reported negative effects on lepidopteran larval growth and development as a result of feeding on foliage holding high levels of IF and low levels of N. Benz (1974) and Baltensweiler *et al.* (1977) reported reduced larval and pupal weights and survival rates for larch budmoth when reared on needles of larch with high IF and low N levels. Bauce and Hardy (1988) found that spruce budworm reared on balsam fir foliage with high IF content had longer mean development times, reduced pupal weight and increased mortality rates compared to budworm fed on low IF content. Although these investigations point to a negative effect of IF on lepidopteran

development and performance, they cannot identify it as the sole cause because of the many constituents contained within foliage besides IF. As is evident from the results of this investigation, the simple addition of powdered cellulose is inadequate at imitating the possible effects of foliar IF on spruce budworm performance.

Varying levels of cellulose generally had little effect on budworm performance but an unexpected positive effect on pupal weight and fecundity occurred by increasing cellulose levels to 15:1, cellulose:casein N, within the treatments containing 100 percent casein of the standard diet. Females reared on the 15:1, cellulose:casein N diet, (diet 2) had 28 percent heavier pupal weights and laid 36 percent more eggs than females on the standard McMorran diet (diet 1) which contains 1:3, cellulose:casein N. From these results it seems possible that an increase in cellulose levels to about 15:1, cellulose:casein N, in the standard McMorran diet could be beneficial to spruce budworm performance. Perhaps this artificial diet is deficient in a dietary requirement for fibre which may be as important to insects as it is to mammals (Burkitt *et al.* 1974: Heaton 1983). Another potential inadequacy in the diet was suggested in experiment 3 where fecundity on the 25 percent casein diet was found to be superior to that on the 100 percent casein diet. Although not strictly comparable because of different IF levels, results on the 25 percent casein diets of this investigation do not corroborate those of the previous experiment. Even so, the data do suggest that there is room for improvement in the McMorran diet for the rearing of spruce budworm.

FUTURE STUDIES

The results of my study were inconclusive that a threshold IF:N exists above which spruce budworm performance declines. Modifications should be made to the methods of experiment 4 to investigate this hypothesis more thoroughly. Intact IF, rather than powdered cellulose, should be added to the artificial diet to accurately assess its effect on budworm fitness. To accomplish this, a technique would have to be developed to ensure complete chemical digestion of foliage that is intact rather than ground into a powder as is currently required by the digestion method of Goering and Van Soest (1970).

A further modification would be to rear spruce budworm from second instar to pupa on treatment diets with varying IF:N. In experiment 3, a lower limit of dietary N was determined below which budworm performance was poor. This was accomplished by observing performance of budworm reared from second instar to pupa on diets with varying casein. These levels should again be incorporated into diets with varying IF:N but spruce budworm should be reared as they were in the preliminary experiment to ensure that the pre-determined lower N limit is relevant to the following experiment. Another option would be to run the varying IF:N experiment as a transfer experiment, as I did, but include two additional runs with the 12% casein diets (15:1 and 50:1, IF:N) upon which budworm are reared from second instar to pupa.

SUMMARY

The dietary relationship that spruce budworm has with its host trees is poorly understood. Spruce budworm have had to adapt to changing food quality which may be a resistance mechanism that protects trees against herbivorous attack (Whitham 1983). Food quality as assessed in this study by water, N, and IF varied little within the crowns of host trees balsam fir, white spruce, and black spruce. This spatial homogeneity of food source is advantageous to the insect, allowing current-year needles to be highly predictable and susceptible to defoliation. Food quality, on the other hand, deteriorated steadily through the growing season as evidenced by declining water and N levels and increasing IF and leaf toughness levels. This temporal heterogeneity may protect host trees by presenting a relatively short period in early summer during which foliage is acceptable for insect consumption.

As early-season defoliators, spruce budworm experience superior fitness on early- compared to late-season foliage. Spruce budworm developed more rapidly, attained greater pupal weights and had higher fecundity when reared on early-season compared to late-season host foliage. Early-season foliage has higher levels of the nutritional components of N and water and lower levels of the allelochemical component of IF, in comparison to late-season foliage. It also has a low IF:N and relatively "soft" needles whereas late-season foliage has a high IF:N and significantly "harder" needles. Leaf toughness is known to be a deterrent to herbivore feeding (Coley 1983, Raupp 1985) and is greater in late-season needles ostensibly because of the IF accumulation associated with

cell wall maturation. I found, however, that for late-season foliage of white and black spruce with similar IF levels, black spruce foliage was significantly tougher than white spruce. Some constituent in addition to foliar IF may be contributing to leaf toughness.

Although I observed that spruce budworm performance was better on early- compared to late-season foliage, I wanted to determine which foliar constituents contributed to this result. Working towards this effort, I chose to vary N and IF:N in artificial diets. Nitrogen is considered to be a limiting factor to insect growth and is positively correlated to insect performance (Scriber and Slansky 1981) while IF is thought to protect plants from insect feeding and negatively affects insect growth and development (Rhoades 1979; Baltensweiler *et al.* 1977). Changes in levels of N are inversely related to changes in levels of IF. Indigestible fibres have always been considered to be constitutive chemical defences (Rhoades and Cates 1976) but several investigations have suggested that they may also be inducible defences that are stimulated by insect feeding (Benz 1974; Bauce and Hardy 1988; Beaudette 1986).

I reasoned that if budworm feeding, at a given defoliation intensity, triggers a relative increase of foliar IF to other nutritional components essential to spruce budworm fitness, then there may be a threshold IF:N above which budworm performance declines. To test this hypothesis, I conducted two experiments. The first was a preliminary experiment in which levels of dietary N were varied to determine a lower limit below which performance would be poor. Using casein as the variable source of available N, budworm were reared from second instar to pupa on diets containing 12, 18, 25, and 100 percent casein in the standard McMorran diet. Budworm reared on the 12 percent casein diet had the poorest survival, fecundity, development and growth, and thus, it was

concluded that this level of casein would be the lowest one used in the second experiment.

Also it was observed that the weight/fecundity relationship identified for spruce budworm by Miller (1957) was not clear in this experiment. Females on the 25 and 100 percent casein diets laid approximately the same number of eggs in spite of pupal weights being significantly lighter on the 25 percent diet. Reducing the casein level in the McMorran diet may be an improvement to the diet which is very N-rich and may not be balanced for optimal egg production. In addition, females on the 18 percent diet laid three times as many eggs as those on the 12 percent diet even though their pupal weights did not vary significantly. This brings the reliability of the weight/fecundity relationship into question which Leather (1988) says should be treated with caution because it is frequently masked by other factors. Leather (1988) asserts that longevity is the single most important factor influencing fecundity within the Lepidoptera.

It should be noted that fecundity data presented in these experiments should be compared to other investigations with caution. The reason is that mating success was not determined and it is unknown whether each of the five females placed per mating jar actually mated. As a result, it is questionable if values of the fecundity variable 'number of eggs laid per female' are true averages of five females. Notwithstanding, I believe that the fecundity results are comparable among trials in this thesis. Technique and conditions of mating moths did not vary among trials and experiments, so relative comparisons are valid.

The fourth experiment was conducted to test if there is a threshold IF:N above which budworm performance declines. Cellulose and casein were manipulated in the artificial diet to mimic low (15:1) and high (50:1) IF:N as found in early- and late-season host foliage, respectively. Spruce budworm

were reared from second to fourth instars on the standard diet and then transferred as fifth instars to the treatment diets. Results from this experiment were inconclusive. Budworm performance was minimally affected by treatment diets with varying cellulose:casein N. In fact, especially on diets with 12 percent casein, performance was superior to that of the preliminary N experiment even though both used exactly the same levels of casein.

One possible explanation for these results is as follows. Rearing techniques differed between the two experiments which changed expected performances on the varying N levels and, in so doing, nullified the lower N limit which had been determined by the preliminary N experiment. Rearing early-instar larvae on the standard diet and then transferring them to treatment diets gave larvae a N-advantage which supported their growth when reared on the deemed protein-deficient diets. This experiment should have been run by either rearing the budworm on treatment diets from second instar to pupa or by including an additional run on the 12 percent casein diet with budworm reared from second instar to end-of-fourth instar.

These results however do suggest strongly that early instars which feed on N-rich tissues would be unaffected by extremely high IF:N as found in foliage later in the growing season. They also suggest that IF may have nothing to do with decreased budworm performance as suggested by Bauce and Hardy (1986); the effect observed may be due to other foliar compounds.

There appeared to be no threshold IF:N ratio above which budworm performance was adversely affected. The reason was probably due to the use of powdered cellulose which does not resemble the tightly bound structure of plant cell walls and does not appear to influence digestion. Instead, it probably acts as an inert diluent to nutrients in the artificial diet as observed by other investigators (McGinnis and Kasting 1967; Peterson *et al.* 1988). It is

recommended that this experiment be conducted again using intact IF rather than powdered cellulose in the artificial diet. Techniques would have to be developed to produce intact IF that would ensure complete nutrient removal. The inclusion of intact IF plus the rearing of budworm on treatment diets through their life cycles would greatly improve this experiment and serve toward an efficient testing of the hypothesis.

An unexpected result of this experiment was the improvement of budworm performance with increased fibre levels in the standard McMorran diet. Both female pupal weight and fecundity increased as a result of additional dietary fibre. An increased fibre level and reduced casein level has implications for improvement of the McMorran diet.

LITERATURE CITED

Alban, D.H. and R.F. Watt. 1981. Fertilization of black spruce on poor site peatland in Minnesota. U.S. Dept. Agric. For. Serv., Res. Paper NC-210, U.S. Dept. Agric. For. Serv., North Central For. Exp. Sta., St. Paul, Minnesota.

Atkins, A.G. and Y.W. Mai. 1979. On the guillotining of materials. J. Mat. Sci. 14:2747-2754.

Baltensweiler, W., G. Benz, P. Bovery and V. Delucchi. 1977. Dynamics of larch budmoth populations. Ann. Rev. Ent. 22:79-100.

Baltensweiler, W. and A.Fischlin. 1988. The larch budmoth in the alps. pp.331-351 *in* Berryman, A.A. (ed.) Dynamics of Forest Insect Populations: Patterns, Causes, Implications. Plenum Press, New York.

Batzer, H.O. 1973. Defoliation by the spruce budworm stimulates epicormic shoots on balsam fir. Envir. Ent. 2: 727-728.

Bauce, E. and Y. Hardy. 1988. Effects of drainage and severe defoliation on the rawfiber content of balsam fir needles and growth of the spruce budworm (Lepidoptera: Tortricidae). Envir. Ent.17: 671-674.

Bauer, L.S. and G.L. Nordin. 1988. Nutritional physiology of the eastern spruce budworm, *Choristoneura fumiferana* , infected with *Nosema fumiferanae*, and interactions with dietary nitrogen. Oecologia 77:44-50.

Beaudette, R. 1986. Variations interannuelles du contenu en fibre brute du feuillage de l'épinette blanche, *Picea glauca* (Moench) Voss, vs la tordeuse des bourgeons de l'épinette, *Choristoneura fumiferana* (Clem.), B.Sc.F Thesis, Laval University.

Benz, G. 1974. Negative Rückkoppelung durch Raum- und Nahrungskonkurrenz sowie zyklische Veränderungen der Nahrungsgrundlage als Regelprinzip in der Populationsdynamik des Grauen Lärchenwicklers, *Zeiraphera diniana* (Guenée) (Lepidoptera: Tortricidae). Z. angew. Ent. 76:196-228.

Berenbaum, M. 1983. Effects of tannins on growth and digestion in two species of papilionids. Ent. exp. appl. 34:245-250.

Berryman, A.A. 1988. Towards a unified theory of plant defense. pp. 39-53 *in* Mattson, W. J., J. Levieux and C. Bernard-Dagan (eds.) Mechanisms of Woody Plant Defenses Against Insects: Search for Pattern. Springer-Verlag, New York.

Blais, J.R. 1952. The relationship of the spruce budworm (*Choristoneura fumiferana*, Clem.) to the flowering condition of balsam fir (*Abies balsamea* (L.) Mill.) Can. J. Zool. 30:1-29.

Blais, J.R. 1953. Effects of destruction of the current year's foliage on the fecundity and habits of flight of the spruce budworm. Can. Ent. 85:446-448.

Blais, J.R. 1957. Some relationships of the spruce budworm, *Choristoneura fumiferana* (Clemens) to black spruce, *Picea mariana* (Moench) Voss. For. Chron. 13:364-372.

Bourne, M.C., J.C. Moyer, and D.B. Hand. 1966. Measurement of food texture by a universal testing machine. Food Technol. 20:170-174.

Brattsten, L.B. 1979. Biochemical defence mechanisms in herbivores against plant allelochemicals. pp 199-270 *in* Rosenthal G. and D. Janzen (ed.) Herbivores: Their Interaction With Secondary Plant Metabolites. Academic Press, New York.

Broadway, R.M., S.S. Duffey, G. Pearce, and C. Ryan. 1986. Plant proteinase inhibitors: a defense against herbivorous insects? Ent. exp. appl. 41:33-38.

Bryant, J.P., F.S. Chapin, III and D.R.Klein. 1983. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. Oikos 40:357-368.

Bryant, J.P., T.P. Clausen, P.B. Reichardt, M.C. McCarthy, and R.A. Werner. 1987. Effect of nitrogen fertilization upon the secondary chemistry and nutritional value of quaking aspen (*Populus tremuloides* Michx.) leaves for the large aspen tortix (*Choristoneura conflictana* (Walker)). Oecologia 73:513-517.

Burkitt, D.P., A.R.P. Walker, and N.S. Painter. 1974. Dietary fiber and disease. J. Am. med. Assoc. 229:1068.

Canadian Council of Forest Ministers. 1993. Compendium of Canadian Forestry Statistics. Canadian Council of Forest Ministers, Ottawa.

Clancy, K.M. 1991a. Western spruce budworm response to different moisture levels in artificial diets. For. Ecol. Manag. 39:223-235.

Clancy, K.M. 1991b. Douglas-fir nutrients and terpenes as potential factors influencing western spruce budworm defoliation. pp. 123-133 *in* Baranchikov, Y., W. Mattson, F. Hain, and T. Payne (eds.) Forest Insect Guilds: Patterns of Interaction with Host Trees. U.S. Dep. agric. For. Serv. Gen. Tech. Rep. NE-153.

Clancy, K.M. 1992. Response of western spruce budworm (Lepidoptera: Tortricidae) to increased nitrogen in artificial diets. Envir. Ent. 21:331-344.

Clancy, K.M., J.K Itami, and D.P. Huebner. 1993. Douglas-fir nutrients and terpenes: potential resistance factors to western spruce budworm defoliation. For. Sci. 39:78-94.

Clancy, K.M., M.R. Wagner and R.W. Tinus. 1988. Variations in nutrient levels as a defense: identifying key nutritional traits of host plants of the western spruce budworm. pp.203-213 *in* Mattson, W.J., J. Levieux and C. Bernard-Dagan (eds.) Mechanisms of Woody Plant Defenses Against Insects: Search for Pattern. Springer, New York.

Cochran, D.G. 1985. Nitrogenous excretion. pp.467-506 *in* Kerkut, G.A. and L.I. Gilbert (eds.) Vol. IV Regulation: Digestion, Nutrition, Excretion. Comprehensive Insect Physiology, Biochemistry and Pharmacology. Pergamon Press, Toronto.

Coleman, J.S. and C.G. Jones. 1990. A phytocentric perspective of phytochemical induction by herbivores. pp. 3-45*in* D.W. Tallamy and M.J. Raupp (eds.) Phytochemical Induction by Herbivores. Wiley, New York.

Coley, P.D. 1983. Herbivory and defensive characteristics of tree species in a lowland tropical forest. Ecol. Monogr. 53:209-233.

Coley, P.D., J.P. Bryant, and F.S Chapin III. 1985. Resource availability and plant antiherbivore defense. Science 230:898-899.

Curtis, H. 1975. Biology. Second Edition. Worth Publishers, Inc. New York, New York.

Dadd, R.H. 1985. Nutrition: Organisms. pp. 313-390 *in* Kerkut, G.A. and L.I. Gilbert (eds.) Vol. IV Regulation: Digestion, Nutrition, Excretion. Comprehensive Insect Physiology, Biochemistry and Pharmacology. Pergamon Press, Toronto.

Danthanarayana, W. 1975. Factors determining variation in fecundity of the light brown apple moth, *Epiphyas postvittana* (Walker) (Tortricidae). Aust. J. Zool. 23:439-451.

Dickman, D.L. and T.T. Kozlowski. 1969. Seasonal variations in reserve and structural components of *Pinus resinosa* cones. Amer. J. Bot. 56:515-520.

Dickson, R.E. 1979. Analytical procedures for the sequential extraction of 14 C-labelled constituents from leaves, bark and wood of cottonwood plants. Physiol. Plant. 45:480-488.

Duchesne, L.C. and D.W. Larson. 1989. Cellulose and the evolution of plant life. BioScience 4:238-241.

Du Merle, P. 1988. Phenological resistance of oaks to the green oak leafroller, *Tortrix viridana* (Lepidoptera: Tortricidae). pp.216-226 *in* Mattson, W.J., J. Levieux and C. Bernard-Dagan (eds.) Mechanisms of Woody Plant Defenses Against Insects: Search for Pattern. Springer, New York.

Emerson, J.D. and M.A. Stoto. 1988. Transforming data. pp. 97-128 *in* Hoaglin, D.C., F. Mosteller, and J.W. Tukey (eds.) Understanding Robust and Exploratory Data Analysis. John Wiley & Sons, Inc., New York, New York.

Ericcson, A., C. Hellqvist, B. Långström, S. Larsson and O. Tenow. 1985. Effects of simulated and induced shoot pruning by *Tomicus piniperda* as related to carbohydrate and nitrogen dynamics in Scots pine. J. Appl. Ecol. 22:105-124.

Faeth, S.H., E.F. Conner, and D. Simberloff. 1981. Early leaf abscission: a neglected source of mortality for folivores. Am. Nat. 117:409-415.

Feeny, P. 1970. Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. Ecology 51:565-581.

Feeny, P. 1976. Plant apparency and chemical defense. Recent Adv. Phytochem. 10:1-40.

Fowler, S.V. and J.H. Lawton. 1985. Rapidly induced defenses and talking trees: the devil's advocate position. Am. Nat. 126:181-195.

Gazelius, D., A. Ericsson, J.E. Hallgren, and L. Brunes. 1981. Effects of bud removal on Scots pine (*Pinus sylvestris*) seedlings. Physiol. Plant. 51:181-188.

Gilbert, N. 1986. Control of fecundity in *Pieris rapae* IV. Patterns of variation and their ecological consequences. J. Anim. Ecol. 55:317-329.

Grisdale, D.G. 1972. An improved method for producing large numbers of second-instar spruce budworm larvae, *Choristoneura fumiferana* (Lepidoptera: Tortricidae). Can. Ent. 104:1955-1957.

Goering, H.K. and P.J. Van Soest. 1970. Forage fiber analysis: apparatus, reagents, procedures and some applications. Agric. Hdbk. No. 379, Agric. Res. Serv., USDA, Wash., D.C.

Greenbank, D.O. 1963. Host species and the spruce budworm. pp. 219-223. *in* Morris, R.F. (ed.) The Dynamics of Epidemic spruce Budworm Populations. Mem. Ent. Soc. Can., No. 31.

Hagen, K.S., R.H. Dadd, and J. Reese. 1984. The food of insects. pp. 80-112 *in* Huffaker, C.B. and R.L. Rabb (eds.) Ecological Entomology. Wiley, New York.

Hall, P.J. and B. Moody. 1994. Forest depletions caused by insects and diseases in Canada, 1982 -1987. Forest Insect and Disease Survey Info. Rep. ST-X-8. Nat. Res. Canada, Can. For. Serv., Ottawa.

Harper, J.L. 1977. Population Biology of Plants. Academic Press Inc. (London) Ltd, London, U.K.

Hartley, S.E. and J.H. Lawton. 1990. Biochemical basis and significance of rapidly induced changes in birch foliage. pp. 105-132 *in* Tallamy, D.W. and M.J. Raupp (eds.) Phytochemical Induction by Herbivores. Wiley, New York.

Harvell, C. 1990. The ecology and evolution of inducible defenses. Quart. Rev. Bio. 65:323-340.

Harvey, G.T. 1974. Nutritional studies of eastern spruce budworm (Lepidoptera: Tortricidae). I. Soluble sugars. Can. Ent. 106:353-365.

Haukioja, E. 1980. On the role of plant defences in the fluctuation of herbivore populations. Oikos 35:202-213.

Haukioja, E. 1990. Induction of defenses in trees. Ann. Rev. Ent. 36:25-42.

Haukioja, E. and S. Neuvonen. 1985. Induced long-term resistance of birch foliage against defoliators: defensive or incidental? Ecology 66:1303-1308.

Haukioja, E. and S. Neuvonen. 1987. Insect population dynamics and induction of plant resistance: the testing of hypotheses. pp. 411-432 *in* Barbosa, P. and J. Schultz (eds.) Insect Outbreaks. Academic, San Diego.

Haukioja, E., J. Suomela, and S. Neuvonen. 1985. Long-term inducible resistance in birch foliage: triggering cues and efficacy on the defoliator. Oecologia 65:363-369.

Heaton, D.W. 1983. Dietary fibre: concepts and definitions. pp.19-21 *in* Wallace, G. and L. Bell (eds.) Fibre in Human and Animal Nutrition. Roy. Soc. N.Z. Bull. 20.

Heron, R.J. 1965. The role of chemotactic stimuli in the feeding behaviour of spruce budworm larvae on white spruce. Can. J. Zool. 43:247-269.

Hough, J.A. and D. Pimental. 1978. Influence of host foliage on development, survival, and fecundity of the gypsy moth. Envir. Ent. 7:97-102.

House, H.L. 1969. Effects of different proportions of nutrients on insects. Ent. exp. appl. 12:651-669.

Hunter, A.F. and M.J. Lechowicz. 1992. Foliage quality changes during canopy development of some northern hardwood trees. Oecologia 89:316-323.

Karban, R. and J. Myers. 1989. Induced plant responses to herbivory. Ann. Rev. Ecol. Syst. 20:331-348.

Klomp, H. 1958. Larval density and adult fecundity in a natural population of the pine looper (*Bupalus piniarius* L.). Arch. Neerl. Zool. 13 Suppl. 1:319-334.

Koller, Clifford N. 1987. Chemical defenses against herbivory on balsam fir (*Abies balsamea* (L.) Mill.): natural variation in foliage nitrogen and condensed tannin, and its effect on survival, growth and development of spruce budworm (*Choristoneura fumiferana* (Clemens)) in the field. Ph.D. Thesis, U. of Minnesota. 140 pp. (Microfilm).

Koller, C.N. and D.E. Leonard. 1981. Comparison of energy budgets for spruce budworm, *Choristoneura fumiferana* (Clemens) on balsam fir and white spruce. Oecologia 49:14-20.

Kozlowski, T.T. and J.J. Clausen. 1965. Changes in moisture contents and dry weights of buds and leaves of forest trees. Botan. Gaz. 126:20-26.

Kramer, P.J. and T.T. Kozlowski. 1960. Physiology of Trees. McGraw-Hill Book Company, Inc., New York.

Larcher, W. 1983. Physiological Plant Ecology. Corrected printing of the 2d Ed. (Trans. from German). Springer, New York.

Lavallée, R. and Y. Hardy. 1988. Étude en laboratoire du développement du *Choristoneura fumiferana* sur l'*Abies balsamea*, le *Picea glauca* et le *Picea rubens*. Phytoprotection 69:79-86.

Leather, S.R. 1988. Size, reproductive potential and fecundity in insects: things aren't as simple as they seem. Oikos 51:386-389.

Leather, S.R., Watt, A.D., and D.S. Barbour. 1985. The effect of host plant and delayed mating on the fecundity and lifespan of the pine beauty moth, *Panolis flammea* (D&S): their possible influence on population dynamics and pest management strategies. Bull. Ent. Res. 7:641-651.

Lewis, N., R. Razal, E. Yamamoto, G. Bokelman, and J. Wooten. 1989. ^{13}C specific labelling of lignin in intact plants. pp. 169-181 *in* Lewis, N.G. and M.G. Paice (eds.) Plant Cell Wall Polymers - Biogenesis and Biodegradation. 3rd Chem. Cong.North America (195th National Meeting of the American Chemical Society), Toronto, Ont., Can., June 5-11, 1988. ACS Symposium Series 399. Am. Chem. Soc., Wash., DC.

Lewinsohn, E., M. Gijzen, and R. Croteau. 1991. Defense mechanisms of conifers: differences in constitutive and wound-induced monoterpene biosythesis among species. Plant Physiol. 96:44-49.

Lieutier, F. and A.A. Berryman, 1988. Elicitation of defensive reactions in conifers. pp.313-319 *in* Mattson, W.J., J. Levieux and C. Bernard-Dagan (eds.) Mechanisms of Woody Plant Defenses Against Insects: Search for Pattern. Springer, New York.

Loader, C. and H. Damman. 1991. Nitrogen content of food plants and vulnerability of *Pieris rapae* to natural enemies. Ecology 72:1586-1590.

Lorio, P.L. 1986. Growth-differentiation balance: a basis for understanding southern pine beetle interaction. For. Ecol. Manag. 14:259-273.

Loveless, A.R. 1961. A nutritional interpretation of sclerophylly based on differences in the chemical composition of sclerophyllous and mesophytic leaves. Ann. Bot. 25:168-184.

Loveless, A.R. 1962. Further evidence to support a nutritional interpretation of sclerophylly. Ann. Bot 26:551-561

Lucas, P.W. and B. Pereira. 1990. Estimation of the fracture toughness of leaves. Functional Ecol. 4:819-822.

Lysyk, T.J. 1989. Stochastic model of eastern spruce budworm (Lepidoptera: Tortricidae) phenology on white spruce and balsam fir. J. econ. Ent. 82:1161-1168.

MacLean, D.A. 1980. Vulnerability of fir-spruce stands during uncontrolled spruce budworm outbreaks: a review and discussion. For. Chron. 56:213-221.

MacLean, D.A. 1985. Effects of spruce budworm outbreaks on forest growth and yield. pp. 148-175 *in* Sanders, C.J., R.W. Stark, E.J. Mullins and J. Murphy (eds.) Rec. Adv. Spruce Budworms Res. Proc. CANUSA Spruce Budworms Res. Symp. Can. For. Serv., Ottawa.

Martin, M.M. and H.M. Van't Hof. 1988. The cause of reduced growth of *Manduca sexta* larvae on a low-water diet: increased metabolic processing costs or nutrient limitation? J. Insect Physiol. 34:515-525.

Mattson, W.J. 1980. Herbivory in relation to plant nitrogen content. Ann. Rev. Ecol. Syst. 11:119-161.

Mattson, W.J. 1985. Role of host plants in the population dynamics of the spruce budworm. pp.124-125 *in* Sanders, C.J., R.W. Stark, E.J. Mullins and J. Murphy (eds.) Rec. Adv. Spruce Budworms Res. Proc. CANUSA Spruce Budworms Res. Symp. Can. For. Serv., Ottawa.

Mattson, W.J. and J.M. Scriber. 1987. Nutritional ecology of insect folivores of woody plants: water, nitrogen, fiber and mineral considerations. pp.105-146 *in* Slansky, Jr. F. and J. Rodriguez (eds.) Nutritional Ecology of Insects, Mites, Spiders, and Related Invertebrates. Wiley, New York.

Mattson, W.J., G.A. Simmons and J.A Witter. 1988a. The spruce budworm in eastern North America. pp 309-330 *in* Berryman, Alan A. (ed.) Dynamics of Forest Insect Populations. Plenum Publishing Corporation.

Mattson, W.J., S. Slocum and C.N. Koller. 1983. Spruce budworm *(Choristoneura fumiferana)* performance in relation to foliar chemistry of its host plants. pp. 55-65 *in* Talerico R. and M. Montgomery (Tech. Coords.) Proc., For. Def. - Host Interactions: A Comparison between Gypsy Moth and Spruce Budworms. Broomall, PA, USA, April 5-7, 1983. USDA For. Serv. NE Sta. Gen. Tech. Rep. NE-85.

Mattson, W. J., R.K. Lawrence, R.A. Haack, D.A. Herms and P. Charles. 1988b. Defensive strategies of woody plants against different insect-feeding guilds in relation to plant ecological strategies and intimacy of association with insects. pp 3-38 *in* Mattson, W. J., J. Levieux and C. Bernard-Dagan (eds.) Mechanisms of Woody Plant Defenses Against Insects: Search for Pattern. Springer-Verlag, New York.

Mattson, W.J., D.A. Herms, J.A. Witter, and D.C. Allen. 1991. Woody plant grazing systems: North American outbreak folivores and their host plants. pp. 53-84. *in* Baranchikov, Y.N., W.J. Mattson, F.P. Hain, and T.L. Payne (eds.) Forest Insect Guilds: Patterns of Interaction with Host Trees. U.S.D.A. For. Serv. Gen. Tech. Rep. NE-153.

McGinnis, A.J. and R. Kasting. 1967. Dietary cellulose: Effect on food consumption and growth of a grasshopper. Can. J. Zool. 45:365-367.

McGugan, B.L. 1954. Needle-mining habits and larval instars of the spruce budworm. Can. Ent. 86:439-453.

McKinlay, K.S. 1981. The importance of dry plant material in the diet of the grasshopper, *Melanoplus sanguinipes* (Orthoptera, Acrididae). Can. Ent. 113:5-8.

McMorran, A. 1965. A synthetic diet for the spruce budworm, *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae). Can. Ent. 97:58-62.

Miller, C.A. 1957. A technique for estimating the fecundity of natural populations of the spruce budworm. Can. J. Zool. 35:1-13.

Miller, C.A. 1958. The measurement of spruce budworm populations and mortality during the first and second larval instars. Can. J. Zool. 36:409-422.

Miller, C.A. 1963. The analysis of fecundity proportion in the unsprayed area. pp. 75-86 *in* R.F. Morris (ed.) The Dynamics of Epidemic Spruce Budworm Populations. Mem. ent. Soc. Can., No. 31.

Miller, C.A. 1977. The feeding impact of spruce budworm on balsam fir. Can. J. For. Res. 7:76-84.

Moody, B.H. and I.S. Otvos. 1980. Distribution of hibernating spruce budworm larvae within crowns of balsam fir trees in Newfoundland. Can. For. Serv. Info. Rep. N-X-182.

Mole, S. and P.G. Waterman. 1987. Tannic acid and proteolytic enzymes: enzyme inhibition or substrate deprivation? Phytochemical 26:99-102.

Morris, R.F. 1963. Forest depletion and the spruce budworm. pp. 223-228 *in* R.F. Morris (ed.) The Dynamics of Epidemic Spruce Budworm Populations. Mem. ent. Soc. Can., No. 31.

Morrison, I.K. and N.W. Foster. 1990. On fertilizing semimature jack pine stands in the Boreal forest of central Canada. pp. 416-431 *in* Gessel, S.P., D.S. Lacate, G.F. Weetman, and R.F. Powers (eds.) Sustained Productivity of Forest Soils. Proc. 7th North Am. For. Soils Conf., Univ. B.C., Fac. For. Publ., Vancouver, B.C.

Mulye, H. and R. Gordon. 1990. A method for rearing the larval stages of the eastern spruce budworm, *Choristoneura fumiferana* Clemens (Lepidoptera: Tortricidae), for physiological studies. Can. Ent. 122:1271-1272.

Myers, J.H. and D. Bazely. 1990. Thorns, spine, prickles and hairs: are they stimulated by herbivory and do they deter herbivores? pp. 325-344 *in* D.W. Tallamy and M.J. Raupp (eds.) Phytochemical Induction by Herbivores. Wiley, New York.

Myers, J.H. and B.J. Post. 1981. Plant nitrogen and fluctuations of insect populations: A test with the cinnabar moth - tansy ragwort system. Oecolgia 48:151-156.

Myers, J.H. and K.S. Williams. 1984. Does tent caterpillar attack reduce the food quality of red alder foliage? Oecologia 62:74-79.

Neuvonen, S. and E. Haukioja. 1984. Low nutritive quality as defence against herbivores: induced responses in birch. Oecologia 63:71-74.

Neuvonen, S. and E. Haukioja. 1990. The effects of inducible resistance in host foliage on birch-feeding herbivores. pp. 277-291 *in* D.W. Tallamy and M.J. Raupp (eds.) Phytochemical Induction by Herbivores. Wiley, New York.

Neuvonen, S. and P. Niemelä. 1991. Do differences in inducible resistance explain the population dynamics of birch and pine defoliators? pp. 103-112 *in* Baranchikov, Y.N., W.J. Mattson, F.P. Hain, and T.L. Payne (eds.) Forest Insect Guilds: Patterns of Interaction with Host Trees. U.S.D.A. For. Serv. Gen. Tech. Rep. NE-153.

Nichols-Orians, C.M. and J.C. Schultz. 1990. Interactions among leaf toughness, chemistry, and harvesting by attine ants. Ecol. Ent. 15:311-320.

Niemelä, P, J. Tuomi, R. Mannila, and P. Ojala. 1984. The effect of previous damage on the quality of Scots pine foliage as food for Diprionid sawflies. Z. angnew. Ent. 98:233-43.

Northcote, D.H. 1989. Contol of plant cell wall biogenesis: an overview. pp.1-17 *in* Lewis, N.G. and M.G. Paice (eds.) Plant Cell Wall Polymers - Biogenesis and Biodegradation. 3rd Chem. Cong.North America (195th National Meeting of the American Chemical Society), Toronto, Ont., Can., June 5-11, 1988. ACS Symposium Series 399. Am. Chem. Soc., Wash., DC.

Paul, D.C., G.Subba Rao, and D.C. Deb. 1992. Impact of dietary moisture on nutritional indices and growth of *Bombyx mori* and concommitant larval duration. J. Insect Physiol. 38:229-235.

Pennings, S.C. and V.J. Paul. 1992. Effect of plant toughness, calcification, and chemistry on herbivory by *Dolabella auricularia*. Ecology73:1606-1619.

Peterson, S.S., J.M. Scriber and J.G. Coors. 1988. Silica, cellulose and their interactive effects on the feeding performance of the southern armyworm, *Spodoptera eridania* (Cramer) (Lepidoptera: Noctuidae). J. Kans. ent. Soc. 61:169-177.

Pharis, R.P. 1967. Seasonal fluctuation in the foliage-moisture content of well-watered conifers. Bot. Gaz. 128:179-185.

Piene, H. 1980. Effects of insect defoliation on growth and foliar nutrients of young balsam fir. For. Sci. 26:654-673.

Piene, H. 1989. Spruce budworm defoliation and growth loss in young balsam fir: recovery of growth in spaced stands. Can. J. For. Res. 19:1616-1624.

Piene, H and K.E. Percy. 1983. Changes in needle morphology, anatomy, and mineral content during the recovery of protected balsam fir trees initially defoliated by the spruce budworm. Can. J. For. Res. 14:238-245.

Prévost, Y.H. and J.E. Laing. 1986. Phenology of spruce budworm, *Choristoneura fumiferana* (Clem.), in relation to the development of cones and foliage of black spruce, *Picea mariana* (Mill.) B.S.P. pp. 225-239 *in* Roques, A. (ed.) Proc. 2nd Conf. Cone and Seed Insects Working Party 52:07-01, Briançon, France Sept.3-5, 1986.

Price, P. 1984. Insect Ecology. John Wiley and Sons, Inc., New York.

Price, P.W., C.E. Bouton, P. Gross, B.A. McPheron, J.N. Thompson, and A.E. Weis. 1980. Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. Ann. Rev. Ecol. Syst. 11:41-65.

Raupp, M.J. 1985. Effects of leaf toughness on mandibular wear of the leaf beetle, *Plagiodera versicolora*. Ecol. Ent. 10:73-79.

Rausher, M.D. 1981. Host plant selection by *Battus philenor* butterflies: the roles of predation, nutrition, and plant chemistry. Ecol. Monogr. 51:1-20.

Raven, P.H., R.F. Evert and S.E. Eichhorn. 1986. Biology of Plants, Fourth Edition. Worth Publishers, Inc., New York.

Reese, J.C. 1979. Interactions of allelochemicals with nutrients in herbivore food. pp. 309-330 *in* Rosenthal G. and D. Janzen (ed.) Herbivores: Their Interaction With Secondary Plant Metabolites. Academic Press, New York.

Reese, J.C. and S.D. Beck. 1978. Interrelationships of nutritional indices and dietary moisture in the black cut worm *(Agrotis ipsilon)* digestive efficiency. J. Insect Physiol. 24:473-479.

Régnière, J. and R.M. Fletcher. 1983. Direct measurement of spruce budworm (Lepidoptera: Torticidae) larval dispersal in forest stands. Env. Ent. 12:1532-1538.

Régnière, J., T.J. Lysyk, and M. Auger. 1989. Population density estimation of spruce budworm *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae) on balsam fir and white spruce from 45-cm mid-crown branch tips. Can. Ent. 121:267-281.

Régnière, J. and M. You. 1991. A simulation model of spruce budworm (Lepidoptera: Tortricidae) feeding on balsam fir and white spruce. Ecol. Mod. 54:277-297.

Renwick, J.A.A. and C.D. Radke. 1982. Ovipositional choice and larval survival of the spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae). Env. Ent. 11:503-505.

Retnakaran, A. 1983. Spectrophotometric determination of larval ingestion rates in the spruce budworm (Lepidoptera: Tortricidae). Can. Entomol. 115:31-40.

Rhoades, D.F. 1979. Evolution of plant chemical defense against herbivores. pp.3-54 *in* Rosenthal G. and D. Janzen (ed.) Herbivores: Their Interaction With Secondary Plant Metabolites. Academic Press, New York.

Rhoades, D. F. and R. G. Cates. 1976. Toward a general theory of plant antiherbivore chemistry. Recent Adv. Phytochem. 10:168-213.

Roland, J and J. Myers. 1987. Improved insect performance from host-plant defoliation: winter moth on oak and apple. Ecol. Ent. 12:409-414.

Royama, T. 1984. Population dynamics of the spruce budworm *Choristoneura fumiferana.* Ecol. Monogr. 54:429-462.

Schultz, J.C. 1983. Habitat selection and foraging tactics of caterpillars in heterogenous trees. pp. 61-90 *in* Denno, R.F. and M.S. McClure (eds.) Variable Plants and Herbivores in Natural and Managed Systems. Academic Press, Inc. New York.

Schultz, J.C., P.J. Nothnagle, and I.T. Baldwin. 1982. Seasonal and individual variation in leaf quality of two northern hardwoods tree species. Amer. J. Bot. 69:753-759.

Scriber, J.M. 1977. Limiting effects of low leaf-water content on the nitrogen utilization, energy budget, and larval growth of *Hyalophora cecropia* (Lepidoptera: Saturniidae). Oecologia 28:269-287.

Scriber, J.M. 1978. The effects of larval feeding speicalization and plant growth form on the consumption and utilization of plant biomass and nitrogen: and ecological consideration. Entomol. Exp. Appl. 24:694-710.

Scriber, J.M. 1979. Effects of leaf-water supplementation upon post-ingestive nutritional indices of forb-, shrub-, vine-, and tree-feeding Lepidoptera. Entomol. Exp. Appl. 25:240-252.

Scriber, J.M. 1982. Food consumption and utilization. pp. 87-163 *in* Kerkut, G.A. and L.I. Gilbert (eds.) Vol. IV Regulation: Digestion, Nutrition, Excretion. Comprehensive Insect Physiology, Biochemistry and Pharmacology. Pergamon Press, Toronto.

Scriber, J.M. 1984. Host-plant suitability. pp.159-202 *in* Bell, W.J. and R.T. Cardé (eds.) Chemical Ecology of Insects. Chapman and Hall, London.

Scriber, J.M. and M.P. Ayres. 1988. Leaf chemistry as a defense against insect. ISI Atlas of Science: Animal and Plant Sciences. pp.117-123.

Scriber, J.M. and P.P Feeny. 1979. Growth of herbivorous caterpillars in relation to feeding specialization and to the growth form of their food plants. Ecology 60:829-850.

Scriber, J.M. and F. Slansky, Jr. 1981. The nutritional ecology of immature insects. Ann. Rev. Ent. 26:183-211.

Schroeder, L.A. 1976. Energy, matter and nitrogen utilization by the larvae of the monarch butterfly *Danaus plexippus*. Oikos 27:259-264.

Schroeder, L.A. 1986. Protein limitation of a tree feeding Lepidopteran. Ent. exp. appl. 41:115-120.

Schroeder, L.A. and M. Malmer. 1980. Dry matter, energy and nitrogen conversion by Lepidoptera and Hymenoptera larvae fed leaves of black cherry. Oecologia 45:63-71.

Shaw, G.G. and C.H.A. Little. 1972. Effect of high urea fertilization on balsam fir trees on spruce budworm development. pp. 589-597 *in* Rodriquez, J.G. (ed.) Insect and Mite Nutrition. North-Holland, Amsterdam.

Shaw, G.G., C.H.A. Little and D.J. Durzan. 1978. Effect of fertilization of balsam fir on spruce budworm nutrition and development. Can. J. For. Res. 8:364-374.

Slansky, F. and P. Feeny 1977. Stabilization of the rate of nitrogen accumulation by larvae of the cabbage butterfly on wild and cultivated food plants. Ecol. Monogr. 47:209-228.

Slansky, F. and J.M. Scriber. 1985. Food consumption and utilization. pp. 87-164 *in* Kerkut, G.A. and L.I. Gilbert (eds.) Vol. IV Regulation: Digestion, Nutrition, Excretion. Comprehensive Insect Physiology, Biochemistry and Pharmacology. Pergamon Press, Toronto.

Slansky, F., Jr., and G.S. Wheeler. 1991. Food consumption and utilization responses to dietary dilution with cellulose and water by velvetbean caterpillars, *Anticarsia gemmatalis*. Physiol. Ent. 16:99-116.

SPSS. 1988. SPSS-X User's Guide. Third Edition. SPSS Inc., Chicago Illinois.

Städler, E. 1974. Host plant stimuli affecting oviposition behavior of the eastern spruce budworm. Ent. exp. appl. 17:176-188.

Steel, R.G.D. and J.H. Torrie. 1980. Principles and Prodecures of Statistics: A Biometrical Approach. Second Edition. McGraw-Hill, Inc., New York.

Swain, Tony. 1979. Tannins and lignin. pp. 657-682 *in* Rosenthal, G. A. and D. H. Janzen (eds.) Herbivores: Their Interaction With Secondary Plant Metabolites. Academic Press, New York.

Tanton, M.T. 1962. The effect of leaf "toughness" on the feeding of larvae of the mustard beetle *Phaedon cochleariae* Fab. Entomol. Exp. Appl. 5:74-78.

Terra, W.R., A. Valentin and C.D. Santos. 1987. Utilization of sugars, hemicellulose, starch, protein, fat and minerals by *Erinnyis ello* larvae and the digestive role of their midgut hydrolases. Insect Biochem. 17:1143-1147.

Thielges, B.A. 1968. Altered polyphenol metabolism in the foliage of *Pinus sylvestris* associated with European sawfly attack. Can. J. Bot. 46:724-725.

Thomas, A.W. 1983. Foliage consumption by 6th-instar spruce budworm larvae, *Choristoneura fumiferana* (Clem.), feeding on balsam fir and white spruce. pp. 47-48 *in* Talerico R. and M. Montgomery (Tech. Coords.) Proceedings, Forest Defoliator - Host Interactions: A Comparison between Gypsy Moth and Spruce Budworms. Broomall, PA, USA, April 5-7, 1983. USDA For. Serv. Northeastern Station Gen. Tech. Rep. NE-85.

Thomas, A.W. 1987. The effect of age of current-year shoots of *Picea glauca* on survival, development time, and feeding efficiency of 6th-instar larvae of *Choristoneura fumiferana*. Ent. exp. appl. 43:251-260.

Thomas, A.W. 1989. Food consumption and utilization by 6th-instar larvae of spruce budworm, *Choristoneura fumiferana:* a comparison on three *Picea* (spruce) species. Ent. exp. appl. 52:205-214.

Timmer, V.R. and L.D. Morrow. 1984. Predicting fertilizer growth response and nutrient status of jack pine by foliar diagnosis. pp. 335-351 *in* Stone, E.L. (ed.) Forest Soils & Treatment Impacts. Proc. 6th N.A. For. Soils Conf., Univ. Tennessee, June 1983. Dept. For., Wildl. and Fish., Univ. Tennessee, Knoxville, Tennessee.

Timmins, W.A., K. Bellward, A.J. Stamp, and S.E. Reynolds. 1988. Food intake, conversion efficiency, and feeding behaviour of tobacco hornworm caterpillars given artificial diet of varying nutrient and water content. Physiol. Ent. 13:303-314.

Tuomi, J., P. Niemelä, F.S. Chapin, III, J.P. Bryant, and S. Sirén. 1988. Defensive responses of trees in relation to their carbon/nutrient balance. pp. 57-72 *in* Mattson, W.J., J. Levieux and C. Bernard-Dagan (eds.) Mechanism of Woody Plant Defenses Against Insects: Search for Pattern. Springer-Verlag, New York. 416 pp.

Tuomi, J., P. Niemelä, E. Haukioja, S. Sirén, and S. Neuvonen. 1984. Nutrient stress: an explantion for plant anti-herbivore responses to defoliation. Oecologia 64:208-210.

Turgeon, J.J. 1986. The phenological relationship between the larval development of the spruce budmoth, *Zeiraphera canadensis* (Lepidoptera: Olethreutidae), and white spruce in northern New Brunswick. Can. Ent. 118:345-350.

Vance, C.P., T.K. Kirk, and R.T. Sherwood. 1980. Lignification as a mechanism of disease resistance. Ann. Rev. Phytopathol. 18:259-288.

Valentine, H.T., W.E. Wallner, and P.M. Wargo. 1983. Nutritional changes in host foliage during and after defoliation, and their relation to the weight of gypsy moth pupae. Oecologia 57:298-302.

Wagner, M. R. 1986. Influence of moisture stress and induced resistance in ponderosa pine, *Pinus ponderosa* Dougl. Ex. Laws, on the pine sawfly, *Neodiprion autumnalis* Smith. For. Ecol. Manag. 15:43-53.

Wagner, M. R. 1988. Induced defenses in ponderosa pine against defoliating insects. pp.141-156 *in* Mattson, W.J., J. Levieux and C. Bernard-Dagan (eds.) Mechanisms of Woody Plant Defenses Against Insects: Search for Pattern. Springer, New York.

Wagner, M. R. and P.D. Evans. 1985. Defoliation increases nutritional quality and allelochemics of pine seedlings. Oecologia 67:235-237.

Wainhouse, D., D.J. Cross, and R.S. Howell. 1990. The role of lignin as a defence against the spruce bark beetle *Dendroctonus micans*: effect on larvae and adults. Oecologia 85:257-265.

Walker, J. R. L. 1975. The Biology of Plant Phenolics. Edward Arnold (Publ.) Ltd, London.

Waring, Richard and William H. Schlesinger. 1985. Forest Ecosystems: Concepts and Management. Academic Press, Toronto.

Watt, A.D. 1992. The influence of crown feeding position on the growth and survival of pine beauty moth larvae (*Panolis flammea* Den. & Schiff.) (Lep., Noctuidae). J. appl. Ent. 114:113-117.

Watt, R.F. and M.L Heinselman. 1965. Foliar nitrogen and phosphorus level related to site quality in a northern Minnesota spruce bog. Ecology 46:357-361.

Webb, W.L. and J.J. Karchesy. 1977. Starch content of douglas fir defoliated by the tussock moth. Can. J. For. 7:186-188.

West, E.S., W.R. Todd, H.S. Mason, and J.T. Van Bruggen. 1966. Textbook of Biochemisty. Fourth Edition. The MacMillan Co., Collier-MacMillan Canada, Toronto.

Wharton, G.W. 1985. Water balance of insects. pp.565-601 *in* Kerkut, G.A. and L.I. Gilbert (eds.) Vol. IV Regulation: Digestion, Nutrition, Excretion. Comprehensive Insect Physiology, Biochemistry and Pharmacology. Pergamon Press, Toronto.

Wharton, G.W. and L.G. Arlian. 1972. Utilization of water by terrestrial mites and insects pp. 153-163 *in* Rodriquez, J.G. (ed.) Insect and Mite Nutrition. North Holland, Amsterdam.

Whitham, T.G. 1983. Host manipulation of parasites: within-plant variation as a defense against rapidly evolving pests. pp. 15-41 *in* Denno, R.F. and M.S. McClure (eds.) Variable Plants and Herbivores in Natural and Managed Systems. Academic Press, Inc. New York.

Williams, L.H. 1954. The feeding habits and food preferences of Acrididae and the factors which determine them. Trans. Roy. Entomol. Soc. London. 105:423-454.

Williams, A.G. and T.G. Whitham. 1986. Premature leaf abscission: an induced plant defense against gall aphids. Ecology 67:1619-1627.

Wilson, B.F. 1970. The Growing Tree. The University of Massachusetts Press, Amherst.

Wilson, L.F. and J.L. Bean. 1963. Site of spruce budworm egg masses on their preferred hosts in the Lake States. J. econ. Ent. 56:574-578.

Wood, J.G. 1933. The physiology of xerophytism in Australian plants. Australian J. Exp. Bio. Med. 11:139-150.

Zimmerman, M.H. and C.L. Brown. 1974. Trees: Structure and Function. Springer-Verlag, New York.

Zar, J.H. 1974. Biostatistical Analysis. Prentice-Hall, Inc. Englewood Cliffs, N.J..

APPENDICES

APPENDIX I

NEUTRAL-DETERGENT SOLUTION

No.	Ingredient	Amount
1	distilled water	1.00 l
2	sodium lauryl sulphate	30.00 g
3	EDTA	18.61 g
4	$Na_2B_4O_7 \cdot 10H_2O$	6.81 g
5	Na_2HPO_4	4.56 g
6	ethylene glycol	10.00 mL

Put some of the distilled water into a wash bottle to spray weighing dishes with.
Use two 500 mL beakers and a large Erlenmeyer flask.
Put the two, 500 mL beakers on two hot plates.

Into Beaker 1
- use ingredients # **1, 3** and **4**
- weigh ingredients **3** and **4** first and put into a beaker with distilled water to dissolve on high heat (it takes awhile to dissolve)

Into Beaker 2
- use ingredients **1** and **5**
- weigh out ingredient **5** and put into beaker with distilled water to dissolve on medium heat (it dissolves quickly)

Into the Erlenmeyer Flask - use ingredients **2** and **6**
- weigh out ingredient **6** and pipette ingredient **2** into flask and add some distilled water to dissolve on heat
- add beaker 1 and 2 to the flask and mix
- add the rest of the distilled water (don't forget water in wash bottle)
- let flask cool down to room temperature before use

To each Kjeldahl flask add:

0.100-0.1500 g of sample
0.125 g sodium sulfite
1.0 mL decahydronaphthalene
25 mL neutral-detergent soln
(I just use the wash bottle runoff)

APPENDIX II

NOTES ABOUT THE CHEMICAL DIGESTION PROCESS

1. The Micro-Kjeldahl Digestor (Figure 1) was used because it was designed for processing micro-samples of nitrogen-containing material. It consists of a glass manifold and a heater mantle with six flask stations. Each station has an individual heater assembly and variable heat control switch. It was necessary to operate the digestor through a voltage regulator because the small samples processed with this study required less heat than was available from the lowest heat control setting. The voltage regulator reduced the power input to the apparatus.

2. The vacuum filtering unit (Figure 2) consisted of a metal frame (9cm x 25cm x 34cm) to which was clamped a hand blown glass vacuum manifold that accommodated six filter stations. Each station was comprised of a one litre sidearm filter flask clamped to the frame and connected to the manifold by rubber vacuum tubing and a stopcock for individual vacuum control. Filtering was accomplished through 50 mL Gooch-type crucibles inserted in modified Walter crucible holders that sat in the filter flasks. A distilling flask and a cold trap were both used to prevent liquid and gaseous residues from entering and damaging the vacuum pump through backstreaming. The distilling flask was fitted with a stopcock that was opened prior to turning the pump on and off to release the vacuum and prevent backstreaming. It also acted as a catch basin for liquid that travelled from the crucibles, through the manifold toward the

pump. Gases were frozen in the cold trap prior to entering the pump; the trap consisted of a glass finger dipped in a thermos flask containing liquid nitrogen.

3. The Kjeldahl flasks required constant monitoring and frequent heat adjustment to maintain an even boil. It was important that all tissue remain in continuous suspension so the flasks were turned frequently and necks washed down to remove adhering particles. A wash bottle containing neutral detergent solution was used for this purpose.

4. I found that spraying water from a fine-nosed wash bottle effectively separated the tissue mat that formed while filtering, thereby, permitting a thorough wash.

5. After each use, the pyrex crucibles were wet ashed to remove residues. Dry ashing at high temperatures for a few hours (as recommended by Goering and Van Soest 1970) was no employed because it caused the pyrex to melt. Instead, the following procedure was developed. In a fume hood, the crucible were brought to a boil in a crystallizing dish containing reagent grad sulphuric acid (H_2SO_4) and the heat reduced until the organic matter ashed. The crucibles were cooled, rinsed and backwashed with chromic acid to remove the ash remaining on the glass filters. The backwash involved setting the crucible upside down on a filtervac diaphragm resting on a bench vacuum. Small amounts of chromic acid were poured into the crucible bottom until the ash disappeared and distilled water then backwashed through the filter. A backwash was employed so as not to pull ash through the filter any further than necessary. The crucible was turned right side up, inserted in a modified Walter crucible holder and filled with distilled water three times. It was then washed in soapy water, rinsed and oven-dried overnight at 100°C. The crucibles were cooled in a desiccator for two hours and weighed in preparation for their next use. Hard forceps were used to handle the crucibles.

APPENDIX III

PROBLEMS ENCOUNTERED WITH THE DIGESTION PROCESS

1. One problem encountered with the digestion process was to maintain a consistent boil in the Kjeldahl flasks at all times. Temperature in the coils on the heating mantle was controlled with a voltage regulator, but a selected voltage that produced a consistent boil one day seldom produced one the next. Variations in room temperature and humidity seemed to affect the boiling process. As a result, constant vigilance was required over the 650 digestions that were conducted. A consistent, one hour boil was required to digest fully all tissue components except IF. If this did not occur then the post-digested IF dry weight included the dry weight of IF plus the dry weight of undigested foliar components. This erroneously high, post-digested tissue dry weight divided by the predigested tissue dry weight would have produced a higher percent IF than actually existed in the sample. Although new samples of the particular collection date were digested repeatedly until results were consistent, this problem may have contributed to some of the variation observed in IF levels among crown positions.

2. Another problem encountered was the occurrence of violent boiling of balsam fir tissue samples, particularly those which had been collected in the early-season. The violent boiling frequently began 30 minutes into the digestion process and actually caused Kjeldahl flasks to jump off the heating mantle and break. Teflon boiling chips reduced the problem somewhat but violent boiling still occurred intermittently which caused tissue and chemicals to shoot out the mouth of the flask, thereby, requiring digestion of the sample

again. The occurrence of violent boiling of early-season tissue seemed to decrease with the length of time that the oven-dried sample sat in the desiccator, i.e. the longer the sample stayed in the desiccator the less the chances of violent boiling.

3. The teflon chips themselves posed a problem because it was frequently required to insert many chips into the flask to reduce boiling. When it came time to remove those chips from the Gooch crucibles after filtering the digestion solution, it was integral that no tissue remain on the chip. It is possible that tissue did remain on the chips on occasion thereby lowering the percent IF values.

4. Another problem was that three different people were responsible for completing the 650 digestions. Some variation in results is likely to happen due to the way that each person applied the technique, although we all tried to be consistent.

APPENDIX IV

SEASONAL FOLIAR MOISTURE CONTENT (% FRESH WEIGHT)

Spp.	Date	Julian	Lower Crown			Middle Crown			Upper Crown		
	d/m	Date	Mean	SD	n	Mean	SD	n	Mean	SD	n
Bf	1/6	152	72.10	1.92	7	71.70	1.71	7	71.79	1.85	7
Bf	5/6	156	71.35	2.39	7	62.23	6.28	5	72.13	1.48	7
Bf	11/6	162	71.19	1.90	7	70.91	1.86	7	72.53	3.25	7
Bf	14/6	165	72.26	1.54	7	72.21	1.86	7	72.58	1.64	7
Bf	21/6	172	74.20	2.45	7	73.12	1.99	7	72.86	1.68	7
Bf	28/6	179	69.28	1.43	5	69.33	0.84	5	63.25	14.13	5
Bf	4/7	185	65.75	10.68	7	68.09	2.14	6	63.84	5.39	7
Bf	31/7	212	57.16	1.51	7	58.74	3.03	7	57.61	2.06	7
Bf	27/8	239	56.71	1.28	6	56.74	1.91	7	56.87	1.15	7
Sw	1/6	152	78.11	1.66	7	77.57	1.62	7	77.44	2.34	7
Sw	5/6	156	80.85	1.50	7	79.31	2.34	7	80.56	2.64	7
Sw	11/6	162	80.57	0.72	7	80.43	0.72	7	80.40	0.90	7
Sw	18/6	169	79.23	1.84	7	79.00	1.80	7	79.31	1.15	7
Sw	25/6	176	73.96	2.51	7	71.86	3.14	7	72.36	2.31	7
Sw	4/7	185	67.19	1.64	7	64.92	4.21	7	68.31	4.04	7
Sw	10/7	191	63.92	2.32	7	62.36	2.32	7	63.11	3.29	7
Sw	31/7	212	60.18	1.54	7	59.53	2.04	7	58.54	1.63	7
Sw	27/8	239	57.16	1.46	7	56.97	1.23	7	56.74	1.17	6
Sb	11/6	162	73.08	2.19	7	73.92	1.89	6	73.02	1.54	6
Sb	14/6	165	76.40	1.94	7	77.02	1.55	7	77.17	0.87	7
Sb	18/6	169	78.77	1.44	7	79.01	0.94	7	78.86	0.74	7
Sb	21/6	172	79.06	1.01	7	78.67	0.67	7	79.17	0.72	7
Sb	25/6	176	78.01	0.73	7	77.44	1.03	7	77.92	0.51	7
Sb	28/6	179	76.93	1.11	7	75.74	1.17	7	76.38	2.54	7
Sb	4/7	185	71.77	5.45	7	73.53	3.29	7	71.91	10.28	7
Sb	10/7	191	68.78	1.04	7	67.71	2.76	7	68.71	2.40	7
Sb	31/7	212	59.22	1.24	7	59.40	1.37	7	59.77	1.80	7
Sb	27/8	239	68.78	1.04	7	67.71	2.76	7	68.71	2.40	7

APPENDIX V

SEASONAL FOLIAR NITROGEN CONTENT (% DRY WEIGHT)

Spp.	Date	Julian	Lower Crown			Middle Crown			Upper Crown		
	d/m	Date	Mean	SD	n	Mean	SD	n	Mean	SD	n
Bf	1/6	152	3.87	0.31	4	3.65	0.28	2	3.58	0.19	4
Bf	5/6	156	2.98	0.26	4	2.63	0.03	2	2.82	0.34	4
Bf	11/6	162	2.30	0.18	4	2.16	0.25	2	2.38	0.15	4
Bf	14/6	165	2.02	0.18	4	2.07	0.10	2	2.13	0.13	4
Bf	21/6	172	1.82	0.17	4	1.68	0.13	2	1.91	0.06	4
Bf	4/7	185	1.39	0.26	4	1.56	0.05	2	1.38	0.10	4
Bf	31/7	212	1.30	0.16	4	1.23	0.13	2	1.22	0.03	4
Bf	27/8	239	1.28	0.08	4	0.96	0.41	2	1.17	0.06	4
Sw	1/6	152	3.87	0.58	4	3.46	0.08	2	3.47	0.51	4
Sw	5/6	156	3.03	0.40	4	2.87	0.16	2	2.82	0.35	4
Sw	11/6	162	2.27	0.28	4	2.00	0.25	2	2.15	0.19	4
Sw	18/6	169	1.77	0.18	4	1.59	0.18	2	1.65	0.16	4
Sw	25/6	176	1.37	0.15	4	1.21	0.10	2	1.33	0.08	4
Sw	4/7	185	1.12	0.10	4	1.03	0.06	2	1.13	0.09	4
Sw	31/7	212	0.97	0.05	4	1.03	0.01	2	0.80	0.31	4
Sw	27/8	239	0.99	0.26	4	0.96	0.13	2	1.08	0.59	4
Sb	11/6	162	3.12	0.33	4	2.69	0.06	2	2.63	0.07	3
Sb	18/6	169	1.99	0.25	4	1.75	0.04	2	2.01	0.05	4
Sb	25/6	176	1.67	0.13	4	1.54	0.09	2	1.71	0.24	4
Sb	4/7	185	1.13	0.19	4	1.57	0.29	2	1.28	0.18	4
Sb	31/7	212	1.13	0.46	4	0.79	0.11	2	1.25	0.30	4
Sb	27/8	239	0.95	0.13	4	0.87	0.01	2	1.06	0.03	4

APPENDIX VI

SEASONAL FOLIAR INDIGESTIBLE FIBRE CONTENT (% DRY WEIGHT)

Spp.	Date	Julian	Lower Crown			Middle Crown			Upper Crown		
	d/m	Date	Mean	SD	n	Mean	SD	n	Mean	SD	n
Bf	1/6	152	33.29	5.14	4	27.69	0.99	2	27.73	1.76	4
Bf	5/6	156	33.70	3.15	4	41.24	0.83	2	33.84	3.43	4
Bf	11/6	162	36.01	4.98	4	33.18	2.41	2	37.56	2.54	4
Bf	14/6	165	37.56	2.72	4	36.92	0.86	2	37.87	2.71	4
Bf	21/6	172	42.77	1.26	3	39.82	2.64	2	41.22	2.74	4
Bf	4/7	185	44.21	6.06	4	40.92	7.72	2	42.29	1.24	4
Bf	31/7	212	45.02	1.50	4	45.34	1.16	2	44.44	3.35	4
Bf	27/8	239	40.58	3.57	4	38.95	0.12	2	38.44	2.30	4
Sw	1/6	152	27.04	9.61	4	27.14	7.14	2	23.83	4.73	4
Sw	5/6	156	27.94	5.17	4	27.16	2.85	2	27.54	6.33	4
Sw	11/6	162	35.33	3.39	4	31.96	8.18	2	32.61	5.62	4
Sw	18/6	169	40.77	3.99	4	40.17	0.78	2	39.77	5.63	4
Sw	25/6	176	46.28	4.33	4	52.15	3.43	2	50.64	4.57	4
Sw	4/7	185	52.01	1.38	4	53.61	0.47	2	49.93	2.79	4
Sw	31/7	212	50.20	2.26	4	50.03	2.38	2	49.42	4.30	4
Sw	27/8	239	48.11	2.34	4	49.53	1.27	2	49.13	1.72	4
Sb	11/6	162	24.52	6.86	4	29.65	1.64	2	27.93	3.19	3
Sb	18/6	169	29.60	3.00	4	32.04	0.93	2	27.02	5.56	4
Sb	25/6	176	26.38	4.12	4	34.76	5.48	2	33.00	2.84	4
Sb	4/7	185	40.53	9.94	4	43.38	1.77	2	41.72	8.32	4
Sb	31/7	212	44.73	2.59	4	48.01	0.25	2	44.79	2.20	4
Sb	27/8	239	46.82	2.81	4	47.75	2.39	2	48.17	3.41	4

APPENDIX VII

PENETROMETER

The penetrometer (below), which was constructed at the Lakehead University Science Laboratory, consisted of two aluminum halves, each of the dimensions 2.5 x 10.01 x 7.4 (h x l x w) cm. The lower half was a die block and the upper half was a punch block. The punch was made of tool steel and was 2 mm in diameter and blunt; it went through a tool steel die supported by a bronze bushing. The aluminum punch pad was 6.3 cm in diameter. The unit was secured with two alignment pins and two hold-down screws.

APPENDIX VIII

STANDARD DIET (McMORRAN 1965)

Ingredient	Amount	Ingredient	Amount
water	66.0 ml	benzoic acid	0.45 g
formalin (37% formaldehyde)	0.15 ml	vitamin	2.75 g
4 M potassium hydroxide	1.5 ml	aureomycin	1.67 g
sucrose	10.5 g	casein	10.5 g
salt	3.0 g	wheat germ	9.0 g
alphacel	1.5 g	agar	7.5 g
methyl-h benzoic acid	1.88 g	water	190.0 ml

1. Measure the agar and wheat germ into separate weigh boats.
2. Measure the room temperature ingredients into one large weigh boat.
3. Measure the cold ingredients into another large weigh boat.
4. Into the blender or a small Erlenmeyer beaker, mix together the first addition of water with the formalin and potassium hydroxide.
5. Measure the second addition of water into a beaker but put some of it into a wash bottle.
6. Heat the water in the beaker on the LOW hot plate setting with a magnetic stirrer. Immediately add the agar and rinse weigh boat with wash bottle.
7. Put a thermometer in the beaker and monitor the agar very carefully. Heat slowly until the temperature has reached 80°C.
8. In the meantime, add the water, formalin, potassium hydroxide mixture into the blender if it is not there already.
9. Add the dry ingredients a little at a time ending with the wheat germ. Blend well between additions and rinse off weigh boats.
11. Add the wash bottle water to the blender. It is not needed for the agar.
12. As soon as the agar hits 90°C, add to the running blender through the hole in the lid. Blend well.

APPENDIX IX

MANN-WHITNEY TESTS FOR FOLIAR TOUGHNESS, IF, AND MOISTURE

Variable	Species	Season of Collection	Mann-Whitney U Test	Z	Number of Cases	P
a Between seasons of collection						
Toughness	Bf	Early vs Late	67.0	-6.21	38,30	0.00
Toughness	Sw	Early vs Late	62.0	-5.81	31,30	0.00
Toughness	Sb	Early vs Late	65.0	-5.69	30,30	0.00
IF	Bf	Early vs Late	8.0		10,10	0.00
IF	Sw	Early vs Late	45.0		10,10	0.00
IF	Sb	Early vs Late	45.0		10,10	0.00
Moisture	Bf	Early vs Late	45.0		10,10	0.00
Moisture	Sw	Early vs Late	45.0		10,10	0.00
Moisture	Sb	Early vs Late	45.0		10,10	0.00
b Between species within season.						
Toughness	Bf vs Sw	Early	63.0	-6.34	38,31	0.00
Toughness	Bf vs Sb	Early	70.0	-6.17	38,30	0.00
Toughness	Bf vs Sw	Late	22.0	-6.33	30,30	0.00
Toughness	Bf vs Sb	Late	26.0	-6.27	30,30	0.00
Toughness	Sw vs Sb	Late	177.0	-4.04	30,30	0.00
IF	Bf vs Sw	Early	17.0		10,10	0.01
IF	Bf vs Sw	Late	45.0		10,10	0.00
IF	Bf vs Sb	Late	45.0		10,10	0.00
Moisture	Bf vs Sw	Early	45.0		10,10	0.00
Moisture	Bf vs Sb	Early	45.0		10,10	0.00
Moisture	Sw vs Sb	Early	45.0		10,10	0.00
Moisture	Bf vs Sb	Late	45.0		10,10	0.00
Moisture	Sw vs Sb	Late	45.0		10,10	0.00

APPENDIX X

MEAN DEVELOPMENT TIMES AND PUPAL WEIGHTS OF FEMALE AND MALE SPRUCE BUDWORM REARED IN EXPERIMENT 2.

Performance Characteristic	Foliage Season	Balsam Fir Mean	SD	n	White Spruce Mean	SD	n	Black Spruce Mean	SD	n
Females										
# of days from L2 to pupa	Early	20.7	2.1	33	17.4	1.3	28	18.3	1.2	15
	Late	33.5	1.1	8	30.4	2.4	7	0	0	0
# of days in pupa	Early	5.3	0.8	30	5.8	0.8	27	5.7	1.2	11
	Late	6.5	0.5	8	6.7	0.5	7	0	0	0
Pupal weight (mg f.w.)	Early	96.4	19.9	33	89.9	18.9	28	86.3	6.6	15
	Late	61.9	10.3	8	40.9	7.6	7	0	0	0
Males										
# of days from L2 to pupa	Early	17.6	1.7	44	16.0	0.9	20	17.4	0.7	11
	Late	31.5	1.6	16	31.9	5.1	8	0	0	0
# of days in pupa	Early	6.2	0.6	37	6.6	0.6	19	6.7	1.2	10
	Late	6.4	1.0	16	6.0	1.1	6	0	0	0
Pupal weight (mg f.w.)	Early	66.2	15.1	44	74.7	11.5	20	70.4	6.7	11
	Late	44.6	10.2	16	32.2	11.5	8	0	0	0

APPENDIX XI

MANN-WHITNEY TESTS FOR DEVELOPMENT DAYS AND PUPAL WEIGHTS FOR FEMALE AND MALE SPRUCE BUDWORM REARED IN EXPERIMENT 2

Variable	Species	Season of Collection	Mann-Whitney U Test	Z	Number of Cases	P
a Between sexes within diet						
Days from L2 to pupa	Bf	Early	170.0	-5.81	33,44	0.00
Days from L2 to pupa	Sw	Early	116.1	-3.63	28,20	0.00
Days from L2 to pupa	Sb	Early	45.0		15,11	0.03
Days from L2 to pupa	Bf	Late	18.5		8,16	0.00
Days in pupa	Bf	Early	236.5		30,37	0.00
Days in pupa	Sw	Early	114.5		27,19	0.00
Pupal weight	Bf	Early	179.5	-5.62	33,44	0.00
Pupal weight	Sw	Early	135.0	-3.03	28,20	0.00
Pupal weight	Sb	Early	7.0		15,11	0.00
Pupal weight	Bf	Late	17.0		8,16	0.00
b Between diets for each sex						
Days from L2 to pupa						
Females	Bf vs Sw	Early	87.5	-5.49	33,28	0.00
Females	Bf vs Sb	Early	82.5	-3.71	33,15	0.00
Females	Sw vs Sb	Early	133.5	-2.01	28,15	0.04
Males	Bf vs Sw	Early	152.0	-4.32	44,20	0.00
Males	Sw vs Sb	Early	26.0		20,11	0.00
Females	Bf vs Sw	Late	8.5		8,7	0.02
Pupal weight						
Females	Bf vs Sb	Early	138.5	-2.42	33,15	0.02
Females	Bf vs Sw	Late	3.0		8,7	0.00
Males	Bf vs Sw	Late	29.0		16,8	0.03

APPENDIX XI (CONTINUED)

Variable	Species	Season of Collection	Mann-Whitney U Test	Z	Number of Cases	P
c Between season of collection for each sex and species						
Days from L2 to pupa						
Females	Bf	early vs late		-4.37	33,8	0.00
Females	Sw	early vs late		-4.17	28,7	0.00
Males	Bf	early vs late		-6.01	44,16	0.00
Males	Sw	early vs late		-4.19	20,8	0.00
Days in pupa						
Females	Bf	early vs late	28.0	-3.57	31,8	0.00
Females	Sw	early vs late	33.5	-1.37	27,7	0.00
Pupal weight						
Females	Bf	early vs late	23.0	-3.59	33,8	0.00
Females	Sw	early vs late		-4.04	28,7	0.00
Males	Bf	early vs late	85.5	-4.46	44,16	0.00
Males	Sw	early vs late		-4.07	20,8	0.00

APPENDIX XII

DEVELOPMENT DAYS AND WEIGHTS OF FEMALE (F) AND MALE (M) SPRUCE BUDWORM REARED IN EXPERIMENT 3

		INSTAR								
		L5			L6			Pupa		
Sex	Diet No.*	Mean	SD	n	Mean	SD	n	Mean	SD	n
a Number of days:										
F	1	4.36	0.50	11	13.25	4.13	8	8.17	1.94	6
F	2	5.27	1.35	22	9.81	1.17	21	7.22	0.88	18
F	3	4.37	0.60	19	9.47	1.81	19	7.06	0.42	18
F	4	3.73	0.55	22	9.55	1.71	22	7.41	0.59	22
M	1	4.61	1.04	18	12.57	6.45	14	7.75	1.39	8
M	2	4.54	0.66	13	8.36	1.12	11	7.92	0.79	12
M	3	3.70	0.98	20	7.70	0.80	20	7.44	0.51	18
M	4	3.72	1.36	18	8.50	2.36	18	7.50	0.62	18
b Weight (mg):										
F	1	6.75	1.20	11	22.32	6.66	11	85.19	12.78	7
F	2	6.46	2.75	22	25.08	6.26	22	90.28	26.13	21
F	3	7.25	1.66	19	24.38	4.47	19	106.18	21.06	19
F	4	9.14	2.21	22	30.61	5.68	22	131.07	19.94	22
M	1	5.81	2.26	19	19.19	5.31	18	50.08	12.97	14
M	2	5.49	1.57	14	17.18	3.51	13	55.46	12.91	12
M	3	8.06	3.29	21	20.18	5.45	20	71.76	19.92	19
M	4	7.57	2.09	18	22.17	3.37	18	74.83	13.33	18

* Diet 1 = 12% casein; Diet 2 = 18% casein; Diet 3 = 25% casein; Diet 4 = 100% casein in standard McMorran diet

APPENDIX XIII

MANN-WHITNEY TESTS FOR DEVELOPMENT DAYS FOR FEMALE AND MALE SPRUCE BUDWORM REARED IN EXPERIMENT 3

Instar	Treatment diet number*	Mann-Whitney U - test	Z	Number of cases	P
a Comparison of development days between sexes within diet					
L5	3	106.00	-2.54	19, 20	0.01
L6	2	44.50	-2.89	21, 11	0.00
L6	3	48.00	-4.14	19, 20	0.00
L6	4	103.50	-2.63	22, 18	0.01
Pupa	2	61.00		18, 12	0.05
b Comparison of development days between diets for each sex					
Number of days in L5					
Female	1, 2	61.50	-2.48	11, 22	0.02
Female	1, 4	58.00	-2.81	11, 22	0.02
Female	2, 3	113.00	-2.75	22, 19	0.01
Female	2, 4	46.00	-4.88	22, 22	0.00
Female	3, 4	102.50	-3.15	19, 22	0.00
Male	1, 3	94.50		18, 20	0.01
Male	1, 4	80.50		18, 18	0.01
Male	2, 3	63.00		13, 20	0.01
Male	2, 4	53.00		13, 18	0.01
Number of days in L6					
Female	1, 2	39.00		8, 21	0.03
Female	1, 3	28.50		8, 19	0.01
Female	1, 4	34.50		8, 22	0.01
Male	1, 2	42.50		14, 11	0.06
Male	1, 3	59.00		14, 20	0.00
Male	1, 4	71.00		14, 18	0.04

* Diet 1=12% casein; Diet 2=18% casein; Diet 3=25% casein; Diet 4=100% casein in the standard McMorran diet

APPENDIX XIV

SIGNIFICANT TESTS FOR WEIGHTS OF FEMALE AND MALE SPRUCE BUDWORM REARED IN EXPERIMENT 3

Instar	Treatment diet number*	Student's t-test	df	P
a Comparison of weight between sexes within diet				
L5	4	2.43	38	0.02
L6	2	4.56	33	0.00
L6	3	2.72	37	0.01
L6	4	5.86	38	0.00
Pupa	1	4.75	19	0.00
Pupa	2	4.34	31	0.00
Pupa	3	5.27	36	0.00
Pupa	4	9.00	38	0.00

b Comparison of weight among diets for each sex

	Treatment diet number	One-way ANOVA F	df	P
Weight at L5				
Female	1, 2, 3, 4	6.51	3, 70	0.00
Male	1, 2, 3, 4	4.73	3, 68	0.00
Weight at L6				
Female	1, 2, 3, 4	6.93	3, 70	0.00
Male	1, 2, 3, 4	3.12	3, 65	0.03
Pupal weight				
Female	1, 2, 3, 4	15.36	3, 65	0.00
Male	1, 2, 3, 4	10.45	3, 59	0.00

* Diet 1=12% casein; Diet 2=18% casein; Diet 3=25% casein; Diet 4=100% casein in the standard McMorran diet

APPENDIX XV

CELLULOSE AND CASEIN LEVELS IN DIETS WITH VARYING CELLULOSE:CASEIN N USED IN EXPERIMENT 4

Cellulose:casein N	Diet Number	% Casein in Standard Diet[a]	Casein (g)	Cellulose[b] (g)
3:1 (Standard)	1	100	10.5	1.50
15:1	2	100	10.5	20.39
50:1	3	100	10.5	74.04
15:1	4	25	2.625	3.14
50:1	5	25	2.625	16.54
15:1	6	18	1.89	1.53
50:1	7	18	1.89	11.19
15:1	8	12	1.26	0.15
50:1	9	12	1.26	6.59

[a] Determinations of levels of casein were as follows: a) the 100% casein diets had 10.5 g casein; b) the 25% casein diets had 10.5 g x 0.25 = 2.625 g casein; c) the 18% casein diets had 10.5 x 0.18 = 1.89 g casein; and d) the 12% casein diets had 10.5 x 0.12 = 1.26 g casein.

[b] Calculations for cellulose determination using diet 2 as an example:

1. The base amount of IF in standard diet was determined to be 2.61 g according to the following method:

Diet component	Dry weight (g) in diet	% IF[c]	g of IF (g x %)
wheat germ	9.0	28.6845	2.58
agar	7.5	0.3826	0.03
			2.61g

[c] Neutral detergent fibre.

APPENDIX XV (CONTINUED)

2. The ratio of 15:1, cellulose:casein N was determined to be 20.39 g cellulose and 10.5 g casein according to the following method:

i. determine g of N in casein (casein has 14.6% N [d])	10.5 g casein x .146 = 1.533 g N
ii. multiply by 15 to obtain 15:1, cellulose:casein N	1.533 g x 15 = 22.995 g IF
iii. subtract base IF from standard diet	22.995 g IF - 2.61 g IF = 20.39 g cellulose

[d] Determined by Lakehead University Instrumentation Laboratory.

3. Conclusion: To obtain a 15:1, cellulose:casein N, in the 100 percent casein in standard diet, it is necessary to add 20.39 g cellulose to the standard diet.

APPENDIX XVI

DEVELOPMENT DAYS FOR FEMALE (F) AND MALE (M) SPRUCE BUDWORM REARED ON THE DIETS WITH VARYING CELLULOSE:CASEIN N OF EXPERIMENT 4

		No. days in L5			No. days in L6			No. days in Pupa		
Sex	Diet No.*	Mean	SD	n	Mean	SD	n	Mean	SD	n
F	1	3.9	0.6	10	9.3	1.2	10	6.8	1.1	10
F	2	3.8	0.6	10	9.2	1.2	10	6.6	0.5	9
F	3	4.0	0.7	10	10.6	2.4	10	6.6	0.5	9
F	4	4.6	0.7	10	8.8	0.7	9	6.4	0.5	8
F	5	4.0	0.5	10	8.9	1.3	10	6.3	0.5	10
F	6	4.3	0.5	10	10.5	2.6	8	6.1	0.4	8
F	7	4.6	1.1	10	9.2	1.5	10	6.1	0.3	9
F	8	3.9	0.8	9	10.8	2.0	7	6.4	0.5	7
F	9	5.1	2.2	10	9.4	1.9	8	6.2	0.4	6
M	1	3.4	0.5	10	7.3	0.5	10	6.8	0.4	10
M	2	3.4	0.5	10	6.9	0.7	10	7.0	0.0	10
M	3	3.7	0.5	9	8.2	1.8	9	6.9	0.3	9
M	4	3.9	0.6	9	7.4	1.1	9	6.7	0.5	9
M	5	3.8	0.6	10	6.6	0.5	10	6.8	0.4	10
M	6	3.9	0.4	8	7.4	1.2	8	7.1	0.4	7
M	7	4.4	0.5	10	7.2	1.5	10	6.8	0.5	8
M	8	4.2	0.8	10	7.6	1.9	10	6.9	0.3	10
M	9	3.8	0.4	10	8.1	2.5	8	6.5	0.5	8

* Refer to Table 7

APPENDIX XVII

MANN-WHITNEY TESTS FOR THE NUMBER OF DEVELOPMENT DAYS FOR FEMALE AND MALE SPRUCE BUDWORM REARED ON THE DIETS WITH VARYING CELLULOSE:CASEIN N OF EXPERIMENT 4

	Comparison of Female and Male Development Days			
Instar	Diet number*	Mann-Whitney U - test	Number of cases	P
L6	1	8.0	10, 10	0.00
L6	2	4.0	10, 10	0.00
L6	3	18.5	10, 9	0.03
L6	4	11.5	9, 9	0.01
L6	5	3.0	10, 10	0.00
L6	6	9.0	8, 8	0.02
L6	7	17.0	10, 10	0.01
L6	8	7.0	7, 10	0.00
Pupa	6	3.0	8, 7	0.00
Pupa	7	13.0	9, 8	0.03

* Refer to Table 7

APPENDIX XVIII

WEIGHTS FOR FEMALE (F) AND MALE (M) SPRUCE BUDWORM REARED ON THE DIETS WITH VARYING CELLULOSE:CASEIN N OF EXPERIMENT 4

		L5 Weight			L6 Weight			Pupal Weight		
Sex	Diet No.*	Mean	SD	n	Mean	SD	n	Mean	SD	n
F	1	7.0	1.8	10	26.1	3.8	10	108.1	32.4	10
F	2	7.0	1.3	10	29.4	7.6	10	138.6	18.9	10
F	3	6.4	1.5	10	30.8	8.0	10	103.2	38.2	10
F	4	6.4	1.5	10	22.1	5.8	10	92.1	16.2	8
F	5	6.5	2.0	10	23.2	8.6	10	102.4	25.2	10
F	6	6.4	1.5	10	22.3	4.0	10	92.8	13.2	8
F	7	6.7	2.0	10	25.9	10.6	10	83.3	26.8	10
F	8	6.7	2.0	10	21.8	6.6	9	76.6	15.4	7
F	9	6.7	1.7	10	29.4	7.6	10	81.5	26.7	9
M	1	5.8	0.8	10	19.4	2.6	10	71.4	5.1	10
M	2	5.8	0.5	10	22.0	4.9	10	79.1	11.2	10
M	3	6.2	1.2	10	19.7	3.4	9	65.9	14.1	9
M	4	6.2	0.9	10	18.3	3.7	10	54.5	15.6	9
M	5	5.8	1.4	10	19.3	3.5	10	55.8	13.2	10
M	6	5.0	1.0	10	17.2	4.5	8	44.9	14.4	8
M	7	5.8	1.7	10	17.4	4.4	10	49.9	11.9	10
M	8	6.2	0.9	10	20.3	3.0	10	50.6	9.0	10
M	9	5.7	0.7	10	18.5	5.4	10	50.8	8.4	7

* Refer to Table 7

APPENDIX XIX

SIGNIFICANT TESTS FOR L5 AND L6 WEIGHTS OF FEMALE AND MALE SPRUCE BUDWORM REARED ON THE DIETS WITH VARYING CELLULOSE:CASEIN N OF EXPERIMENT 4

Instar	Diet number*	Test Statistic	df	P
a Comparison of weight between sexes within diet				
L5	2	t=2.80	18	0.01
L5	6	t=2.32	18	0.03
L6	1	t=4.68	18	0.00
L6	2	t=2.82	18	0.01
L6	3	t=4.19	17	0.00
L6	6	t=2.68	16	0.02
L6	7	t=2.51	18	0.02
L6	9	t=4.24	18	0.00
b Comparison of weight among diets for each sex				
Test - One-way ANOVA				
L6 weight-females on 15:1	8, 6, 4, 2	F = 3.49	3, 35	0.03
Test - Student's t-test				
L6 weight - females on 12% casein	8, 9	t =2.31	17	0.03

* Refer to Table 7

APPENDIX XX

SIGNIFICANT TESTS FOR PUPAL WEIGHTS OF FEMALE AND MALE SPRUCE BUDWORM REARED ON THE DIETS WITH VARYING CELLULOSE:CASEIN N OF EXPERIMENT 4

	Diet number*	Student's t-test	df	P
a Comparison of weight between sexes within diet				
Pupa	1	2.23	18	0.04
Pupa	2	8.26	18	0.00
Pupa	3	2.52	17	0.02
Pupa	4	4.93	15	0.00
Pupa	5	4.55	18	0.00
Pupa	6	5.41	15	0.00
Pupa	7	3.28	18	0.00
Pupa	8	4.48	15	0.00
Pupa	9	2.87	14	0.01

b Comparison of weight among diets for each sex

	Diet Number	One-way ANOVA F	df	P
Pupal weight				
females on 15:1	8, 6, 4, 2	24.50	3, 29	0.00
males on 15:1	8, 6, 4, 2	13.72	3, 33	0.00
males on 50:1	9, 7, 5, 3	3.14	3, 32	0.04
females on 100% casein	1, 2, 3	2.90	2, 27	0.05
males on 100% casein	1, 2, 3	4.35	2, 26	0.01

* Refer to Table 7