

**The activity of the phytochemical defenses of Red
Maple (*Acer rubrum* L.) and Sugar Maple (*Acer
saccharum* Marsh.) against the Forest Tent
Caterpillar (*Malacosoma disstria* Hübner)**

Thesis submitted to the School of Graduate Studies in partial fulfillment of the requirement
for the degree Master of Science.

by Robert W. Nicol

At the University of Ottawa, May 1996.



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Abstract

The forest tent caterpillar (*Malacosoma disstria* Hübner) is a serious economic pest of many deciduous trees throughout North America. This insect avoids the foliage of red maple (*Acer rubrum* L.). An investigation of the phytochemical activity of the foliage of *A. rubrum* against the forest tent caterpillar (FTC) was undertaken to attempt to identify any antifeedant secondary compounds and to quantify the toxicity or growth and development inhibiting properties of these compounds. During preliminary research, it was discovered that the foliage of sugar maple (*Acer saccharum* Marsh.) which is generally considered to be a food host tree of the FTC, could also contain secondary chemicals that harm this insect. This maple species was then added to the study. Standard methods such as life cycle and nutritional utilization bioassays were used to quantify anti-insect activity of the phytochemicals, with compound isolation being completed at the Canadian Forest Service, Sault Ste. Marie.

When fed foliage of both maples, the FTC grew at a significantly reduced rate than those larvae that were fed the foliage of the preferred host, trembling aspen (*Populus tremuloides* Michx). All larvae that were fed red maple foliage were dead within two weeks. It was demonstrated that the primary mode of action of the foliage of sugar maple was one of internal toxicity (growth inhibition) whereas red maple foliage was severely antifeedant and possibly also internally toxic.

Feeding bioassays with extracts and purified compounds from the maples incorporated into artificial diet at sub-ecological levels, revealed that both species are chronically toxic and cause concentration dependent reductions in larval growth and pupal weight, and increases in larval mortality. An increase in the time needed to reach pupation and adulthood for males in the red maple treatment was also observed. The extract of *A. saccharum* leaves was actually more active against the forest tent caterpillar than was the species of maple that the insect always avoided.

A study of the variability of the maple defensive phytochemicals showed that although some variation in anti-FTC activity between different growing sites and within individual trees from the same growing site existed, it was not great enough to support the hypothesis that certain sugar maple trees possessed relatively lesser phytochemical defenses, which would allow for selective defoliation by *M. disstria* larvae.

The research concluded by suggesting that nutritional differences in the leaves of red maple and sugar maple may alone, or in conjunction with the secondary chemicals, influence FTC response to the foliage.

Résumé

La livrée des forêts (*Malacosoma disstria* Hübner) est un ravageur important de plusieurs espèces décidues de l'Amérique du Nord. Cependant, il est notoire que l'érable rouge (*Acer rubrum* L.) ne fait pas partie des essences attaquées par ce défoliateur. Les objectifs initiaux de cette étude étaient donc de 1) déterminer la présence de composés secondaires antiappétants contre la livrée des forêts dans les feuilles d'érable rouge et 2) de quantifier la toxicité et les propriétés inhibitrices de la croissance et du développement de ces composés chez la livrée des forêts. À l'issue d'expériences préliminaires, il a été établi que les feuilles d'érable à sucre (*Acer saccharum* Marsh.), une essence généralement considérée comme hôte de la livrée des forêts, contenaient également des composés secondaires délétères contre la livrée des forêts. Nous avons donc inclu cette essence dans notre recherche avec les mêmes objectifs que pour l'érable rouge. Les méthodes courantes d'étude du cycle vital des larves de livrées des forêts nourries sur le feuillage des deux essences ainsi que de mesure des indices d'utilisation de la nourriture à partir de diètes artificielles contenant des fractions isolées du feuillage des deux essences ont été utilisées. L'isolement des fractions a été complétée dans les laboratoires du Service Canadien des Forêts, à Sault-Ste-Marie.

Lorsque nourries sur du feuillage des deux essences d'érable, les taux de développement des larves étaient significativement moins élevés que sur du feuillage de peuplier faux-tremble (*Populus tremuloides* Michx), l'hôte préféré de la livrée des forêts. Dans les faits, aucune larve n'a pu compléter son développement sur le feuillage d'érable rouge et toutes étaient mortes à l'intérieur d'une période de deux semaines. Il a été démontré que le principal mode d'action des composés présents dans le feuillage d'érable à sucre était d'inhiber la croissance des larves (toxicité), tandis que celui des composés de l'érable rouge était

d'inhiber de façon marquée la consommation même du feuillage (action antiappétante). Dans ce dernier cas, il est également possible qu'une action toxique soit présente lorsque le feuillage est ingéré.

Les expériences menées avec des diètes artificielles contenant des extraits ou des composés isolés du feuillage à des concentrations moindre que celles normalement rencontrées en condition naturelle ont démontré que les deux essences d'érable causaient une toxicité chronique dépendante de la concentration se traduisant par un diminution du taux de croissance larvaire et du poids atteint à la pupaison ainsi que par une augmentation de la mortalité larvaire. Des augmentations significatives du temps de développement larvaire et du temps pour atteindre le stade adulte ont également été observées pour les mâles élevés sur la diète contenant les extraits d'érable rouge. Cependant, les extraits d'érable à sucre étaient plus actifs que ceux d'érable rouge, l'essence qui est normalement évitée par la livrée des forêts.

Par ailleurs, l'étude de la variation des composés secondaires de l'érable à sucre a permis de démontrer que, bien qu'il existe des différences entre sites et à l'intérieur d'un même arbre, celles-ci ne sont pas suffisantes pour suggérer que la livrée des forêts puisse sélectionner des arbres ou des sites avec des niveaux de défenses phytochimiques moindre.

En conclusion, cette recherche suggère que les différences dans la qualité nutritionnelle du feuillage des deux essences d'érable peuvent, isolément ou en relation avec les composés secondaires présents, expliquer la réponse observée de la livrée des forêts, bien que d'autres travaux dans ce domaine soient nécessaires pour obtenir une confirmation.

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CHAPTER I: GENERAL INTRODUCTION:

1.1 Background

1.1.1 Chemical Ecology

The field that the following work encompasses is multidisciplinary in nature, and as compared to other branches of science, relatively new. Broadly defined, chemical ecology is the study of naturally occurring chemicals that mediate interactions between organisms. These interactions can be mutualistic or antagonistic, and the participating organisms can range from belonging within the same taxonomic genus, as in pheromonal communication between social insects (Smith and Wenzell 1988, Melathopoulos et al. 1996), to being members of separate kingdoms, as in chemical defenses of plants that ward off fungal attack (Tattar et al. 1971). This thesis investigates the defensive role of plant chemicals (phytochemicals) in plant-insect interactions.

In co-evolutionary theory, phytochemicals arose because they in some way conferred an adaptive advantage on the individuals that possessed them. An obvious way in which these chemicals are advantageous can be seen in the general interaction of insect herbivory with plants. Humans have known for many thousands of years that plants contain substances that are bioactive (beneficial as medicine or deleterious as toxins). Some of the phytochemicals that arose would have also been active against specific, or whole classes, of insects. This would result in reduced frequency or severity of herbivory on the plant, and would give the individual plant that metabolized them an advantage over other individuals in its population, and perhaps, ecosystem. Reduced levels of herbivory would mean that the individual plant would have more available energy for growth and reproduction, through a combination of avoidance of the loss of photosynthetic potential and the loss of resource acquisition, and by not having to replace damaged tissue. In this way, such phytochemicals could be seen as defensive in nature, and any genotypes that had

such effective defenses would gain an advantage over other genotypes that lacked the specific phytochemicals and subsequently, through natural selection, come to dominate their respective species.

These phytochemicals that arose had no known metabolic or primary function (and hence are also referred to as secondary compounds) but were harmful to herbivores by acting as growth inhibitors, acute toxins, juvenile hormone analogues (ecdysteroids), or as substances that cause the herbivore to cease consuming the plant tissue (antifeedants).

All secondary compounds are not defensive, although the disproportionate amount of research done in this area of chemical ecology may give that impression. Many phytochemicals mediate a diverse array of plant-insect interactions (Harborne 1993, Harborne and Baxter 1993). These include actions that benefit the plant, such as the attraction of insects for pollination and in some cases for their nutritional value. Conversely, insects can utilize phytochemicals by cueing on them to locate appropriate host plants for food and ovipositing, as well as the direct use of the compounds via sequestration for insect defense or incorporation into mate-attracting pheromones. Due to the numerous roles secondary substances play within ecosystems, it is beneficial to learn as much as possible in this area for two main reasons. First, the knowledge obtained from such research has a practical value, that is, it can sometimes be applied to control pests in a more environmentally conscious manner. These types of studies also have ecological significance, as the opportunity is present to gain a better understanding of how these novel chemicals interact with organisms and what kind of roles they play within ecosystems.

1.1.2 Phytochemicals as Plant Defenses

Over the last thirty years of work in this area of research, many secondary compounds have been isolated and tested for bioactivity against herbivores. The sheer volume of research completed seems to indicate that Fraenkel (1959) was correct in assuming that the *raison d'être* for plant secondary compounds is to repel and attract insects (and other organisms that pose a threat, or a benefit, to the plant such as pathogenic

viruses, microorganisms and other plants). The literature indicates that a disproportionate number of these chemicals act as allomones rather than as kairomones (phytochemicals that are useful to the receiving organism, [Reese 1979]). Some of this unevenness may be due to investigator bias, as applied research is emphasized in the present environment. However, it is evident that the majority of these compounds are probably defensive in nature.

Many phytochemicals have been isolated, tested and shown to be active against insect herbivores, via different modes of action. For example, one secondary chemical that is extremely potent is rocaglamide, from *Aglaia odorata* Lour. This benzofuran has been shown to significantly reduce larval growth in the European corn borer at a scant level of 0.05 ppm in artificial diet, and to drastically increase larval mortality at 0.1 to 0.2 ppm (Ewete et al. 1996). Alpha-terthienyl, isolated from the Asteraceae, is toxic to insects only after being activated by light. This phototoxin, when combined with near UV irradiation or naturally occurring sunlight, proves fatal to mosquito larvae (LD₅₀ of 19 ppb), blackfly larvae (LD₅₀ of 28 ppb), and the tobacco hornworm (LD₅₀ of 10 µg/g in contact application) (Arnason et al. 1989). Some plants forgo the direct route altogether. Herbivory on the leaves of the lima bean induces systemic production of volatiles that attract predators of the feeding insect, thereby exerting pest control by “calling” for help from the third trophic level (Dicke et al. 1993). Other plants disrupt metabolism of the herbivore by means of toxic amino acids (e.g. canavanine in the Leguminosae), or have ecdysteroids in their foliage that interfere with normal insect development (e.g. some species of fir, sweet basil and bracken fern) (Harborne 1993). The list can go on *ad infinitum*, but the point is that many of the secondary chemicals have been shown to be too potent, novel and specific to have evolved as anything else but plant defenses. The emphasis in the preceding sentence should be placed on “many” as some secondary metabolites have undoubtedly arisen in the absence of herbivore pressure, and in response to other biotic factors.

1.1.3 Application of Theory

Due to the antibiotic activity of the secondary compounds, an enormous practical opportunity exists in the field of chemical ecology. The phytochemicals have arisen over millions of years to keep bacteria, viruses, fungi and insects at bay and are therefore candidates for new and new models of pesticides and medicinal drugs.

The control of insect pests in North America has always been crucial under current agricultural and forestry practices. Forest managers need to control pests to protect valuable timber species and to prevent yield losses due to herbivory. Because of the size and value of the forestry industry, insect pests have the potential to create large economic losses. For example, in 1988, the value of forestry products was \$4.3 billion or 3.6% of the Canadian GDP which translated into 770 000 jobs depending directly or indirectly on the forestry sector (Forestry Canada 1990). The dollar amount in 1988 represented 21% of the total world trade in forestry products (Forestry Canada 1990).

It is evident from an economic standpoint, that forest resources must be protected against losses due to insects. The challenge now is to find new methods of accomplishing this task. New emphasis has been placed on finding "natural" pesticides from botanical sources that have "softer" modes of action, that do not persist in ecosystems and have a wider public acceptance. The Canadian Forest Service has recently decided to officially pursue alternatives to synthetic pesticides (Forestry Canada 1990, Canadian Council of Forest Ministers, 1992). This search for alternative pest control methods was the catalyst for the work presented herein, namely, to investigate the phytochemical properties of a deciduous tree that had been observed in the field to be resistant to defoliation from a pest of many economically important hardwood trees, the forest tent caterpillar (FTC).

1.2 Dramatis Personæ

1.2.1 The Forest Tent Caterpillar

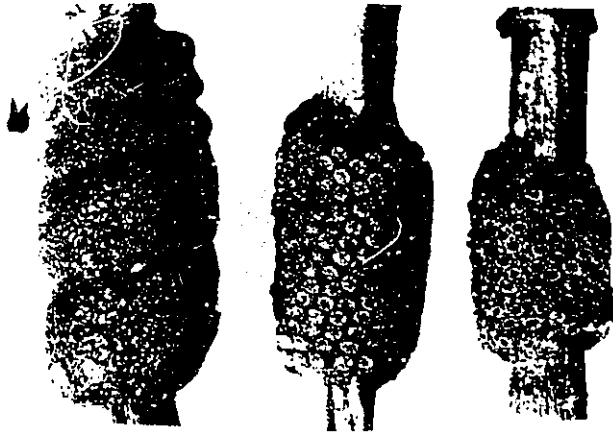
Forest tent caterpillars represent the larval stage of the lepidopteran *Malacosoma disstria* (Hübner) and eventually complete their life cycle as adult moths. This insect belongs in the Lasiocampidae family which has 30 representative species in North America (Davidson and Prentice 1967), with the FTC and other *Malacosoma* spp. being easily identified because of the silken tents or mats they create. They are found throughout Canada and their natural range extends as far south as Louisiana (Martineau 1984).

Larvae emerge from eggs (Figure 1.1a) in the early spring, coinciding with the leaf flush of many of their food hosts, deciduous trees. The larvae (Figure 1.1b) of *M. disstria* are voracious folivores that prefer the leaves of trembling aspen (Hodson 1941, Prebble 1974) but will defoliate many hardwoods such as ash, red oak, sugar maple, birch and black and red gum during an outbreak (Davidson and Prentice 1967, Batzer and Morris 1971). The larvae are gregarious by nature, and feed and live in colonies. If host foliage is completely stripped within an area, the FTC move on to other areas or begin to consume other plants that are not usually considered food hosts.

The larvae go through five or six instars, which takes three to four weeks. They then pupate inside silken webs on trees and metamorphosize into adult moths. The moths mate and the female (Figure 1.1c) lays eggs in formations of bands (Figure 1.1a), covered by a silver-grey spumilin, around twigs and small branches of trees. The adults will then die, with the entire portion of the larval to moth stages of the life cycle taking about six weeks. The FTC embryos overwinter in the egg stage and hatch the following spring, thereby starting over the life cycle for another season.

The population dynamics are cyclical, with low levels of the insect persisting between outbreaks of a regular frequency. Outbreaks of this pest usually occur at intervals of 10 to 13 years (Hodson 1941, Myers 1993) and usually last no more than 3 to 4 years (Sippell 1962), with the earliest outbreak in North America being recorded in 1791 (Martineau 1984). FTC population dynamics are related to climate (Wellington et al. 1952,

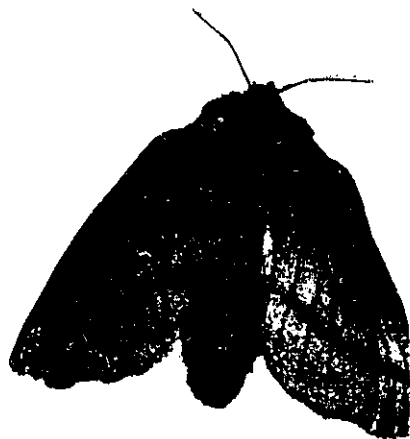
Figure 1.1: Representative stages in the life cycle of *M. disstria* (excluding pupal stage). Egg bands (a) around poplar twig covered with spumilin in first band and with spumilin weathered off in final band revealing individual eggs. Fifth instar larva (b) and adult female moth (c). Modified from Prebble 1971.



a, egg bands



b, 5th instar larva



c, female adult moth

Wetzell et al. 1973, Raske 1975) with spring temperatures affecting the phenology and hatching of neonates of *M. disstria*.

Parasitism also plays a large role in FTC outbreaks, with the occurrence of a pathogenic fungus, and a nuclear-polyhedrosis virus of the larval stage (Stairs 1972) and a fly parasitic to the pupae, *Sacrophaga aldrichi* Park. influencing the frequency and severity of outbreaks. Many researchers (Caesar 1915, Sippell 1957a, Witter and Kulman 1979) found that *S. aldrichi* sometimes parasitized up to 90% of FTC pupae. Modern forestry harvesting and agricultural practices may influence the duration of an outbreak, as fragmented forest habitats have been correlated to an increase in FTC outbreak duration (Roland 1993) via the alteration of the interactions between the above mentioned parasites and the FTC.

The extent of damage caused by FTC in the given “worst” recent year throughout Canada can be seen in Table 1.1. More current statistics reveal that during the last *M. disstria* outbreak in Ontario, this pest defoliated more than 18 000 000 ha at its peak in 1991 (National Forestry Database 1994).

1.2.2 Host and Non-Host Trees

As mentioned above, FTC prefer trembling aspen foliage, but will eat the leaves of many different species. One species whose leaves the larvae will not eat is the red maple (*Acer rubrum* L.) (Hodson 1941, Oliver 1964). Forest researchers have reported that during *M. disstria* outbreaks when many hectares of different trees are stripped of foliage, red maple is left virtually untouched. Preliminary leaf disk bioassays completed at the Canadian Forest Service (CFS) in Sault Ste. Marie, Ontario, confirmed that the larvae would starve to death before eating the red maple disks. The foliage of this tree species may therefore contain secondary compounds that in some way act as phagodeterrents and/or as inhibitors of insect development.

The foliage of two other deciduous tree species will also be examined, to better

Table 1.1: The total area of forest (in hectares) moderately to severely defoliated by the forest tent caterpillar (FTC) in Canadian regions from 1980 to 1987. Only the single year with the greatest amount of area defoliated is reported for each area.

| Region | Year | Area Defoliated (ha) | Reference |
|------------------|------|----------------------|--------------------------|
| Maritimes† | 1982 | 1 412 500 | Magasi, 1995 |
| Québec | 1980 | 780 800 | Lachance, 1995 |
| Ontario | 1987 | 1 649 977 | Howse, 1995 |
| Prairies‡ | 1981 | 20 400 000* | Cerezke and Volney, 1995 |
| British Columbia | 1986 | 93 425 | Van Sickle, 1995 |

†Includes New Brunswick, Nova Scotia and Prince Edward Island.

‡Includes Manitoba, Saskatchewan and Alberta.

*Some damage attributed to aspen tortix (*Choristoneura conflictana* Walk.) and other aspen defoliators.

realize the way in which red maple can withstand defoliation from FTC and to broaden the understanding of the food preferences of this forest pest.

The first comparison species is the sugar maple (*Acer saccharum* Marsh.), as this is a related tree, in the same genus as red maple, but it is known to be defoliated by FTC (Hodson 1941, Davidson and Prentice 1967, Gross 1991). Only 10 of the approximately 150 species of maple that are known in the world are native to Canada (Hosie 1990). The two maple species investigated in this project share similar ranges, with that of sugar maple being slightly more restricted than that of red maple. *A. rubrum* is found from Newfoundland to the Ontario/Manitoba border, whereas *A. saccharum* grows from Nova Scotia to the western shore of Lake Superior (Hosie 1990).

The second comparison species is *M. disstria*'s preferred food host, *Populus tremuloides* Michx, commonly known as the trembling aspen. *P. tremuloides* is known as a prolific pioneer species that usually gives way to more shade tolerant trees. It is pancontinental and can be found from Newfoundland to Vancouver Island (Hosie 1990) and south into the alpine regions of Mexico (Elias 1980).

Investigating the secondary chemistry of the three species and the growth and developmental effects when foliage of the three are fed to FTC, should be efficacious in determining the mechanisms by which red maple escapes herbivory by *M. disstria* larvae.

The FTC cause serious economic damage to the two susceptible species when outbreaks occur due to the length of the outbreaks (three to five years) (Sippell 1962 and Myers 1993) and as they are colonial and voracious polyphages. The severe defoliation that is concomitant with an infestation of *M. disstria* larvae decreases growth, (and hence reduces yield of wood and pulp), and can reduce the quality and quantity of sugar maple sap (Division of Timber Management Research 1965, Batzer and Morris 1971). It was estimated by Cerezke (1991) that radial stem growth of aspen was reduced in FTC outbreaks such that a tree may only reach 10% of its normal growth. This growth

reduction translated into annual wood volume losses of up to 4.5m³/ha during an infestation and could occur for several consecutive years (Cerezke 1991).

Tree mortality does not often happen because of damage by *M. disstria* larvae (Cerezke and Volney 1995), but it can occur. In an FTC outbreak in the late 1970's, approximately 14 400 ha of sugar maple forest that were attacked in the Algonquin and Southwestern regions of Ontario experienced more than 25% tree mortality (Howse 1995). In other instances, death can occur when a tree already stressed by herbivory faces other stresses such as adverse climatic conditions or subsequent attack by other pathogenic organisms. For example, aspen becomes more susceptible to other insects (especially those belonging to the wood boring guild) and the incidence of hypoxylon canker increases after an FTC infestation, which can lead to tree mortality (Anonymous 1986).

Table 1.2 represents the known phytochemicals in red and sugar maple and trembling aspen. One phytochemical that is conspicuous is the indole amine gramine, which can usually be found in members of the grass family, Gramineae, (Glasby 1991). However, the levels that have been isolated from red maple are quite low (0.001% of dry weight, Barbosa et al. 1990) and so far, it has been found only in tissue that the FTC does not feed upon (i.e. the maple "keys" and autumn foliage, Table 1.2). Tissues of all three species contain phenolics, which as a class of phytochemicals occur in the majority, if not all, of the tree species in North America, and are known to be active against a wide range of phytophagous insects.

Table 1.2: The secondary compounds present in the three relevant tree species.

| Tree Species | Compound(s) | Isolated From: | Reference |
|-----------------------|---|----------------|-----------------------------------|
| | caffeic, ferulic, vanillic and p-coumaric acids | dormant buds | Thakur 1977 |
| | gallic acid, catechin | wood, bark | Tattar and Rich 1973 |
| <i>A. saccharum</i> | quercetin, chlorogenic acid | foliage | Hatano et al. 1990 |
| | geraniin, davidiin | foliage | Ibid. |
| | catechin, epicatechin, cyanidin | wood, bark | Tattar and Rich 1973, Parker 1974 |
| | cyanidin glycosides | foliage | Ji et al. 1993 |
| | gramine | fruits | Pachter 1959 |
| <i>A. rubrum</i> | gramine | fall leaves | Barbosa et al. 1990 |
| | gallic acid, catechin | wood, bark | Tattar and Rich 1973 |
| | cyanidin glycosides | foliage | Ji et al. 1993 |
| <i>P. tremuloides</i> | salicin, salicortin, tremuloidin, tremulacin | foliage | Lindroth et al. 1987 |
| | tremulone | heartwood | Abramovitch and Micetich 1962 |

1.3 Hypotheses and Objectives

The following project was designed to elucidate how the red maple (*A. rubrum*) escapes herbivory by the forest tent caterpillar. After deleterious effects against the larvae of *M. disstria* were obtained in initial tests with *A. saccharum*, it was decided to also undertake a comprehensive study of this species.

Hypotheses

The foliage of *A. rubrum* contains secondary phytochemicals that are antifeedant and growth inhibitory, and the foliage of *A. saccharum* contains secondary phytochemicals that inhibit growth of the larvae of the forest tent caterpillar, (*M. disstria*) but that are not antifeedant.

Objectives to establish the validity of the hypotheses

- 1.) Quantify the growth and development of *M. disstria* in feeding bioassays using the foliage of the two maples and of the preferred food host (*Populus tremuloides* Michx.), and extracts and purified compounds from *A. rubrum* and *A. saccharum*.
- 2.) Classify, in broad terms via nutritional bioassays, the modes of action of the maple extracts and isolated phytochemicals.
- 3.) Investigate the geographic and interspecific variability of the larval growth-reducing ability of red maple and sugar maple's secondary chemicals.
- 4.) Investigate the antifeedant properties of the foliage of *A. rubrum* in collaboration with the Canadian Forest Service.

In summary, this project aimed to relate feeding behaviour and growth and development of the FTC to phytochemical aspects of the tested tree species. The investigations were first completed at the general level of the consumption and subsequent growth of the FTC on the foliage of trembling aspen, sugar maple and red maple. They continued by using known phytochemical methods to isolate and test the secondary constituents of the maples. These extracts and purified compounds were fed to *M. disstria* larvae, via bioassays using artificial diet, to obtain growth and life cycle effects. Tests

were then completed to determine the general mechanisms by which the secondary compounds exerted their action. During the course of this thesis it was found that the foliage of the maple species that is considered a food host of the FTC (*A. saccharum*) caused detrimental growth and development effects in the forest tent caterpillar. As this result was unexpected, the variability of defensive activity was completed. This was done to test the hypothesis that varying levels of anti-FTC activity in sugar maple could allow this tree to be selectively defoliated by *M. disstria*. Finally, the nutritional differences between the foliage of *A. rubrum* and *A. saccharum* were briefly addressed, as feeding preferences and deleterious effects may not be related to secondary chemicals alone.

CHAPTER II: FOLIAGE BIOASSAYS:

2.1 Introduction

The logical starting point for this project was to quantify the consumption and growth of the larvae when they feed on the foliage of the three species in question. A life cycle and nutritional bioassay were undertaken on the FTC using leaf disks of trembling aspen, sugar maple and red maple.

P. tremuloides, *A. saccharum* and *A. rubrum* are deciduous trees that are native to North America. Sugar maple is the most important species economically, the wholesale value of its food products alone was worth over \$82 million in 1992 (National Forestry Database 1994), but all three are important via different end uses. The wood of most maples is known to be hard and strong, and as such, is prized in the furniture, flooring and veneer industries (Lauriault 1989). The sap of *A. saccharum* is also renowned worldwide as the starting material for maple syrup. *P. tremuloides* is used in the pulp and paper industry (Lauriault 1989).

This chapter presents experiments that simulate the growth and development that would be experienced in nature by FTC feeding on the three species. This was accomplished by running a life cycle bioassay with the *M. disstria* larvae where foliage from poplar, sugar maple and red maple were used as the food source. A nutritional indices bioassay was then completed in order to explain any differences in life cycle parameters in terms of the mode(s) of action of the deleterious characteristics of the foliage.

A life cycle bioassay allows the researcher to quantify insect growth and development after chronic dietary exposure to the test material, (in this case foliage, but later, isolated phytochemicals added to artificial diet), whereas the nutritional bioassay analyzes certain physiological factors of the insect (ingestion, digestion, excretion) and attempts to discern the mechanism by which the foliar defensive chemicals exert their

effects. Phytochemicals may act as internal toxins that disrupt metabolism and hence growth, or they may act at a more basic level as antifeedants, wherein the insect's growth is slowed or arrested due to reduced food intake. Put more succinctly, if the first test exposes differences in growth when the insect feeds on different material, the second test is done to find out how this happens.

2.2 Materials and Methods

2.2.1 Foliage Life Cycle Bioassay

The following bioassay was completed under the supervision of Dr. Blair Helson, CFS Sault Ste. Marie. Foliage from the three tree species: red maple; sugar maple; and trembling aspen was collected in the morning from a woodlot in Sault Ste. Marie, Ontario (46.3°N latitude and 84.1°W longitude). The collection dates (June 3 to July 22, 1994) were phenologically timed to coincide with the life cycle of *M. disstria*.

Ten neonate laboratory reared FTC were placed in a 1 liter Mason jar containing a sprig of the test foliage held in a vial containing water. Five replicates were used for a total of fifty larvae per treatment for each year. The larvae were allowed to feed at will and the foliage was changed weekly or more frequently as necessary. Growth was assessed by weekly larval weight determination and weekly observations of the amount of foliage consumed were recorded. Jars were stored in a controlled environment chamber set at 21°C and 70% relative humidity and a 16:8 hour photoperiod.

2.2.2 Dietary Utilization Study

Foliage was collected in the same manner as above, but as the test was short-term, the foliage used in this bioassay was collected on one date, June 2, 1995. Leaf disks of each species were cut using a number 13 cork borer and three such disks were placed in the bottom of a glass vial along with a newly molted fourth instar FTC. The vial was stoppered with a moist cotton ball to reduce dehydration of the disks and the larvae were permitted to eat at will for three days. Vials were stored in a Convicon® incubator at the

standard (University of Ottawa) environmental regime (25°C, 80% relative humidity, 18:6 photoperiod).

Amount eaten, frass produced and weight gained over the time period were all recorded as dry weight, with the disks and frass being collected daily and oven dried at 60°C for 72 hours. A preliminary lot of larvae was also dried in the same manner, to estimate the initial dry weight of the test larvae.

The bioassay ran for three days, at which time the larvae were not exposed to food for 4 hours to allow them to pass any remaining frass. The nutritional indices: relative consumption rate (RCR); relative growth rate (RGR); assimilation efficiency or approximate digestibility (AD); efficiency of conversion of ingested food (ECI) and efficiency of conversion of digested food (ECD) were then calculated according to Waldbauer (1968) (Appendix I).

As *P. tremuloides* is known to be the preferred food host of *M. disstria*, the foliage of this tree was used in the manner of a positive control for both bioassays.

2.2.3 Statistical Analysis

The results obtained in this chapter, and all other chapters unless otherwise noted, were analyzed in the following manner using the general linear model procedures on the SAS® (SAS 1982) software package. Data was first subjected to an ANOVA test to identify if treatments caused significant ($P < 0.05$) effects. If this turned out to be true either a multiple means comparison, (Student-Neuman-Keuls or SNK), or a Student's T-test, was employed to identify which treatments differed significantly from control. Distributions that were found to not approximate a normal distribution curve or were found to be heteroscedastic, were log transformed before analysis which corrected the violations of assumptions.

2.3 Results

2.3.1 Life Cycle Bioassay

Figures 2.1-2.2: Growth dynamics of the larvae of *M. disstria* after feeding on the foliage of *P. tremuloides* or *A. saccharum* in a life cycle bioassay. Larval weights were recorded until the time of first pupation. All larvae in the *A. rubrum* treatment died within two weeks. Error bars are standard errors of the means (top). The number of *M. disstria* larvae that successfully pupated after feeding on *P. tremuloides* or *A. saccharum* foliage, and the amount of time needed to achieve pupation (bottom).

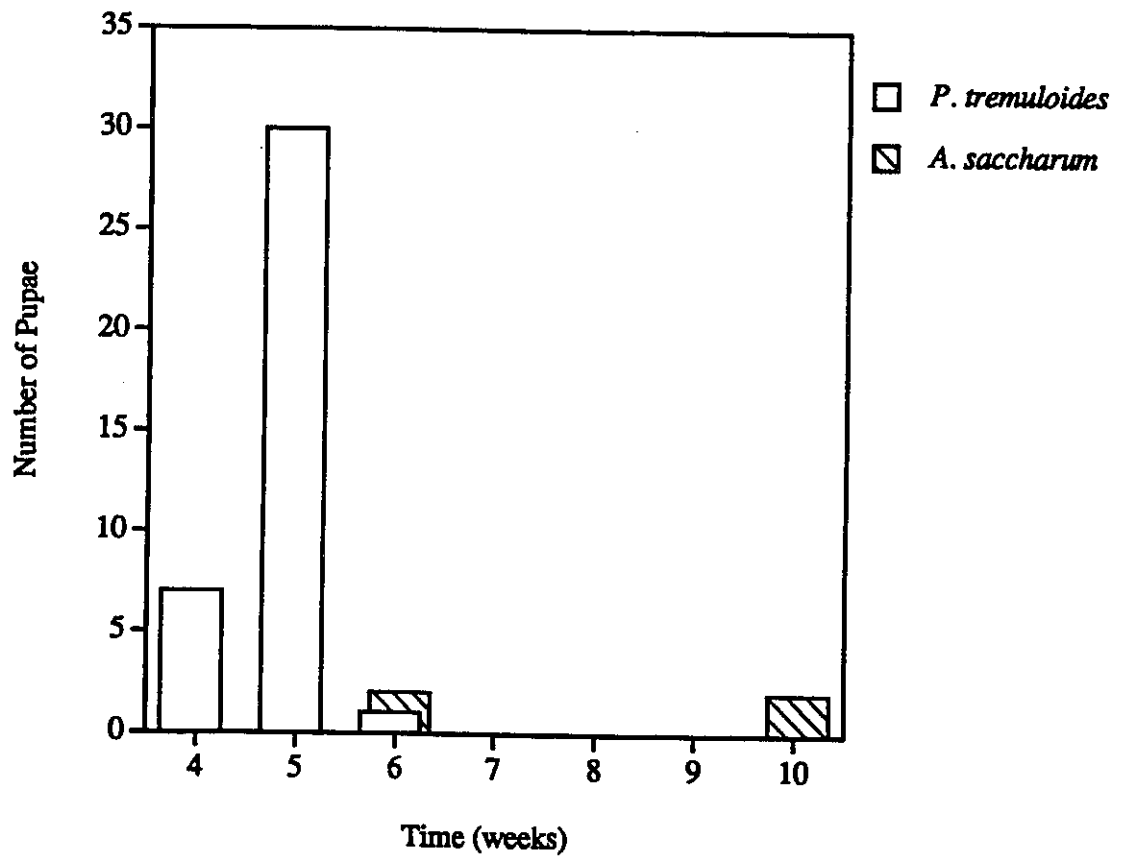
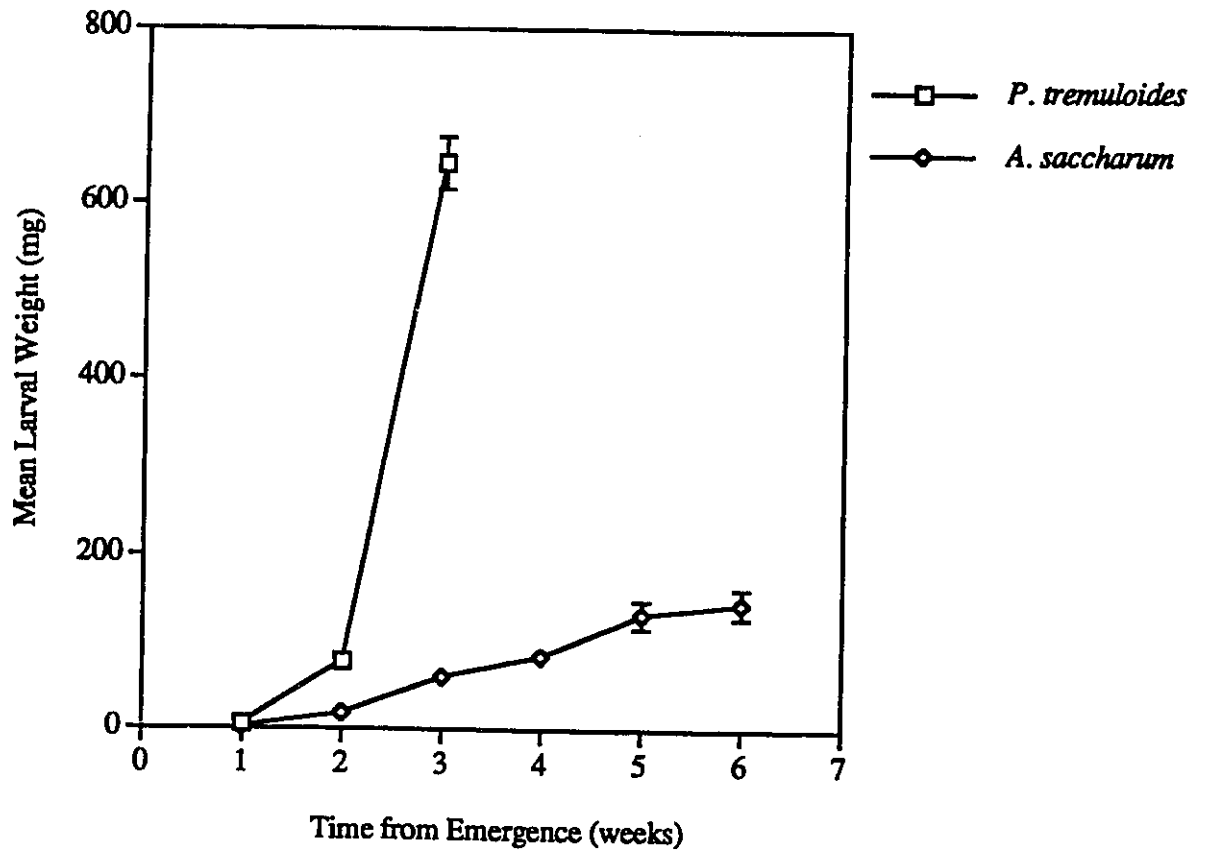


Figure 2.1 illustrates the growth dynamics of the larvae in two of the three treatments. Weekly mean weights were not plotted after the appearance of the first pupa per treatment. One can see the dramatic differences in growth performance between foliage. It is obvious why trembling aspen leaves are the preferred food host as the *M. disstria* larvae grew exponentially between weeks 2 and 3 (Figure 2.1). By the third week of the bioassay, the aspen fed larvae had, on average, grown to 22.6 times the size of the larvae fed the leaves of *A. saccharum*. The differences in average larval weight gain per treatment were very large, and as expected, significantly different (Table 2.1).

Larvae fed red maple leaves, did not survive. The growth dynamics of the replicates in this treatment could not be plotted, as only 17 larvae were alive in the first week averaging a meager 0.25 mg each. All had died by the end of the second week (Table 2.1). Mortality (and all other analyzed test parameters), in the other two treatments followed the trend established in the larval growth stage. Larval mortality was 3.7 times lower in the aspen treatment than in the sugar maple treatment (Table 2.1).

The increased growth rates and lower incidence of mortality translated into a better pupal performance for the FTC that were reared on aspen leaves. The replicates in this treatment began to pupate two to three weeks ahead of their sugar maple fed counterparts (Figure 2.1, Figure 2.2) and enjoyed a much higher rate of successful pupation (77.6% versus 8.5%) (Table 2.1). All successful larvae had pupated by the sixth week in the aspen treatment, whereas 2 of the 4 recorded pupae in the sugar maple treatment pupated during the tenth week (Figure 2.2) and three replicates were still in the larval stage at the cessation of the experiment (after 10 weeks). No larvae survived to pupation in the red maple treatment.

Mean pupal weight also differed significantly between the sugar maple and aspen treatments. The pupae from sugar maple fed larvae that survived to pupation weighed about 436 mg less on average than their aspen fed counterparts (Table 2.1).

2.3.2 Nutritional Utilization Bioassay

Table 2.1: Larval and pupal parameters of FTC reared on the foliage of one of three tree species in a life cycle bioassay.

| Tree Species | Mean Larval Gain per Jar (mg) | Larval Mortality (%) | Time to Appearance of First Pupa | Pupal Attainment (%) | Mean Pupal Weight (mg) |
|-----------------------|-------------------------------|----------------------|----------------------------------|----------------------|------------------------|
| <i>P. tremuloides</i> | 6263.8±339.6 a† | 18.4 | 3 to 4 weeks | 77.6 | 507.3±34.4* a |
| <i>A. saccharum</i> | 277.6±94.1 b | 64.1 | 7 weeks | 8.5 | 71.5±41.0 b |
| <i>A. rubrum</i> | --- | 100 | --- | 0 | --- |

† Means followed by the same letter within a column do not differ significantly in a Student-Newman-Keuls (SNK) test at P<0.05.

* Values are ± standard error.

The nutritional indices demonstrated that the deleterious effects caused by the ingestion of red and sugar maple leaves were due to different mechanisms (Table 2.2).

The leaves of red maple definitely exhibited an antifeedant property. This can be seen in the relative consumption rate in Table 2.2, where the larvae fed the foliage of *A. rubrum* ate significantly less plant material (13.3 times less than those in the aspen treatment) and gained significantly less weight than those larvae in both the sugar maple and aspen treatments. In the consumption values from both bioassays, one can see that red maple foliage has an antifeedant property and this reduction in consumption resulted in significantly reduced growth rates for the FTC.

Although the other indices (AD, ECI and ECD) had a large and significant difference from the corresponding indices in the other two treatments, they may be less reliable because of the small amount of material measured in determining the percentages. For most replicates in the red maple treatment, the amount eaten and amount excreted were very small and in most cases, larval weight gain or amount eaten were negative numbers. The foliage of red maple may contain internally toxic compounds, but the reductions in the efficiencies may be artificially inflated due to problems associated with reduced food intake. This idea is expanded upon in the discussion.

The indices for the sugar maple treatment indicate that these leaves have an internally toxic component and that this toxicity was presumably responsible for the slow growth rate, high mortality and subsequent reduction in pupal parameters experienced in this treatment. The FTC in this treatment ate more than the larvae in the aspen treatment, relative to body weight. However, they gained significantly less weight than the aspen fed FTC (Table 2.2). This reduction in growth was a result of a decreased ability to convert the foliage into biomass rather than any change in the actual digestibility of the foliage. Therefore, compounds in the foliage of *A. saccharum* act against the FTC as internal toxins (disrupting metabolism, causing internal damage or using energy for detoxification

Table 2.2: The nutritional indices for FTC fed leaf disks of either trembling aspen, sugar maple or red maple in a three day bioassay.

| Treatment (species) | RCR* mg mg ⁻¹ day ⁻¹ | RGR mg mg ⁻¹ day ⁻¹ | AD (%) | ECI (%) | ECD (%) |
|-----------------------|--|---|--------------|-------------|-------------|
| <i>P. tremuloides</i> | 1.6±0.1 [†] b [‡] | 0.38±0.0 a | 39.1±1.1 a | 23.6±0.7 a | 61.2±3.2 a |
| <i>A. saccharum</i> | 1.9±0.1 a | 0.24±0.0 b | 25.3±2.0 a,b | 11.6±0.4 b | 49.4±4.4 b |
| <i>A. rubrum</i> | 0.12±0.1 c | -0.03±0.0 c | 18.9±14.9 b | -17.4±9.4 c | -1.4±18.4 c |

[†]Values are ± standard error.

[‡]Means followed by the same letter within a column do not differ significantly in a Student-Newman-Keuls (SNK) test at P<0.05.

*RCR=Relative Consumption Rate, RGR=Relative Growth Rate, AD=Approximate Digestibility, ECI=Efficiency of Conversion of Ingested food, ECD=Efficiency of Conversion of Digested food.

enzymes) which in turn causes growth and developmental problems.

The nutritional indices demonstrate the suitability of *P. tremuloides* as a food host for the FTC. The larvae in this treatment and in the life cycle bioassay, were able to grow rapidly because of the high rate of conversion of the food into biomass. These larvae grew the fastest not by simply outeating those in the sugar maple treatment, but rather by having high ECI's and ECD's

2.4 Discussion

2.4.1 Larval Performance on the Three Species

The reports stating that the FTC do not eat the foliage of red maple in the field agrees with the quantitative data presented here. Observations of feeding behaviour during the life cycle bioassay noted that during the first week (when some larvae were still alive) leaf consumption was “almost no feeding to no feeding”. Both tests demonstrated that the non-existent growth and 100% mortality was a function of the larvae simply not eating.

Although the nutritional indices indicate that there were dramatic and significant reductions in ECI and ECD for the larvae in this treatment, it is difficult to discern internal toxicity that has been caused by secondary compounds from the reductions in efficiencies of internal processes that are sure to accompany the decreases in consumption seen here. That is, when a larva's consumption of food drops, the efficiencies of converting food to biomass can decrease regardless of toxic compounds due to reduced metabolic rates. For example, Schroeder (1976) has demonstrated that reductions in food intake can affect the internal digestive efficiencies as well as the assimilation (approximate digestibility).

The poor performance of the larvae in the sugar maple treatment relative to the aspen suggests a toxic component of this foliage. The increased food consumption, coupled with reduced ECD, may indicate that the larvae are engaging in compensatory feeding behaviour (i.e., they are trying to accumulate more nutritional factors to outweigh

the growth disrupting effects of the phytochemicals). This occurs in other lepidopteran larvae (Scriber and Slansky 1981).

The antifeedant results with the red maple foliage were expected, but the large differences of larval fitness between the sugar maple and aspen treatments were not anticipated. The same life cycle bioassay was run again in 1995 at CFS Sault Ste. Marie, and virtually identical results were obtained (all larvae in the red maple treatment were dead by the second week, and the greatest larval growth and fastest development time was in the aspen treatment).

2.4.2 Why do FTC Eat Sugar Maple?

One aspect of these tests that is of great interest and is of a seemingly paradoxical nature, is the comparison between the laboratory results and field observations of feeding on sugar maple foliage. It is quite obvious from the work presented here that sugar maple is not a suitable source of food for this insect even though the incidence of sugar maple as a food host for the FTC is widely reported (Hodson 1941, Craighead 1950, Davidson and Prentice 1967, Stehr and Cook 1968, Batzer and Morris 1971, Prebble 1974, Retnakaran 1978, Grisdale 1985, Gross 1991). The above results indicate that the vast majority of the larvae would not live to reproduce if these insects ate only sugar maple in the field. The question now is why or (more appropriately, how) do they eat the leaves of *A. saccharum* in nature? Three possible answers to this question are discussed here, and one possibility is explored experimentally.

Firstly, the larvae of *M. disstria* are voracious and polyphagous. They defoliate vast areas of forest and attack many different species during infestations. As food becomes limited during outbreaks the larvae either migrate into new areas or consume plants that are normally not utilized as a food source. This consumption of irregular food sources may explain the differences between field observations and the laboratory results reported here. The reports of FTC eating sugar maple may be based on observations made during times of food shortage.

There is evidence of *M. disstria* eating inappropriate plant material. Magasi (1995) reported that during the New Brunswick outbreak of the early 1980's, FTC initially defoliated hosts that were considered "traditional" such as, trembling aspen and fruit trees in the Rosaceae, and then went on to consume foliage of many species that were not usually utilized. The report states that "sugar maple was defoliated on a large scale for the first time and ash, Manitoba maple, alder, larch, white spruce, Colorado blue spruce, ground cover and garden vegetables were all defoliated". Hodson (1941) reported a similar phenomenon when he wrote that "In some areas the caterpillars were so numerous that they fed upon nearly all green foliage including garden crops and even larch, *Larix laricina*". The realization that an insect pest of deciduous trees would eat conifers and ground cover lends credence to the idea that the FTC eat inappropriate food sources at certain times during population explosions and that these food sources would probably lead to reductions in fitness like the reductions seen in the above bioassays.

Another explanation could be related to insect phenology. FTC may switch to sugar maple at later larval stages. Older insects are generally stronger and more resistant to damage from both natural and synthetic chemicals. If an *M. disstria* larva switched from a primary food host to sugar maple at a later instar, it may be strong enough or close enough to the pupal stage to escape the negative effects seen here. Also, a switch by the FTC at a later date in the season could be concurrent with a drop in the amount of defensive compounds in this species (e.g. hydrolyzable tannins decrease as the season progresses [Baldwin et al. 1987]). This could mean that the dramatic reductions in larval fitness seen here are caused by the experimental design. The reduced growth rates and increased mortality could be due to the starting of the neonates on sugar maple and that these effects would not be seen, or would be seen to a lesser degree if the insects were reared on aspen foliage and then put on the other foliage at a later instar.

Thirdly, phenotypic differences in the relative activity of the defensive compounds over different geographical areas may account for the disparity of the reports of sugar

maple being a food host for the FTC and the negative consequences for the FTC when they consume this foliage. Biotic and abiotic factors in the environment are known to cause changes in the secondary profiles of many plant species (Nowacki et al. 1976, Waterman and Mole 1989, Nichols-Orians 1991). Differences in the habitats between the Sault Ste. Marie growing site and the sites where FTC defoliation have been reported may influence the levels of allelochemicals and hence *M. disstria* performance. Also, variation in the secondary profile between individual *A. saccharum* trees may allow *M. disstria* to preferentially defoliate those trees that have relatively lower activity of secondary compounds. These ideas are addressed in Chapter IV.

In summary, it is apparent from these results why the FTC's preferred food host is aspen as seen by the dynamic larval growth and strong pupation parameters. Red maple's foliage repels the FTC, and this antifeedancy was the primary mode of action that caused the complete larval mortality in two weeks. There may be compounds present in red maple that are internally toxic to *M. disstria* larvae but this conclusion cannot be drawn at this point because of the aforementioned implications of reduced food intake. The foliage of sugar maple apparently contains secondary chemicals that are growth inhibitory, not antifeedant. This was demonstrated by the increased consumption over the aspen fed larvae, relative to body weight and the reduced ECI's and ECD's that led to a significantly lower growth rate and increased mortality.

The trials using foliage are crucial because of the need to compare these results with the subsequent experiments using extracted foliar secondary compounds. The comparison allows one to make a distinction between ecologically and theoretically relevant information and practical applications. The nutritional ecology of the insect is valuable from the theoretical aspect of ecological interactions in forest ecosystems and the practical standpoint of pest management. For example, the more that is known of the feeding and growth of insect pests, the more likely the possibility of disrupting the insect's feeding behaviour in nature, and hence avoiding or lessening economic damage.

CHAPTER III: MERIDIC DIET BIOASSAYS:

3.1 Introduction

To determine if plant defensive chemicals cause the growth and development inhibiting-effects reported in the second chapter, standard phytochemical methods were employed to extract the secondary compounds from the foliage of *A. rubrum* and *A. saccharum*. This allows the researcher to test the secondary compounds for anti-herbivore activity in isolation from other foliage characteristics, such as leaf toughness, morphology, and nutritional content. It also allows a to direct test of the toxicity of the secondary defences of *A. rubrum* in the absence of the strong antifeedancy of the foliage and to compare the growth inhibiting effects with those of *A. saccharum*. It was shown in Chapter II that something in the leaves of *A. rubrum* acts as an antifeedant, but it is not known if any defensive compounds are active against *M. disstria* after ingestion. If extracts of the foliage prove to influence the growth and development of FTC, one can conclude that phytochemicals found in the foliage are responsible for this effect.

Extracts of the foliage of the two maples were incorporated into artificial diet and tested in life cycle bioassays. One purified compound, gramine, was tested concurrently with the extracts, as it was hypothesized that this compound may be responsible for the effects observed in the tests with red maple foliage, and because it was readily available from a commercial source, (Sigma-Aldrich, Mississauga, ONT.). The concentrations used were at or below ecologically relevant levels (the levels that were estimated to be present in the leaf at the time in the season when *M. disstria* would be feeding) to ensure that the larvae would eat the diet and ensure that toxicological data could be collected.

After deleterious effects were observed in the life cycle of *M. disstria*, nutritional indices were completed with the extracts in order to deduce the mechanisms by which they operate in comparison with the unaltered foliage results. The subsequent and final step in

the investigations presented in this chapter was an attempt to identify which specific secondary compounds were involved in the anti-herbivore observations and to again test their modes of action. A phytochemical analysis of the foliage of both *A. rubrum*, and *A. saccharum* was completed by Dr. Mamdouh Abou-Zaid at CFS. Four purified compounds from red maple were then tested in the nutritional bioassay.

3.2 Materials and Methods

3.2.1 Extraction Procedure and Phytochemical Isolation

Leaf material of *A. rubrum* and *A. saccharum* was collected in June of 1993 and 1994 from mature trees in Sault Ste. Marie Ontario by Dr. M. Abou-Zaid. Pressed voucher specimens were deposited in the CFS herbarium: CFS-SSM #'s 1001-1010 (red maple) and CFS-SSM #'s 1015-1025 (sugar maple).

The extracts and purified secondary compounds used in this chapter and the relative amounts of the compounds were completed by Dr. Abou-Zaid at CFS Sault Ste. Marie. Two kilograms of fresh leaf material for each species were extracted in two steps. Leaves were first ground in a large Waring commercial blender and extracted with 95% ethanol and double distilled water. The macerated leaf material was then filtered out, and the filtrates were evaporated under reduced pressure. The extract so obtained was then freeze dried.

The freeze dried extracts were then fractionated using different solvent and column systems, with pure compounds from the active fractions being identified by standard chromatographic and spectrophotometric procedures.

3.2.2 Life Cycle Bioassays

Stock solutions of red maple extract (RME) and sugar maple extract (SME) were made by dissolving a known weight of the powdered extract into a known volume of solvent (3 parts 95% ethanol to 1 part double distilled H₂O). The stock solutions were then incorporated into liquid modified Addy's (1969), artificial diet (Appendix II) at the concentrations of 250, 500, 1000, or 2000 µg/g of maple extract in diet for the first trial

and 25, 50, 100 or 200 $\mu\text{g/g}$ of extract in diet for the second trial, by thoroughly stirring the extract solution into the warm (40°C) liquid diet. For the control, 1 ml of the ethanol:water solvent was added to the diet, with all treatments receiving the same volume of solvent. The modified diet is only slightly different from the original, with levels of cholesterol, alphacel, and agar being different and the deletion of wheat germ oil as an ingredient and the addition of the preservative methyl paraben. The life cycle bioassay was run twice as too great a concentration of extract was used in the first trial and therefore, the test was repeated with lower amounts of extract. The diet was then poured into cube shaped molds, allowed to cool and then removed and stored in a refrigerator at approximately 4°C . For gramine, 3-(dimethylaminomethyl) indole, a known quantity of the compound was dissolved into a known volume of 95% ethanol, and incorporated into the artificial diet as above, at concentrations of 30, 100 and 300 $\mu\text{g/g}$.

Five neonate *M. disstria* larvae were placed in a petri dish with a diet cube containing either one of the concentrations of the foliage extracts, gramine, or a control diet cube (ethanol and water only for the maple extracts or 1 ml of ethanol for the gramine treatment). Fifteen plates were used for each concentration in the first trial and twelve plates per concentration were used in the second trial totalling 75 and 60 larvae per concentration respectively. Initial larval numbers changed accordingly with the availability of *M. disstria* egg bands from CFS.

Larvae were weighed as groups of five every three to five days, at which time the diet cube and frass were removed and replaced with a fresh diet cube. Larval mortality was also recorded at this time. Weights were recorded until the first larva pupated. Pupae and adults were then sexed (Muggli 1974) with the pupal weights and amount of time taken to reach pupation and adult eclosion being recorded.

3.2.3 Dietary Utilization Study

3.2.3.1 Maple Extracts

A nutritional indices bioassay was completed with extracts of both red and sugar maple in the same manner as described in Section 2.2.2, except that the food source was different. Instead of foliage, the larvae were fed artificial diet that had been infused with ethanolic solutions of RME and SME, as in the preceding section, at a concentration of 3000 µg/g.

3.2.3.2 Purified Compounds from *A. rubrum*

The same bioassay was completed with purified compounds from the foliage of *A. rubrum* in an attempt to identify the actual chemicals involved with any deleterious effects seen in the life cycle bioassays and to find out how they operate. Gallic acid (3,4,5-trihydroxybenzoic acid), methyl gallate, ellagic acid (4,4',5,5',6,6'-hexahydroxydiphenic acid 2,6,2',6'-dilactone) and quercetin 3-O-rhamnoglucoside (rutin) were incorporated into the artificial diet at a concentration of 5000 µg/g and 15 larvae were used per treatment.

In the nutritional indices bioassay with gramine, 20 larvae were placed in individual glass vials with a diet cube containing ethanol (control) or 1500 µg/g of gramine for 4 days. Larvae in all experiments were kept in a Conviron[®] incubator with an 18:6 photoperiod, a temperature of 25°C and a relative humidity of about 80%. The larvae, diet and frass were all prepared and weighed in the same manner as in section 2.2.2 in order to calculate the nutritional indices (Appendix I).

3.2.4 Statistical Analysis

Time data was analyzed using the Kruskal-Wallis chi-squared approximation test for non-parametric data on SYSTAT[®] (SYSTAT 1992) as this data was heteroscedastic. Mean weight gain values were first log transformed because of violations of the assumptions of normality and homoscedasticity and then analyzed by ANOVA. The means were then separated using the Student-Neuman Keuls (SNK) multiple means comparison procedure using SAS[®] (SAS 1982). Mortality data was analyzed using a log probit analysis (Finney 1952).

3.3 Results

3.3.1 Secondary Profile of *A. rubrum* and *A. saccharum*

The list presented in Table 3.1 represents the secondary compounds that were identified in the active fractions of the extracts of the two species by M. Abou-Zaid, CFS, and the relative amount at which they were present in the foliage. Quantitative measurements had not been completed at the time of writing the thesis, but approximate levels at which they were present relative to each other are presented. Gramine was not found in the spring foliage, although it has been found in other tissues and at a different time in the growing season (Table 1.1, Barbosa et al. 1990, Pachter 1959). The most noticeable difference in the secondary chemical profiles between the two species is the relative absence of the ellagi- and gallotannins in *A. saccharum*, although some of the hydrolytic products of this class may be represented in the four unidentified phenolics. Bate-Smith (1972) classified *A. saccharum* as a low gallic acid maple. All of the compounds identified in the active fraction of the two species are phenolic compounds (Table 3.1).

3.3.2 First Life Cycle Bioassay

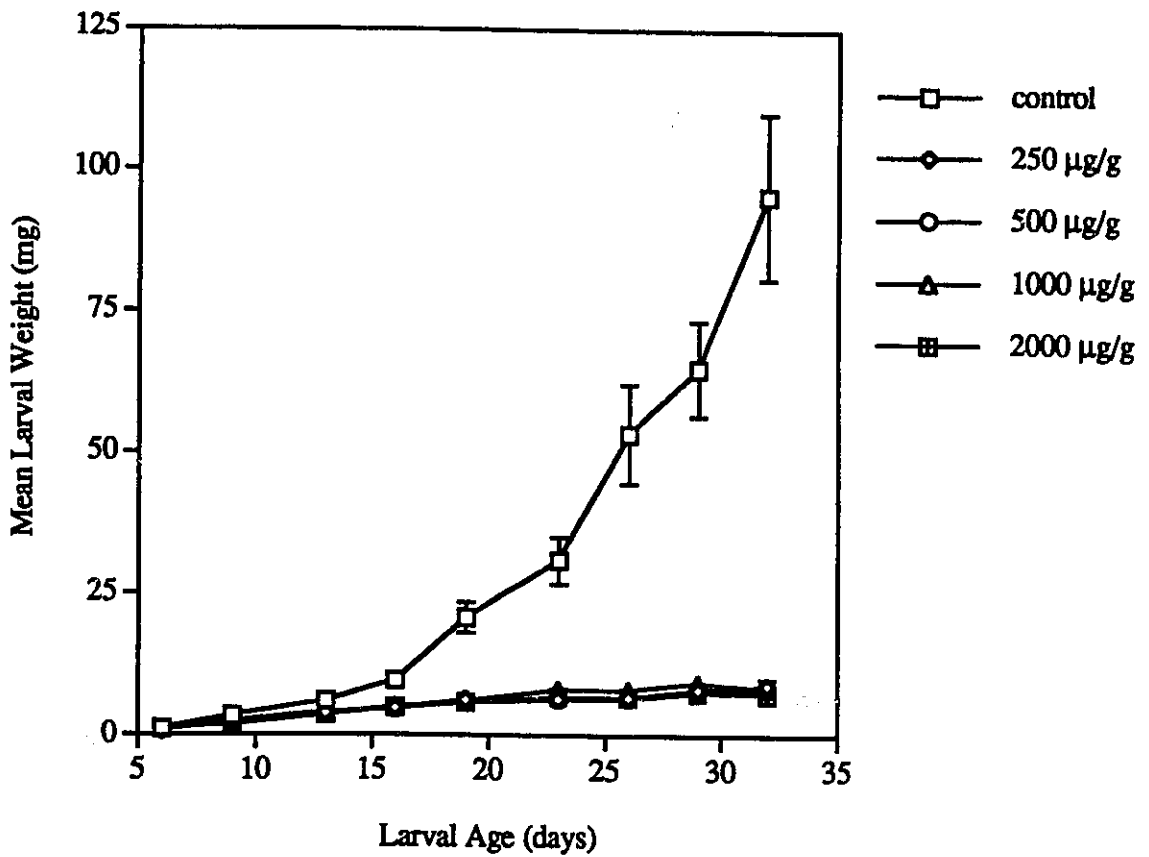
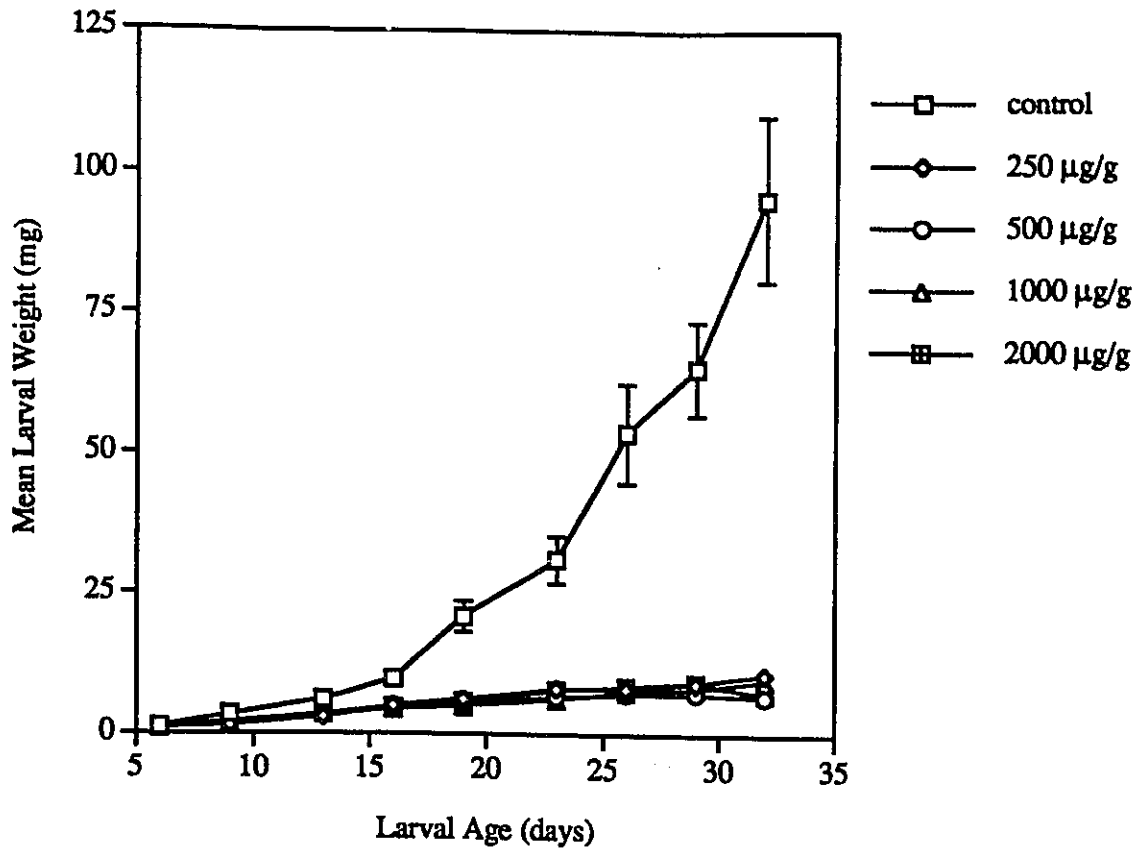
Both red and sugar maple have secondary defences that harm the FTC (Figures 3.1 and 3.2). It is also obvious from Figures 3.1 and 3.2 that the amount of extract used for the first trial was very high, as no concentration dependent action was observed. In a toxicological manner, the larval growth should depend inversely on the extract concentration. As 250 µg/g of both extracts reduced larval growth to the same extent as concentrations that were an order of magnitude higher, it was concluded that although the extracts were active against the FTC, the bioassay should be repeated with lower amounts of extract in the diet. The larvae in all concentrations of the RME treatment were observed to be feeding on the artificial diet so the growth reductions were not due to antifeedancy. Because the concentration of the extracts was too high, no extrapolations could be made regarding the relative activity of RME versus SME.

Table 3.1: A direct comparison of the relative amounts of phytochemicals isolated from red and sugar maple leaves. (from Abou-Zaid et al. 1996).

| Compound | Relative Amounts | |
|---|------------------|---------------------|
| | <i>A. rubrum</i> | <i>A. saccharum</i> |
| Gallic acid (3,4,5-trihydroxybenzoic acid) | ++ | - |
| Methyl gallate (methyl 3,4,5-trihydroxybenzoate) | +++ | - |
| Gallic acid ethyl ester (ethyl 3,4,5-trihydroxybenzoate) | +++ | - |
| m-Galloylgallic acid (3,4-dihydroxy-5-[(3,4,5-trihydroxybenzoyl)oxy]benzoic acid) | ++ | - |
| Diethylgallate | + | - |
| Ellagic acid (4,4',5,5',6,6'-hexahydroxydiphenic acid 2,6,2',6'-dilactone) | + | - |
| 1-Gallyol-glucose | ++ | - |
| 1-Galloyl-rhamnose | ++ | - |
| Kaempferol 3-O-β-D-glucoside (astragalin) | + | - |
| Kaempferol 3-O-β-L-rhamnoside | ++ | ++ |
| Kaempferol-3-O-rhamnoglucoside (nicotiflorin) | + | ++ |

| | | |
|---|-----|-----|
| Quercetin 3-O-β-D-glucoside (isoquercitrin) | + | + |
| Quercetin 3-O-β-L-rhamnoside (quercitrin) | +++ | +++ |
| Quercetin 3-O-rhamnoglucoside (rutin) | + | ++ |
| Quercetin 3-O-glycoside | - | + |
| Quercetin 3-O-diglycoside | - | + |
| Quercetin 3,7-diglycoside | - | tr. |
| Kaempferol 3-O-glycoside | - | + |
| Kaempferol 3-O-diglycoside | - | + |
| Phenolic acid A | - | ++ |
| Phenolic acid B | - | + |
| Phenolic acid C | - | + |
| Phenolic acid D | - | + |

Figures 3.1-3.2: Growth dynamics of the larvae of *M. disstria* after feeding on artificial diet containing four concentrations of either an extract of sugar maple leaves (SME) (top), or an extract of red maple leaves (RME) (bottom), or on a control diet containing ethanol and water, in a life cycle bioassay. Larval weights were recorded until the time of first pupation. Error bars are \pm standard error of the mean.



Due to the high larval mortality experienced in this trial (96.0% to 98.7%, Table 3.4) the remaining life cycle data, namely pupal and adult parameters, could not be collected.

3.3.3 Second Life Cycle Bioassay

A second set of extract bioassays was completed with concentrations below the threshold of 250 $\mu\text{g/g}$ established in the first series. RME and SME were tested again.

The results obtained in the second set of tests confirmed what had been suspected throughout the course of the project, namely, that both red and sugar maple contain secondary chemicals that interfere with the growth and development of *M. disstria* larvae. Feeding both maple extracts to the *M. disstria* larvae via artificial diet proved to again reduce larval growth, this time in a concentration-dependent manner (Figures 3.3 and 3.4). Larvae ceased to grow at the highest concentration of RME (200 $\mu\text{g/g}$) and the two highest concentrations for SME (100 and 200 $\mu\text{g/g}$). The weight gained by the FTC up until the time of pupation was significantly different from control at all concentrations of both maple extracts (Table 3.2).

The extract of sugar maple leaves had a greater effect on larval growth than did the red maple leaf extract. For example, the threshold where larval growth was arrested was reached at a lower concentration in the SME treatment than in the RME treatment (100 $\mu\text{g/g}$ versus 200 $\mu\text{g/g}$, Figures 3.3 and 3.4). The larvae in the SME treatment also seemed to gain less weight than their RME counterparts (Table 3.2), although these means were compared to the control only, so the efficacy as a *M. disstria* growth inhibitor of one extract compared to the other can not be determined from the statistical analysis represented in this Table. Table 3.3 shows the results of a two-way ANOVA completed on the larval weight gain after exposure to SME and RME. This table demonstrates that the type of extract had a significant effect upon the dependent variable of larval weight gain ($P < 0.05$), and that there was no significant interaction between extract and concentration.

Figures 3.3-3.4: The second trial of the life cycle bioassay using concentrations of maple extracts that were a magnitude of order lower than those used in the first attempt. Growth dynamics of *M. disstria* larvae after feeding on artificial diet containing either an extract of sugar maple leaves (SME) (top), an extract of red maple leaves (RME) (bottom), or on a control diet containing ethanol and water. Larval weights were recorded until the time of first pupation. Error bars are \pm standard error of the mean.

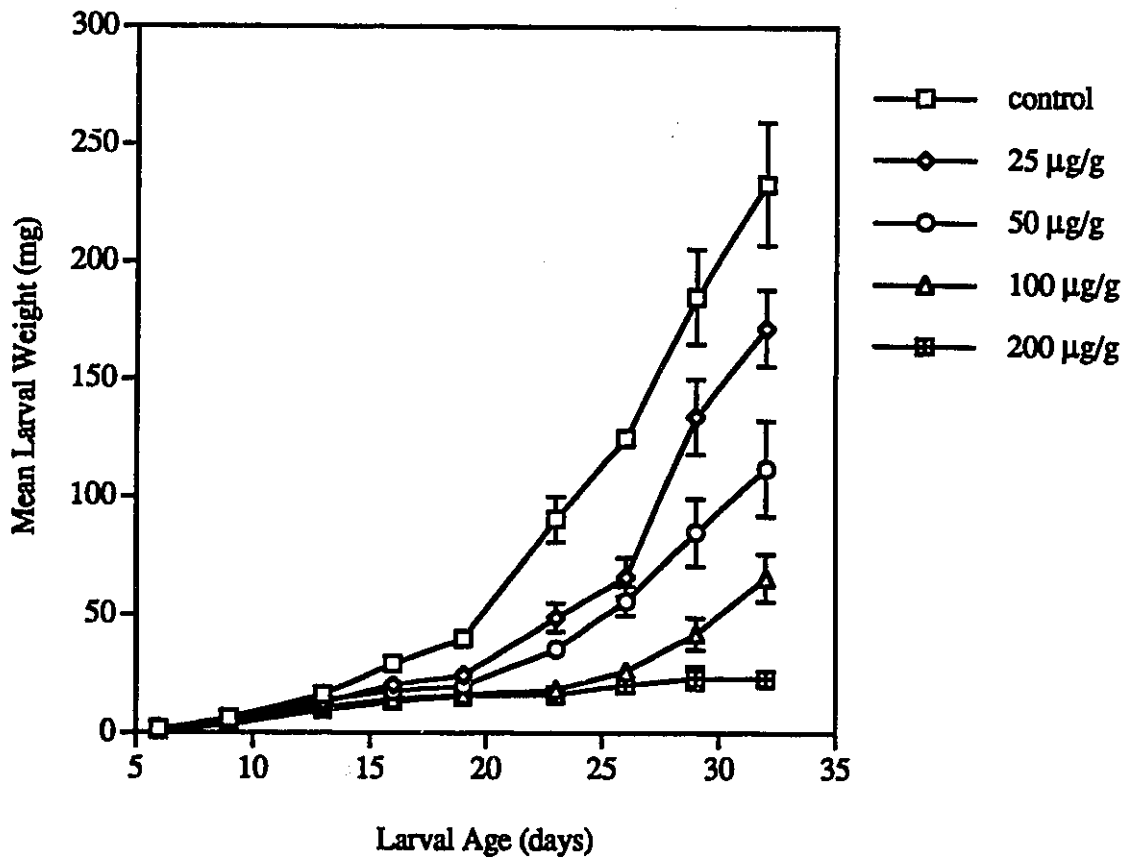
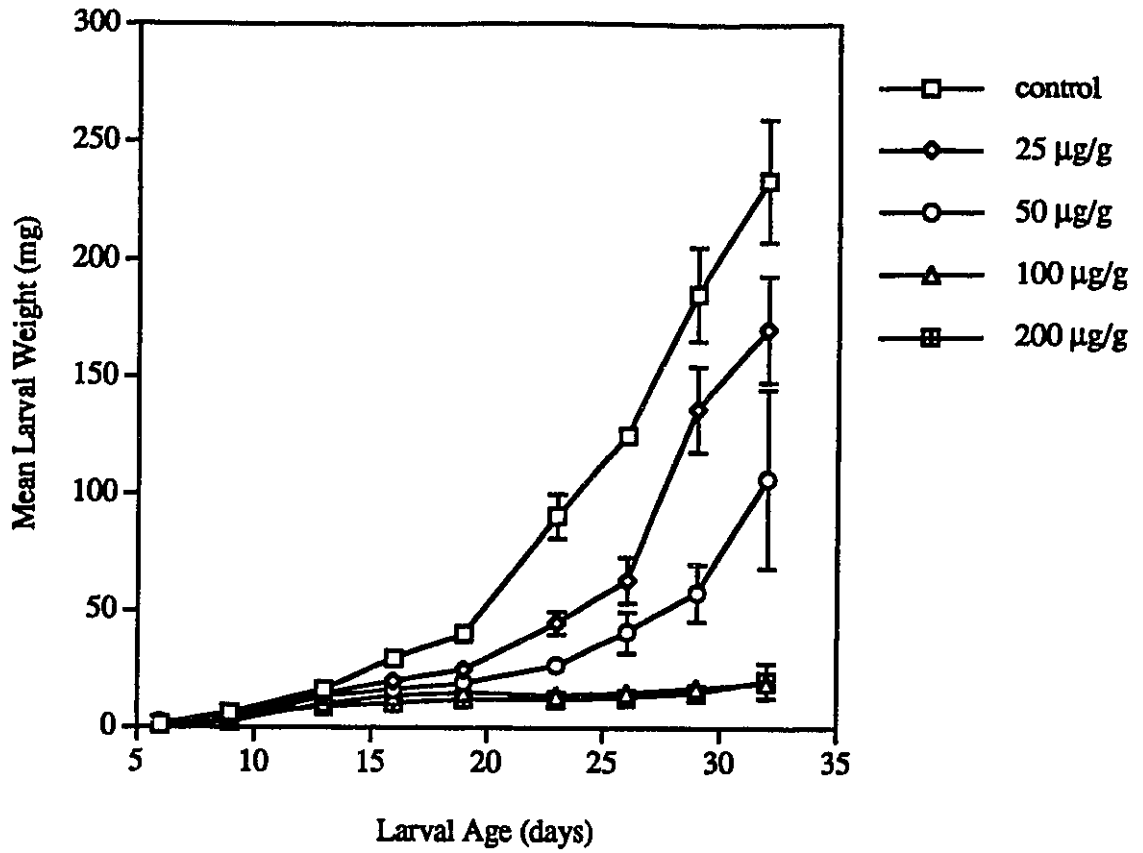


Table 3.2: Average weight gained by replicates *, after 32 days in a life cycle bioassay.

| Treatment | Concentration (µg/g) | Mean Weight Gain (mg) |
|-----------|----------------------|---|
| Control | --- | 779.6±121.3 [†] a [‡] |
| | 25 | 374.9±71.6 a |
| RME | 50 | 168.5±30.1 b |
| | 100 | 82.9±25.1 b |
| | 200 | 28.4±5.4 c |
| | 25 | 345.4±66.8 b |
| SME | 50 | 177.4±47.2 b |
| | 100 | 31.3±9.5 c |
| | 200 | 16.5±8.0 c |

[†] Means are ± standard error.

[‡] Means followed by the same letter, within a column, do not differ significantly in a Student-Newman-Keuls (SNK) test at P<0.05.

* Replicates refers to the mean weight gain per petri dish rather than individual larvae, as *M. disstria* are gregarious and feeding by one larva will influence feeding behaviour of the other larvae

Table 3.3: Values for the two-way ANOVA completed on the interaction between the maple extracts (RME and SME) and concentration, upon the dependent variable (log larval weight gain).

| Source | Degrees of Freedom | Mean Square | F Value | P Value |
|-----------------------|--------------------|-------------|---------|---------|
| Extract | 1, 78 | 3.99, 0.99 | 4.05 | 0.048 |
| Concentration | 3, 78 | 30.57, 0.99 | 30.98 | <0.0005 |
| Extract*Concentration | 3, 78 | 1.27, 0.99 | 1.29 | 0.285 |

Larval mortality was also observed to increase in a concentration dependent manner (Table 3.4). RME and SME caused increased mortality over those in the control. A threshold is seen whereby large increases in extract concentration do not result in increases in mortality. An order of magnitude increase of RME and SME concentration did not increase larval mortality from the 96 to 98% range.

3.3.3.1 Pupal and Adult Parameters

Larvae in the second bioassay survived to adulthood, and therefore, data were obtained on the effects of the extracts on pupal and adult parameters, that were not obtained when the larvae failed to survive to the completion of their life cycle. Both maple extracts influenced the pupal parameters. The number of larvae that successfully pupated, the weight of these pupae and the time needed to achieve pupation were all negatively influenced by the maple extracts in a treatment and concentration dependent manner. SME reduces total numbers of pupae to the greatest extent. Larvae survived to pupation at only the two lowest concentrations, and the total number of pupae was greatly reduced (Figure 3.5). Fewer of the larvae in the RME treatment pupated than in the control, with larvae surviving to pupation in only the three lowest RME concentrations (Figure 3.5). Both maple extracts reduced the mean pupal weight, but only the male pupae in the RME treatment at 100 $\mu\text{g/g}$ were significantly different from control (Table 3.5).

It is of interest to note that although the concentrations of 25-50 $\mu\text{g/g}$ significantly reduced larval weight gain (Table 3.2) the same concentrations did not seem to have the same effect upon pupal weight in either the males or females. SME is generally more active against *M. disstria* than the RME in larval parameters such as growth, weight gain and mortality. However, RME may be more effective in delaying the onset of adulthood. Larvae fed this extract took the longest to pupate and become adult moths out of all of the extract treatments (Figures 3.6-3.7). This trend in the RME treatment is significant in time to pupation and adulthood for males, and relatively close to significant ($P < 0.07$) in time to pupation for females (Table 3.6). Generally, males took longer to reach pupation and

Table 3.4: Cumulative mortality of FTC over 36 days, after dietary exposure to three foliage extracts, for both life cycle bioassays. LC_{50} is the concentration needed to cause 50% mortality within the treated group and was calculated using a Finney (1952) probit analysis.

| Treatment | Concentration ($\mu\text{g/g}$) | Total Mortality (%) | LC_{50} ($\mu\text{g/g}$) |
|-------------|-----------------------------------|---------------------|-------------------------------|
| Control (1) | 0.0 | 32.0 | |
| Control (2) | 0.0 | 38.7 | |
| | 25 | 62.7 | |
| | 50 | 72.0 | |
| | 100 | 88.0 | |
| RME | 200 | 90.7 | $4.4 < 14.0 < 23.1^\dagger$ |
| | 250 | 96.0 | |
| | 500 | 97.3 | |
| | 1000 | 96.0 | |
| | 2000 | 96.0 | |
| | 25 | 68.0 | |
| | 50 | 78.7 | |
| | 100 | 84.0 | |
| SME | 200 | 97.3 | $3.8 < 12.2 < 20.3$ |
| | 250 | 96 | |
| | 500 | 98.7 | |
| | 1000 | 96 | |
| | 2000 | 98.7 | |

† Values are shown with fiducial limits.

Figure 3.5: The per cent of *M. disstria* larvae that successfully pupated (in comparison to initial larval numbers) after dietary exposure to four concentrations of either red maple extract (RME), sugar maple extract (SME), or a control diet containing ethanol or water.

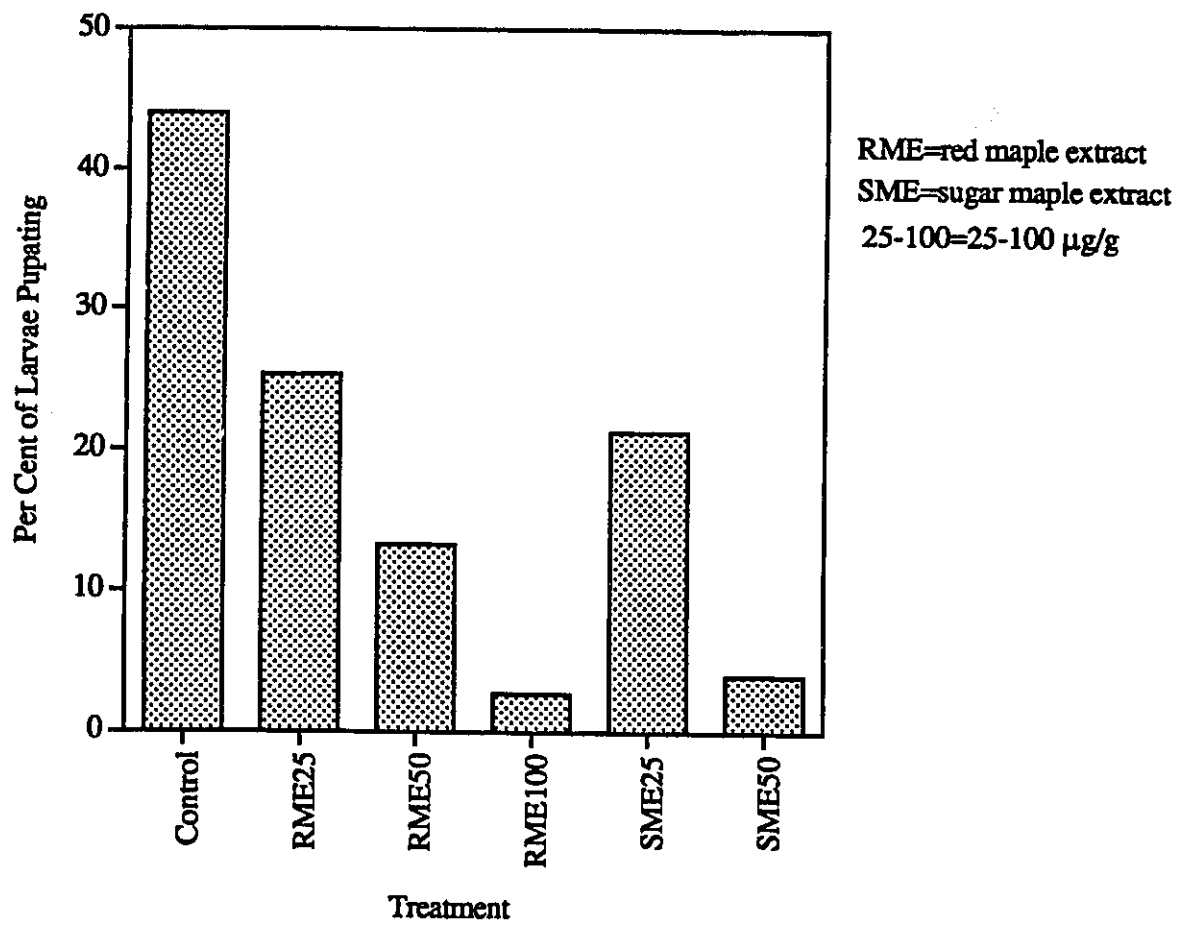


Table 3.5: Average weight of FTC pupae, by sex, after dietary exposure to two maple foliage extracts, red maple extract (RME) or sugar maple extract (SME).

| Treatment | Concentration (µg/g) | Mean Male Pupal Weight (mg) | Mean Female Pupal Weight (mg) |
|-----------|----------------------|--|-------------------------------|
| control | 0 | 237.3±13.5 [†] a [‡] | 353.9±19.3 a |
| RME | 25 | 243.2±13.5 a | 313.5±47.2 a |
| | 50 | 194.7±15.1 a,b | 334.3±47.1 a |
| | 100 | 126.2±0.0 b | 209.0±0.0 b |
| SME | 25 | 218.8±14.2 a | 370.5±40.0 a |
| | 50 | 217.8±0.0 a | 345.6±115.5 a |

[†]Means are ± standard error.

[‡]Means followed by the same letter, within a column, do not differ significantly in a Student-Newman-Keuls (SNK) test at P<0.05.

Figures 3.6-3.7: The mean time needed for *M. disstria* larvae to pupate (top) and reach adulthood (bottom) after dietary exposure to four concentrations of either red maple extract (RME) or sugar maple extract (SME), or a control diet containing ethanol or water. Means are \pm standard error. Those values that have no error bars represent only one surviving larva (with no error in measurement).

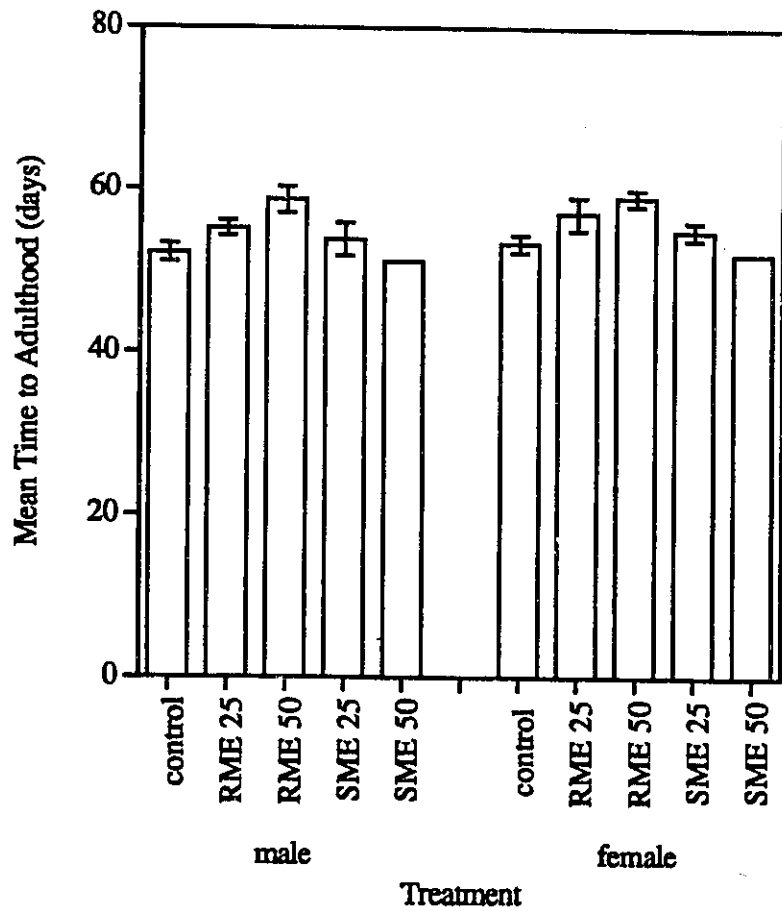
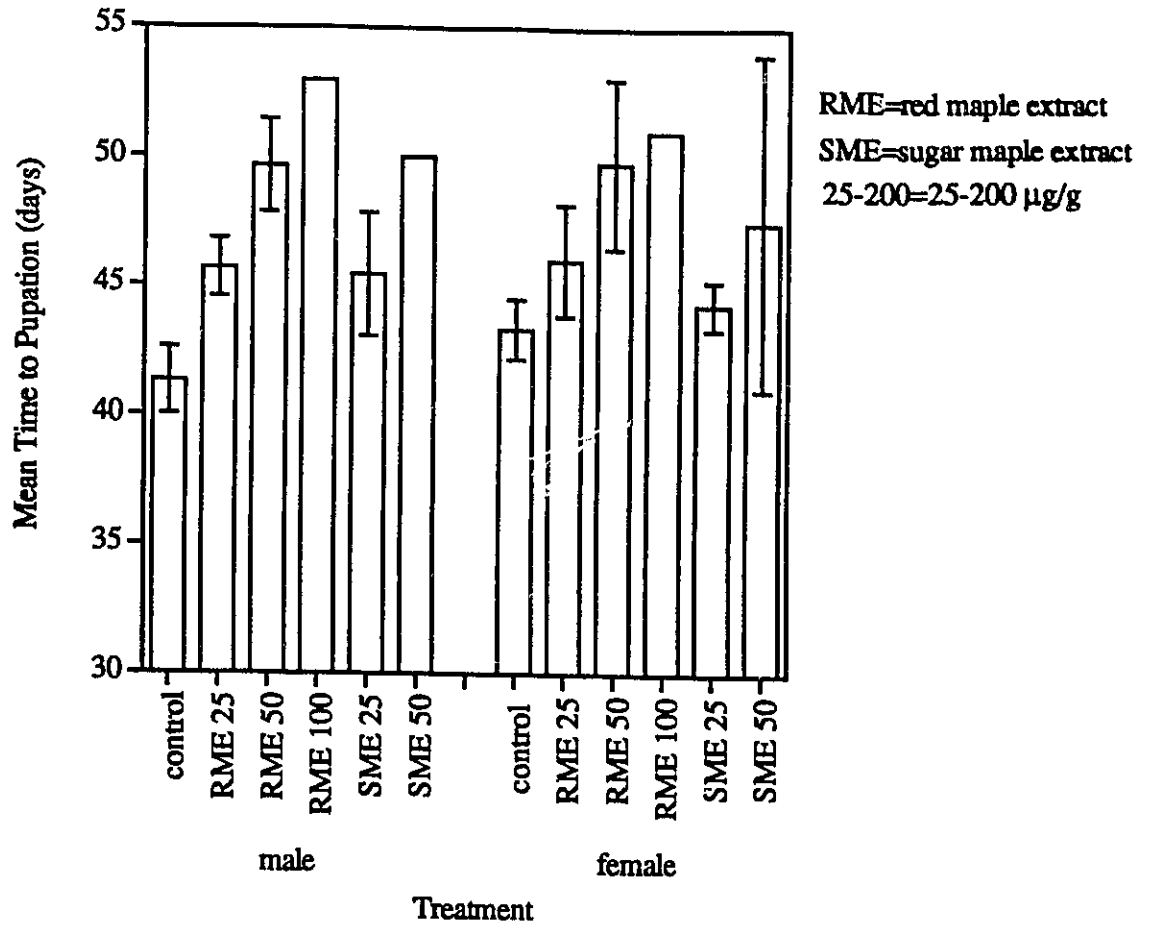


Table 3.6: Statistical analysis of the effect of the maple extracts upon time to pupation and time to adulthood for *M. disstria* larvae.

| Life Stage | Source | Degrees of Freedom | Kruskal-Wallis Test Statistic | P Value |
|------------|---------------------------|--------------------|-------------------------------|---------|
| Pupal | RME [†] , Male | 3 | 12.17 | 0.007 |
| | RME, Female | 3 | 7.17 | 0.067 |
| | SME [‡] , Male | 2 | 3.85 | 0.15 |
| | SME, Female | 2 | 0.80 | 0.67 |
| | Male vs. Female (control) | 1 | 102.0* | 0.22 |
| Adult | RME, Male | 2 | 7.27 | 0.026 |
| | RME, Female | 2 | 4.10 | 0.13 |
| | SME, Male | 2 | 0.97 | 0.62 |
| | SME, Female | 2 | 0.69 | 0.71 |
| | Male vs. Female (control) | 1 | 100* | 0.45 |

[†]RME=Red Maple Extract.

[‡]SME=Sugar Maple Extract.

*The Kruskal-Wallis test reverts to a Mann-Whitney U-Test, when there are only two comparison groups.

adulthood than females in both maple treatments, but this effect was not significant (Table 3.6).

3.3.4 Dietary Utilization Study with Extracts

The maple extracts in artificial diet caused the same kind of reductions in the nutritional indices as seen in the results with unaltered foliage (Table 2.2), but not to the same extent or significance due to the below-natural concentrations employed. The SME treatment caused reductions in the efficiencies of converting food to biomass (Table 3.7), and although these decreases caused a reduction in the RGR, it was not significant. However, the actual percentage reduction in growth is comparable to the results obtained in the foliage nutritional indices bioassay, with larvae fed the sugar maple leaves experiencing a 36.8% drop in growth as compared to control, and the larvae in the SME in diet treatment experiencing a 32.0% drop. The RME treatment reduced growth, consumption, and the internal efficiencies, but not to a significant extent. One noticeable difference between the nutritional indices with the foliage and diet is the approximate digestibility (AD). Artificial diet is more digestible than the leaves with the values for the two controls (diet with ethanol versus leaves of *P. tremuloides*) being 67.2% (Table 3.7) versus 39.1% (Table 2.2).

3.3.5 Dietary Utilization with Compounds

Two of the four compounds present in the foliage of *A. rubrum* caused greater deleterious effects for the FTC than did the red maple extract nutritional indices bioassay. This could be a function of the higher concentration used in this test (5000 µg/g) and because purified compounds are usually (other than in cases of synergism or potentiation) more active than extracts. These results demonstrate two compounds that are candidates for the toxicological action of the secondary profile of *A. rubrum* and they could also play a part in the antifeedant action. The compounds are ellagic acid, and methyl gallate. Both of these phytochemicals significantly reduced the relative consumption rate and reduced the efficiency of converting food into biomass (Table 3.8). The altered RCR, ECI and ECD

Table 3.7: The nutritional indices for FTC fed red maple extract (RME) and sugar maple extract (SME) at 3000 µg/g.

| Treatment | RGR mg mg ⁻¹ day ⁻¹ | RCR mg mg ⁻¹ day ⁻¹ | AD (%) | ECI (%) | ECD (%) |
|-----------|---|---|------------|------------|------------|
| Control | 0.25±0.03† a‡ | 0.934±0.1 a | 67.2±1.6 a | 25.6±2.9 a | 38.5±2.8 a |
| RME | 0.19±0.02 a | 0.73±0.1 a | 75.5±3.4 a | 24.9±2.4 a | 33.9±3.2 a |
| SME | 0.17±0.03 a | 0.89±0.2 a | 73.9±2.4 a | 17.5±2.2 b | 24.2±3.2 b |

†Means are ± standard error.

‡Means followed by the same letter, within a column, do not differ significantly in a Student-Newman-Keuls (SNK) test at P<0.05.

Table 3.8: The nutritional indices for FTC fed four compounds isolated from red maple (*A. rubrum*) at a concentration of 5000 µg/g.

| Compound | RGR (mg mg ⁻¹ day ⁻¹) | RCR (mg mg ⁻¹ day ⁻¹) | AD (%) | ECl (%) | ECD (%) |
|----------------|--|--|--------------|--------------|--------------|
| Control | 0.22±0.0 [†] a [‡] | 1.01±0.1 a | 70.9±1.4 a,b | 22.1±1.7 a,b | 31.7±2.6 a,b |
| Gallic Acid | 0.25±0.0 a | 0.96±0.1 a,b | 64.9±1.6 b | 26.6±2.4 a | 42.4±1.7 a |
| Rutin | 0.20±0.0 a,b | 0.91±0.0 a,b | 74.1±1.6 a | 21.3±1.3 b | 29.1±2.0 b |
| Methyl Gallate | 0.14±0.0 b,c | 0.80±0.1 b | 68.1±2.3 a,b | 15.6±3.6 c,d | 23.9±5.3 b |
| Ellagic Acid | 0.09±0.0 c | 0.79±0.1 b | 72.4±2.4 a | 8.6±4.0 d | 13.4±5.4 c |

[†]Means are ± standard error.

[‡]Means followed by the same letter, within a column, do not differ significantly in a Student-Newman-Keuls (SNK) test at P<0.05.

led to a significant and large reduction in the relative larval growth rate. Methyl gallate and ellagic acid reduced RGR, as compared to control, by 36.4% and 59.1% respectively, with no significant effect being found at the tested concentration for gallic acid and rutin.

3.3.6 Life Cycle Bioassay with Gramine

Gramine, at a concentration as low as 30 $\mu\text{g/g}$, obviously reduced *M. disstria* larval growth (Figure 3.8), but again, concentrations used were above those required for concentration dependent effects. This compound also increased larval mortality over control (Figure 3.9).

3.3.7 Nutritional Bioassay with Gramine

As gramine obviously has the ability to hinder larval growth, a nutritional indices study (Table 3.9) was undertaken in order to determine if the compound worked internally or via an antifeedant effect. The results demonstrate that gramine clearly acts as a post digestive toxin in the FTC. Larvae over the four day period significantly gained less weight in the treatment than in the control, but at the same time consumed more food (Table 3.9). The AD was higher in the treated group, whereas the ECD was lower, indicating that gramine acts as an internal toxin in the *M. disstria* larvae rather than as an antifeedant or digestibility reducer.

Figures 3.8-3.9: Growth dynamics of the larvae of *M. disstria* after feeding on artificial diet containing three concentrations of either gramine or on a control diet containing ethanol and water, in a life cycle bioassay (top). Larval weights were recorded until the time of first pupation. Larval mortality of *M. disstria* after dietary exposure to gramine (bottom). The LC₅₀ value is the concentration of gramine needed to cause a fatality in 50% of the test larval population, as calculated by a Finney (1952) probit analysis.

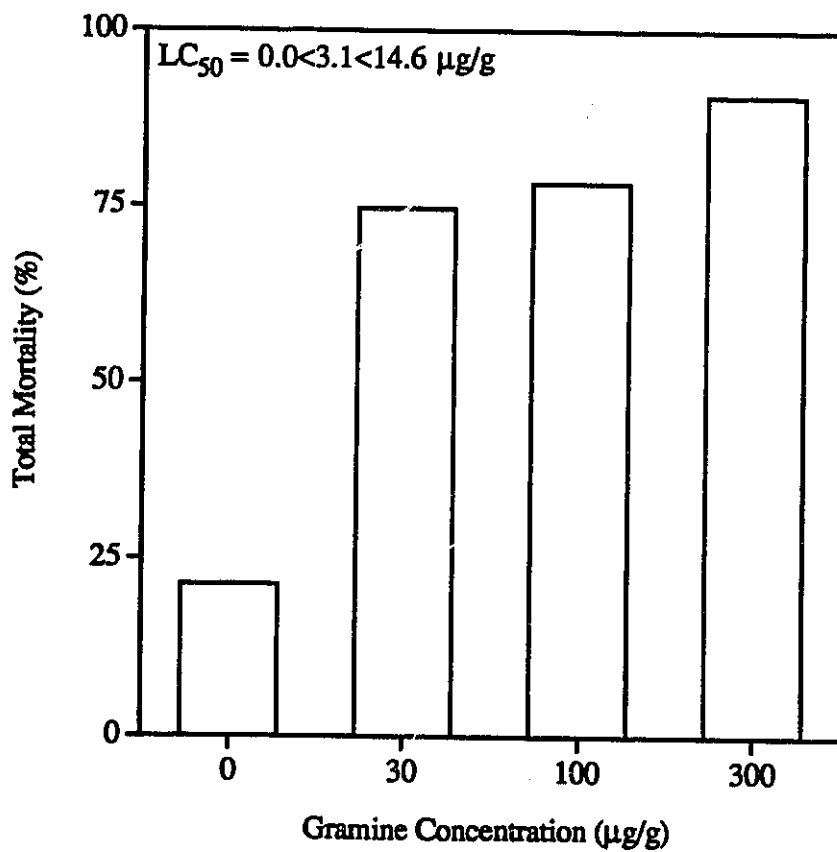
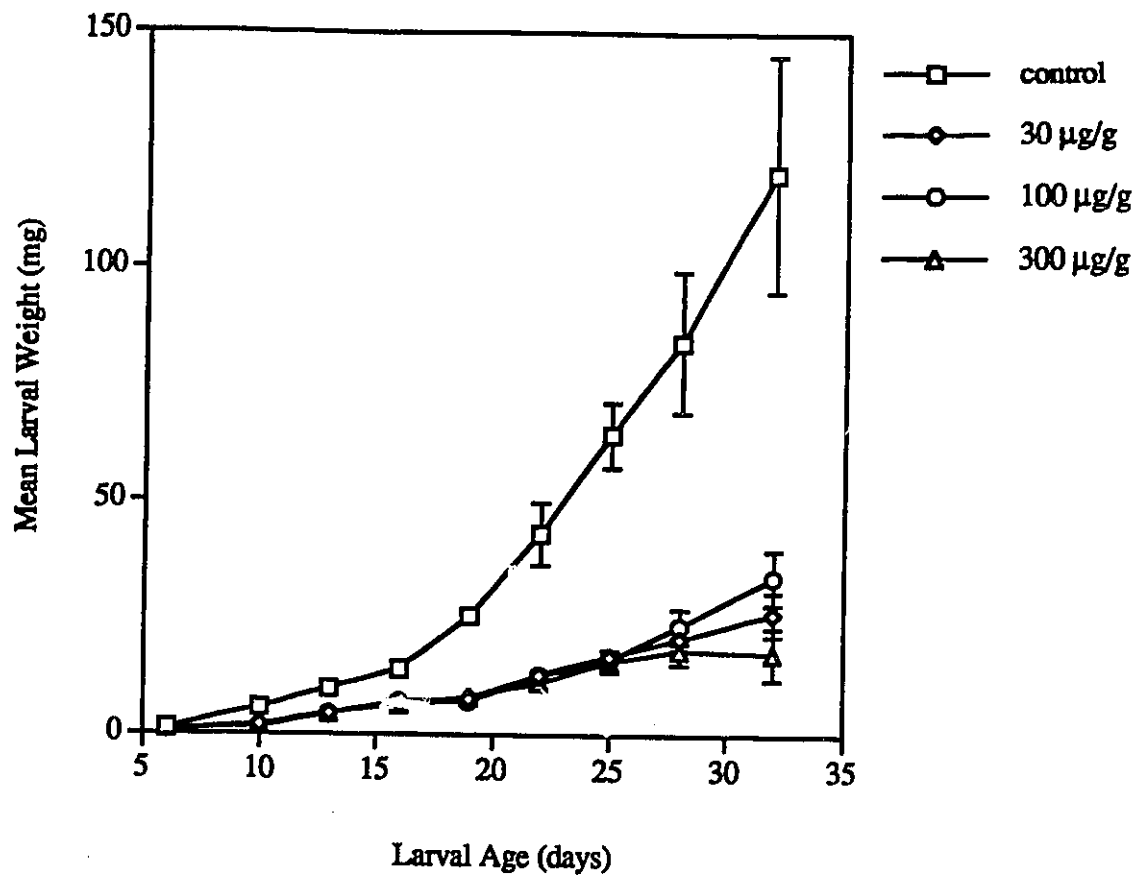


Table 3.9: The nutritional indices for FTC exposed to 1500 µg/g of gramine in artificial diet.

| Compound | RGR (mg mg ⁻¹ day ⁻¹) | RCR (mg mg ⁻¹ day ⁻¹) | AD (%) | ECI (%) | ECD (%) |
|----------|--|--|------------|------------|------------|
| Control | 0.20±0.1 [†] a [‡] | 1.44±0.1 b | 74.4±2.1 b | 13.5±1.1 a | 20.6±2.1 a |
| Gramine | 0.11±0.0 b | 1.89±0.1 a | 83.6±1.8 a | 6.7±1.3 b | 8.1±1.6 b |

[†]Means are ± standard error.

[‡]Means followed by the same letter, within a column, do not differ significantly in a t-test (Student's test of means) at P<0.05.

3.4 Discussion

3.4.1 Observed Effects Upon Larval Growth and Development

The secondary profiles of both maples have constituents that reduce larval growth, increase larval mortality, and create reductions in pupal and adult parameters. However, contrary to the results presented in Chapter 2.0, the activity of the SME in artificial diet is greater than that of RME for most tested parameters. When fed foliage, the FTC suffered the greatest reductions in fitness in the red maple treatment. With extracts of the foliage in diet, larvae in the SME treatment reached the threshold of growth and mortality effects at a lower concentration than those in the RME treatment. Figures 3.3 and 3.4 demonstrate that SME does indeed exert a greater negative effect on larval growth than does RME. As 100 and 200 $\mu\text{g/g}$ SME reduced larval growth to the same extent, (did not support growth) and the same effects were seen in RME at 200 $\mu\text{g/g}$ only, the upper limit for FTC tolerance to SME in artificial diet is between 50 and 100 $\mu\text{g/g}$. In artificial diet containing RME, the tolerance level is somewhere between 100 and 200 $\mu\text{g/g}$. *M. disstria* larvae in the SME treatment also reached the mortality threshold at a lower concentration than those in the RME treatment. In light of these observations coupled with the results of the two-way ANOVA, one can conclude that the secondary defences of *A. saccharum* are more active against the FTC than are those of *A. rubrum*.

Both extracts exerted a greater influence on the time taken to reach pupation rather than adulthood. By the completion of the life cycle, the extract that was most potent (and significant) at creating increases in pupal and adult parameters was the RME. This extract increased the time to reach adulthood and pupation, but only significantly so in the males. The P values become less significant (farther away from 0.05) from the time to adulthood as compared to the time to pupation, and a general trend towards decreased differences between control and all treatments is observed in the mean time to adulthood from the mean time to pupation (Figures 3.6 and 3.7). This may represent a homeostatic ability of the

insect to compensate for lost development time incurred in the larval stage by expediting the time spent in pupal development.

The nutritional indices in the SME treatment agree with the nutritional indices obtained with the foliage. Namely, the secondary profile of *A. saccharum* acts to reduce fitness in *M. disstria* via post-digestive toxicity (as seen in the reductions of ECD in the tests with foliage and with extracts). These reductions in fitness resulted in lower larval growth, and although this number was not significant in the bioassay with artificial diet, it is comparable to the significant reduction obtained using foliage. The lower relative activity of the extracts is expected as the concentrations used were below ecological levels (those levels that are estimated to occur in nature). The results obtained with RME indicate that the extract in the diet at 3000 µg/g, is slightly toxic and slightly antifeedant and that these actions were the cause of the reduced growth rate. A greater post-digestive toxicity would be expected at higher concentrations. This test was done over three days, whereas the longer duration of the life cycle bioassay (approximately one month), would allow for the magnification of the reductions in consumption and efficiency of converting the food into biomass, and the cumulative effects would be sufficient to explain the inhibited larval growth. Reductions in internal efficiencies were obtained with the foliage experiments, and it can now be stated (after the problems associated with severe repellency have been eliminated) that the secondary profile of *A. rubrum* does contain toxic, and hence larval growth-inhibiting, elements.

3.4.2 Differences Between Foliage and Foliage Extracts

The reason why SME is more potent in diet than RME is that the primary mode of action of the foliage of *A. rubrum* was purposely not represented to the same degree in the diet bioassays. It has already been established that the primary mode of action of the intact leaves of *A. rubrum* is one of antifeedancy. These tests were designed to test the toxicity of the extracts and the growth and development effects upon *M. disstria* of this toxicity. In

the absence of a strong antifeedant effect with the extracts in diet, one is comparing the internal toxicity of the two maple extracts. Both trees contain a secondary profile that inhibits growth of FTC, but that of *A. saccharum* is more active (as seen in the reduced ECI's and ECD's, higher mortality and lower growth inhibiting threshold in the life cycle bioassays). Not eating is more harmful and dramatic, in terms of growth and mortality, to *M. disstria* than either of the toxic components of the two maples. When an antifeedant action is present, as in the foliage tests, the FTC grow the least in the red maple treatment, but when there is no, or a weaker, antifeedant action (as in the diet bioassays), the larvae are affected the most by the relatively higher toxicity of the SME. A toxicological analogy will be used to enhance the understanding of the difference between the action of the red maple leaves as compared to the red maple extract. The leaves of *A. rubrum* are acutely antifeedant and this results in the *M. disstria* larvae experiencing immediate and severe growth effects (i.e. no growth and 100% mortality by the second week of the foliage tests), whereas the extract is chronically toxic and antifeedant. In this instance, the combined chronic action of toxicity and antifeedancy causes the deleterious effects seen over time in the life cycle bioassay. The strong antifeedant nature of the *A. rubrum* foliage creates greater growth reductions in the *M. disstria* larvae than the toxic (growth inhibitory) nature of the foliage of *A. saccharum*.

The relative abundance of the phytochemicals present in the foliage of the two (Table 3.1) is a useful tool as a starting point in the effort to deduce which phytochemicals are responsible for the two different primary modes of action. These differences in secondary chemistry are responsible (solely, or possibly together with nutritional factors) for the two distinct modes of action of the two species and for the different specific effects on developmental parameters. The gramine results compared to those of the isolated *A. rubrum* compounds are a good example of different compounds having different modes of action. Although reported as an antifeedant for aphids (Zúñiga et al. 1985), gramine is obviously an internal toxin with no antifeedant action against *M. disstria*, whereas methyl

gallate and ellagic acid have toxic and antifeedant properties (Table 3.6). It is logical to assume that the phytochemicals present in *A. rubrum* and not present in *A. saccharum* play a role in *A. rubrum*'s antifeedancy and that there is an internal metabolic cost to the ingestion of these compounds by FTC. Methyl gallate and ellagic acid were specifically chosen because of their relative abundance in *A. rubrum* and absence in *A. saccharum*. In addition, ellagic acid had been previously shown to cause growth inhibition of another polyphagous lepidopteran larvae (Klocke et al. 1986) via reduction of the conversion efficiencies.

3.4.3 Active *A. rubrum* Compounds and Modes of Action

The most active compound, at the concentration tested, in terms of post-digestive toxicity was ellagic acid. This phenolic dilactone is probably responsible (with methyl gallate) for a large portion of the chronic anti-herbivore toxicity displayed in the life cycle bioassay because of the results of the nutritional indices bioassay (where in three days the larvae grew to less than half as much as the controls per day), and because the reduced RCR, ECI and ECD follow the same trend as established with the extract of red maple, but to a larger and more significant degree. However, not all of the compounds listed in Table 3.1 were tested for toxicity, so it cannot definitively be said that methyl gallate and ellagic acid are solely responsible for the chronic toxicity, although that both cause post digestive toxicity and are antifeedant lends credence to the idea that they are two of the "major" players. Likewise, no compounds isolated directly from sugar maple were tested (although rutin is present in both maples Table 3.1), and therefore the actual toxic components in this species cannot be identified at this time. It would be interesting for future work to test mixtures of these compounds for potentiation and synergism. Synergism of anti-insect compounds occurs when one of two compounds in a mixture has insecticidal activity, whereas the other is non-toxic (Metcalf 1967, Bernard and Philogène 1993) and the outcome on the insect is greater-than-additive. Potentiation refers to a greater-than-additive activity of a mixture of two or more compounds regardless of the toxicity of the constituent

compounds (Raffa and Priester 1985). Therefore, methyl gallate and ellagic acid could be tested together for a potentiation of toxic action upon *M. disstria*, and these two plus gallic acid could be tested for synergistic antifeedancy.

The nutritional indices give a description of how the compounds work in broad terms. They reveal to the researcher if the phytochemicals are causing growth inhibition because of a reduction in food intake, or nutrient availability, or if they are somehow working internally. It was outside the scope of this project to undertake actual physiological bioassays to precisely determine how the defensive compounds work internally, but other work done in this area allows for some deductions to be made. Appel (1993) has done a great deal of work in the area of the physicochemical environment in the gut lumen of herbivorous insects and the role this plays in the toxicity of phenolic compounds. She has theorized that several internal conditions of many lepidopteran larvae are conducive to the autooxidation of phenolic acids. When phenolics are converted to quinones, several types of reactive oxygen radicals are formed which may disrupt membrane integrity and metabolism in the gut epithelium (Appel 1993). Gut lesions that have been previously reported in larvae that had ingested tannins (Bernays 1978, Berenbaum 1984, Steinly and Berenbaum 1985) may then be the result of the oxidation of the plant phenolics that are the hydrolytic products of tannins. One of the prime conditions for autooxidation of these compounds in lepidopteran larvae is an alkaline gut pH. As the pH of the midgut of *M. disstria* is approximately 10 (Karowe 1989) it is very likely that the internal toxicity caused by the tested phenolic compounds above, is a result of oxidative damage on the larval gut epithelium. It has recently been demonstrated that phenolics and tannic acid are indeed oxidized in the gut of *M. disstria* (Barbehenn and Martin 1994). It has also been recently shown (Summers and Felton 1994), that the decrease in fitness experienced by another lepidopteran insect with an alkaline midgut was due to oxidative damage after ingestion of phenolic acids. This was determined by the measurement of

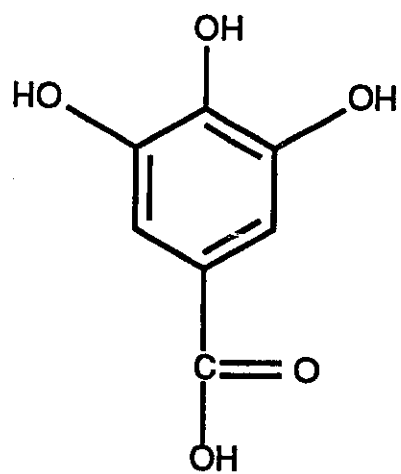
increased lipid peroxidation and protein oxidation products and by the observation that the addition of an antioxidant, ascorbic acid, mitigated the detrimental growth effects.

Ellagic acid may cause the internal damage in the FTC through two distinct modes of action: oxidative damage and inhibition of detoxification enzymes. Lee (1991) has shown that some plant phenols can inhibit the enzyme that makes toxic compounds water soluble, and hence excretable. This enzyme, glutathione transferase (GST), aids in the metabolic detoxification of compounds that possess a carbonyl group, such as simple phenolic acids and quinones (Waldleigh and Yu 1987, Lindroth 1989). The inhibition of GST by ellagic acid has been demonstrated to occur, but in non-Insecta systems (Das et al. 1984, Kurata et al. 1992). Das et al. (1984) found that ellagic acid was over twice as active as other phenolic compounds in inhibiting GST, with I_{50} values (inhibitory concentration) of $8.3 \times 10^{-5}M$, $20.0 \times 10^{-5}M$ and $22.0 \times 10^{-5}M$ for ellagic acid, chlorogenic acid, and ferulic acid respectively. These researchers characterized gallic acid as being non-inhibitory. If ellagic acid is able to inhibit the phase II detoxification (the conjugation of the primary metabolite to a water soluble endogenous group) systems *in vivo* in the FTC, then it is likely that it may exert its effects by simultaneously causing oxidative damage and by suppressing the insect's efforts to detoxify the offending phytochemicals.

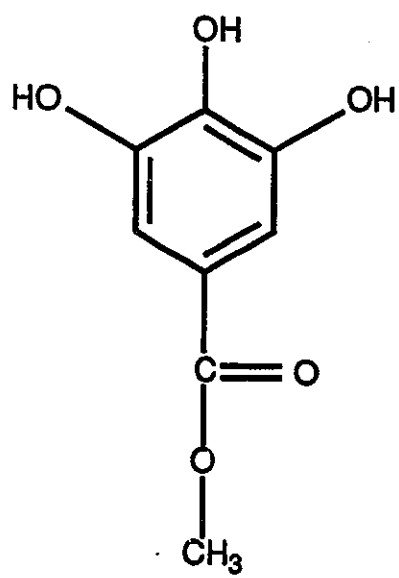
In feeding behaviour tests completed at CFS with isolated *A. rubrum* compounds, it was found that gallic acid and methyl gallate exhibited the greatest antifeedant activity (ellagic acid may be tested in the future). At the concentration tested here, gallic acid exhibited no internal action, and in fact was not a growth reducer (Table 3.5). This is comparable to Klocke et al.'s (1986) finding that the concentration of gallic acid needed to obtain a growth EC_{50} (whereby growth of *Heliothis virescens* Fabr. was reduced to 50% of control) was 8.6 times higher than the concentration of ellagic acid needed to achieve the same growth reduction. That one of the primary antifeedant compounds does not exhibit a larval growth inhibitory action is of great theoretical interest. One would expect that an insect would avoid compounds that create internal toxicity, but this is not always the case

(Harley and Thorsteinson 1967, Bernays and Chapman 1987). Cottee et al. (1988) also demonstrated a lack of a relationship between oral toxicity and deterency in eight secondary metabolites fed to a specialist and generalist insect. Without invoking theoretical evolutionary reasons for the occurrence of this phenomenon (i.e. avoidance responses may have evolved outside of the selective pressures caused by toxicity [Cottee et al. 1988]), there are two possibilities. Both are related to the detectability of the compounds. When lepidopteran larvae bite into a food source, they code the chemical profile largely by two main gustatory organs: the maxillary sensilla (comprised of the lateral and medial styloconica); and the epipharyngeal organ. The presence of sugar sensitive receptor cells in the sensilla styloconica has been demonstrated (Blom 1978, Mitchell and Gregory 1979), as well as the presence of cells that are stimulated the strongest by salt, amino acids and other nutritional compounds (Dethier and Kuch 1971, Dethier 1973, Schoonhoven 1986). In addition to the specific nutritive receptors, cells in the lateral and medial sensilla styloconica have been found that react the strongest to secondary metabolites (Ishikawa 1966, Dethier 1980a, Clark 1981, Schoonhoven 1982) and have been termed "deterrent receptors" (Schoonhoven 1986). The FTC may use the occurrence of a non-toxic deterrent compound, in this case gallic acid, as a "cue" to identify an unsuitable food host. The specific phytochemical may not pose a major physiological threat to the insect, but it may be easily detected by the insect by virtue of a strong receptor response or by its relative abundance, and thereby is used to identify by association, a plant that has other unrelated toxic or development-inhibiting components. Conversely, as methyl gallate has a similar chemical structure to gallic acid (it is the methyl ester of gallic acid Figure 3.10), the FTC may not be able to differentiate between the two compounds. These deterrent receptor cells may "fire" in the same manner for both compounds, causing the *M. disstria* larvae to change its feeding behaviour and reject the food.

Figure 3.10: Chemical structure of gallic acid (ethyl 3,4,5-trihydroxybenzoate), (1) and its methyl ester, methyl gallate (methyl 3,4,5-trihydroxybenzoate), (2).



1



2

3.4.4 Toxicity of *A. saccharum*

No pure compounds from *A. saccharum* were tested but based on the internal toxicity seen with the foliage and the foliar extract, some speculations can be made. Summers and Felton (1994) proposed that toxicity of the *o*-dihydroxy type of phenolic acid is due to oxidative damage to insect midgut tissues (as in Appel). It was demonstrated that the superoxide radicals generated by the degradation of this type of compound caused *in vivo* protein oxidation and lipid peroxidation in the midgut of another generalist lepidopteran herbivore, *Helicoverpa zea* Boddie. One *o*-dihydroxy phenolic acid that caused the most oxidative damage was chlorogenic acid, which has previously been isolated from the leaves of sugar maple (Hatano et al. 1990). This compound could play a role in the observed SME toxicity to FTC through oxidative damage. This idea is bolstered when one compares the deleterious effects demonstrated in the above bioassays with *M. disstria* to those exhibited in *H. zea* after feeding upon artificial diet containing chlorogenic acid. After chronic exposure to chlorogenic acid *H. zea* larvae experienced significantly reduced growth, substantial mortality and the time required for development was also increased (Summers and Felton 1994). Chlorogenic acid is just one of the phenolic acid conjugates present in *A. saccharum* that could cause internal oxidative damage.

Because of performance differences between larvae fed the aspen leaves and control diet fed larvae, no direct comparisons should be made between the results obtained with foliage and those obtained with the extracts incorporated into the artificial diet. It had been speculated before the initiation of this project that although the Addy artificial diet would support the growth and development of *M. disstria*, it was not an ideal food source. Upon comparison with the results obtained with the foliage of *P. tremuloides*, one can see that this is true as the larvae that were fed artificial diet did not consume as much food (RCR's of 1.6 mg/day for poplar leaf compared to 0.89 mg/day for the diet), did not experience the rapid growth in the second to third week of development as seen in Figure 2.1 and needed more time to complete their life cycle. It is known that in some instances the ratio of

nutrients and allelochemicals can influence the feeding behaviour and/or performance of insects (Slansky 1993). The results of the investigation of the main nutritional differences between *A. rubrum* and *A. saccharum* are presented in a following chapter, as these nutritional differences, coupled with the secondary compound differences, may explain the varying feeding behaviour of the FTC.

After examining the results obtained so far, it can be stated with confidence that both maples possess phytochemicals that inhibit growth of the FTC and generally interfere with development. However, two issues remain that could be related. Firstly, a more comprehensive explanation of the way *A. rubrum* escapes herbivory by *M. disstria*, is needed, and secondly there is still the question of the feeding behaviour of the FTC when presented with the foliage of the two maples. The following two chapters deal with these two issues. The inconsistency of the field reports of the larvae of *M. disstria* eating the foliage of sugar maple and the negative developmental results presented here could be due to variability in defensive chemical expression. The variability in maple defensive activity between sugar maple trees growing in different geographic areas, and between individual trees within the same population, will be investigated in Chapter IV. If no interspecific or geographic influence on phytochemical activity is found, differences in foliar nutrients between the red and sugar maple may explain why the FTC chose to strongly reject the red maple leaves and accept the leaves of the species that has now been shown to cause greater larval growth reductions. The primary nutritional components of the leaves of the trembling aspen, sugar maple and red maple will be addressed in Chapter V.

**CHAPTER IV:
INTRASPECIFIC AND GEOGRAPHIC VARIABILITY OF MAPLE
DEFENSIVE ACTIVITY:**

4.1 Introduction

To clarify the dissimilarity of the experimental results of sugar maple extracts causing growth inhibition in FTC and the many publications that report defoliation of sugar maple by FTC (Hodson 1941, Gross 1991, Howse 1995) and that categorize this tree species as a food host of *M. disstria* (Davidson and Prentice 1967, Batzer and Morris 1971, Retnakaran 1978), the variability of maple defenses against *M. disstria* was quantified. Two aspects of secondary profile differences were tested: the variation between individual trees (within species); and a comparison of the variation between groups of representatives from different locations (geographic). It was hypothesized that variability of the defensive compounds may account for the reports of the defoliation of sugar maple by *M. disstria* because the field reports could have come from geographic areas where the natural population of *A. saccharum* had lower relative levels of phenolic compounds or other classes of defensive phytochemicals, or alternatively, selective defoliation of *A. saccharum* due to variable defensive levels within individuals of the same population could also lead to observations of the larvae defoliating sugar maple. In both cases, either different populations or individuals with relatively lower defensive activities could be attacked, resulting in researchers observing sugar maple defoliation by FTC, and classifying sugar maple as a "food host" for this insect.

There is some evidence that selection of trees by the FTC could be related to variation in foliar defensive compounds. It was noted in Lindroth and Bloomer (1991) that individual trembling aspen trees were not uniformly susceptible to FTC defoliation. Lindroth et al. (1987) also demonstrated that two of the most abundant aspen defensive phytochemicals exhibited high within species variability. Robinson and Raffa (1994)

found that hybrid *Populus* spp. had significantly variable tolerances to *M. disstria* defoliation, and that the larvae in turn had different foliage preferences and experienced a divergent larval performance. It was therefore hypothesized that the same type of phenomenon could be occurring in *A. saccharum*, namely, that variable foliar defenses, in individual trees, allowed for variable FTC defoliation in *A. saccharum*.

The phenotypic plasticity of phytochemical defenses is also a plausible scenario that could result in *M. disstria* larvae consuming sugar maple foliage in only certain regions, thereby leading to this tree species being labelled as a FTC food host. Environmental conditions are known to influence the amount of defensive chemicals produced by a plant, with biotic and abiotic factors of a plant's habitat affecting the quantity and quality of secondary chemicals (Lokar et al. 1987, Stuhlfauth et al. 1987, Mole et al. 1988, Mihaliak and Lincoln 1989). To investigate the possibility that the growing site at Sault Ste. Marie, was conducive to the production of high levels of defensive phytochemicals, a comparison between the anti-FTC activity of maple extracts produced from foliage samples taken approximately 700 km apart was undertaken.

4.2 Materials and Methods

4.2.1 Plant Extractions

Foliage of *A. rubrum* was collected from the green belt by Conroy Road in Ottawa Ontario, on June 2, 1995. Five individual trees were located and the foliage subsequently removed from the trees at approximately the same heights (as far as could be reached). Leaves were stored in green garbage bags with holes punched in the bottom to allow for air flow and ensure maximum leaf freshness. Foliage of *A. saccharum* was collected in the same way on June 4 1995 from the Sandy Hill area of Ottawa. The individual trees for both species were estimated to be between 25 and 30 years old.

The leaves of both trees were brought back to the Chemical Ecology laboratory at the University of Ottawa where the petioles and small branches were removed. The foliage

of all four individuals for both species was kept separate throughout the entire procedure to test for biological variability of phytochemical activity. Two different solvents were used for each individual, as a comparison between the standard ethanolic extraction and one designed to optimize the removal of classes of compounds that are difficult to extract (e.g. tannins) was planned. This would allow for a better representation of all of the classes of compounds that are present in the intact foliage, and provide a safeguard against the possibility that the ethanolic extraction procedure was leaving biologically active components behind. The leaves were cut into pieces and split into two lots, one for each extraction procedure. The foliage was weighed and then placed in warm (55°C) solvent. The plant material from each replicate was placed in either a solvent system designed to preferentially remove tannins: 70% acetone and 30% double distilled water (Hagerman 1988 Hagerman and Butler 1991, Porter 1989), with the rest of each lot being placed in 8 parts 95% ethanol to 2 parts double distilled water. Acetone may be an effective solvent for the removal of tannins because it is believed to prevent tannin from binding to leaf protein after maceration (Hagerman and Robbins 1987). The leaves were stirred with the solvent and placed in a water bath of approximately 40°C, for 4 hours, and then were placed in a dark storage area. The foliage was allowed to soak for 72 hours, after which time the extraction procedure continued.

After three days of soaking, the leaves were removed by placing Ahlstrom qualitative filter paper onto a Buchner funnel and pouring the leaf/solvent mixture into the funnel under vacuum filtration. The filtrate was then placed into a 1 liter flask and attached to a rotary evaporator, where the solvent was evaporated off until only a dark green residue remained in the flask. The flasks containing the resin were rolled in liquid nitrogen until frozen and attached to a freeze drier for approximately three hours. After this time the flasks were scraped with a spatula to remove the now powdered foliage extracts. The extracts were weighed and the final ratio of leaf to extract (yield), was determined (Appendix III).

4.2.2 Diet Bioassays

The life cycle bioassay was done in the same manner as in section 3.2.2, except that 12 petri dishes were used for a total of 60 larvae per treatment. The concentration of extract used for the treatments was 100 µg/g. The experimental design then, had larvae being fed diet treated with extracts from four replicates of two species with two extraction procedures and the respective controls, for a total of 17 treatments (15 extracts plus two controls). One of the red maple ethanol extracts was not used because it was lost in the water bath of the rotary evaporator. The growth response of the above experiment was used to identify interspecific variability of defensive compounds, and the weight gain after 20 days was compared to the data obtained with the Sault Ste. Marie extracts to test for geographic biovariability.

The effect of the two solvent systems (ethanol and acetone) upon *M. disstria* growth was also compared to determine if any differences in activity between the two procedures existed. If the acetone extract had a higher relative activity, then unextractable or difficult to extract compounds may be responsible for some of the effects seen in the intact leaf, and may be underrepresented in the ethanol extracts.

4.2.3 Data Analysis

An analysis of co-variance (ANCOVA) was completed on the FTC growth response to the extracts from red and sugar maple and from both extraction procedures. First, the data was log transformed as the distribution of the residuals was found to be not normal and/or homoscedastic. The assumption that a significant interaction between the covariant and the treatment did not exist (the homogeneity of the slopes) was then tested using SYSTAT[®] (SYSTAT 1992). When this assumption was found to be true, an ANCOVA was completed on the growth curves of *M. disstria* larvae fed either sugar maple or red maple ethanol or acetone extract by using the general linear model procedure in SYSTAT[®] (SYSTAT 1992). A Tukey's pairwise comparison was then completed to quantify the divergence of the variable growth responses.

The mean larval weight gain was calculated after 20 days, and used to compare the relative activity of each solvent system, within each species (i.e. is one extraction procedure “better” at reducing larval growth). A one-way ANOVA was completed to identify any significant difference in the two solvent systems.

For the comparison of relative activity between the two sites, no statistical analysis was completed as the *M. disstria* culture at CFS had become infected with microsporidia (and problems with growth and survivorship were present). The results are therefore presented as the per cent value of which the treated larvae grew to those larvae in the control, thereby facilitating a comparison between the uninfected and infected replicates.

4.3 Results

4.3.1 Within Species Variation: Ethanol Extracts

Figures 4.1 and 4.2 demonstrate that the intraspecific variability in ethanol extract activity between the individual trees is very low. Larvae fed both maple extracts from distinct trees all grew more slowly than those in control, but as all of the growth curves appear to increase in the same manner over time, it seems that there is very little difference in activity between the analyzed maple tree replicates. Table 4.1 shows that there is no statistically significant difference in the larval response to the ethanol RME, and some variability in the ethanol SME. The Tukey’s pairwise comparison reveals that there is a relatively little variation in the larval response to this treatment, in the sense of the number of individuals that differed, with Tree 2 being different from Tree 1 and Tree 3 (Table 4.2).

4.3.2 Within Species Variation: Acetone Extracts

The acetone/water extracts of all trees reduced larval growth for both *A. saccharum* and *A. rubrum* (Figures 4.3 and 4.4). Upon cursory inspection of these graphs, one can notice that the spread between the FTC growth curves appear to be greater than those in Figures 4.1 and 4.2. The ANCOVA demonstrates that, with this extraction procedure,

Figures 4.1-4.2: Intraspecific variation of the activity of the maple extracts against the larvae of *M. disstria*. Growth dynamics of the larvae after feeding on artificial diet containing ethanol extracts from either individual sugar maple trees (top), or individual red maple trees (bottom). The extracts were incorporated into the diet at a concentration of 100 $\mu\text{g/g}$, with the control diet containing ethanol and water only. Error bars are \pm standard error.

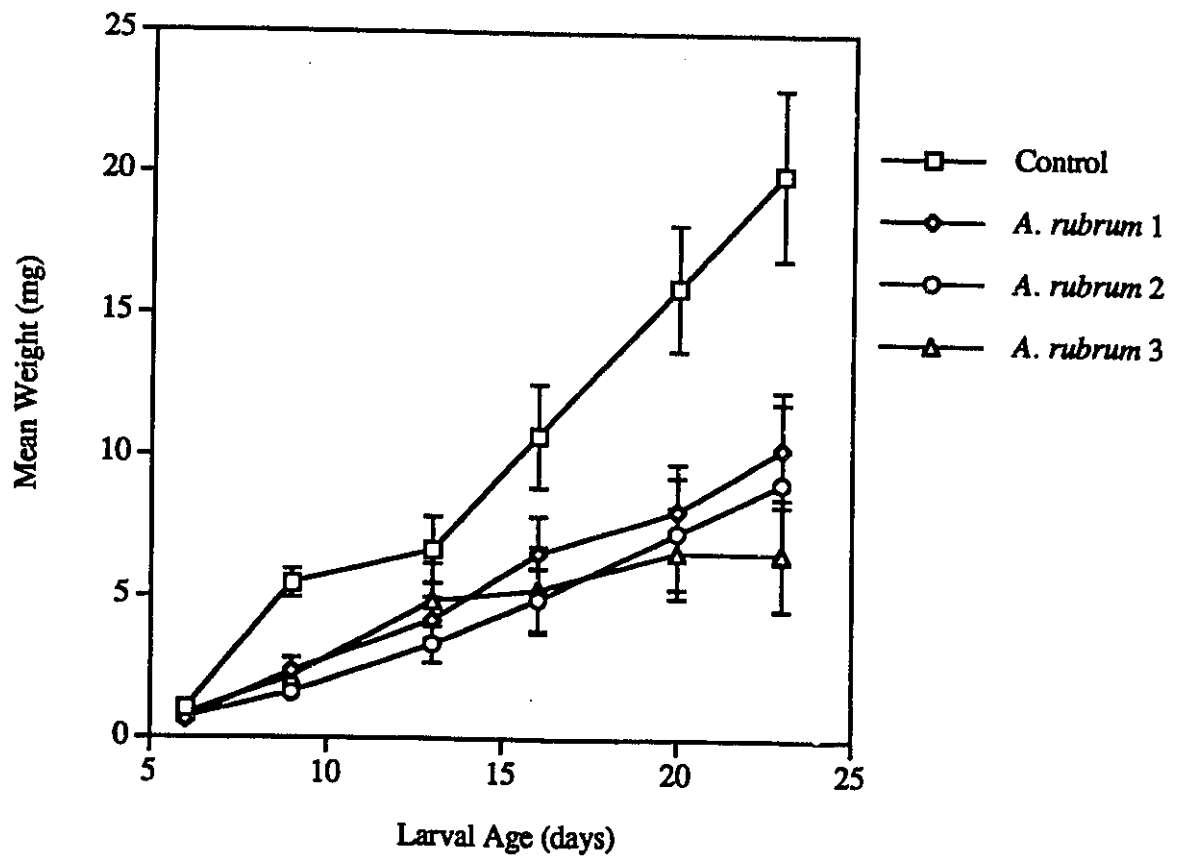
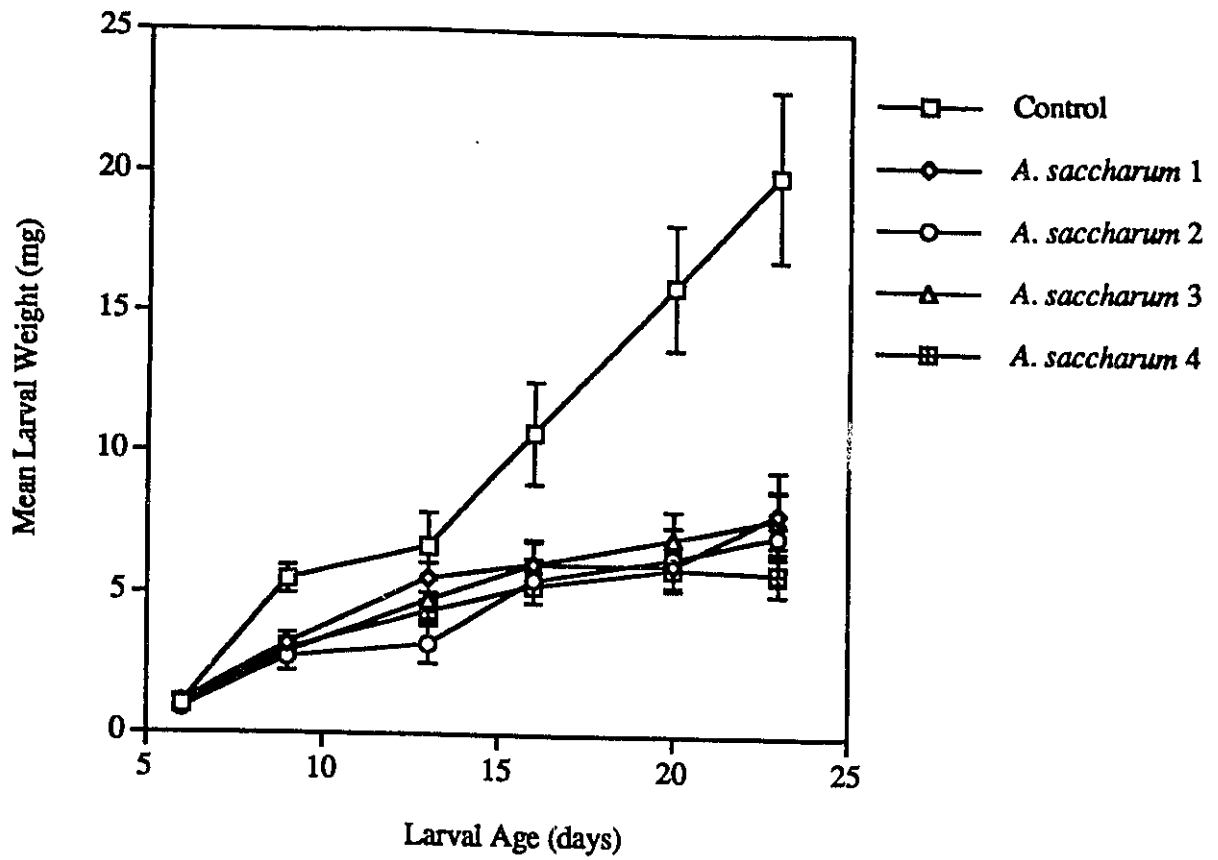


Table 4.1: Values for the ANCOVA completed on the *M. disstris* larval growth response (Figures 4.1 to 4.4) to extracts made with two solvent systems, from the foliage of either individual sugar maple or red maple trees. The "Figure 4.x" in the Species column refers to the graphs on which the ANCOVA was performed.

| Species | Source (Solvent System) | Degrees of Freedom | Mean Square | F Value | P Value |
|-------------------------------------|--------------------------------|-----------------------|-------------|---------|---------|
| <i>A. saccharum</i> (Figure 4.1) | Ethanol and Water [†] | 3, 244 | 0.23 | 3.23 | 0.02 |
| (Figure 4.3) | Acetone and Water [‡] | 3, 256 | 0.99 | 30.74 | <0.0005 |
| <i>A. rubrum</i> (Figure 4.2) | Ethanol and Water | 2, 155 | 0.02 | 0.21 | 0.81 |
| (Figure 4.4) | Acetone and Water | 3, 257 | 1.63 | 34.56 | <0.0005 |

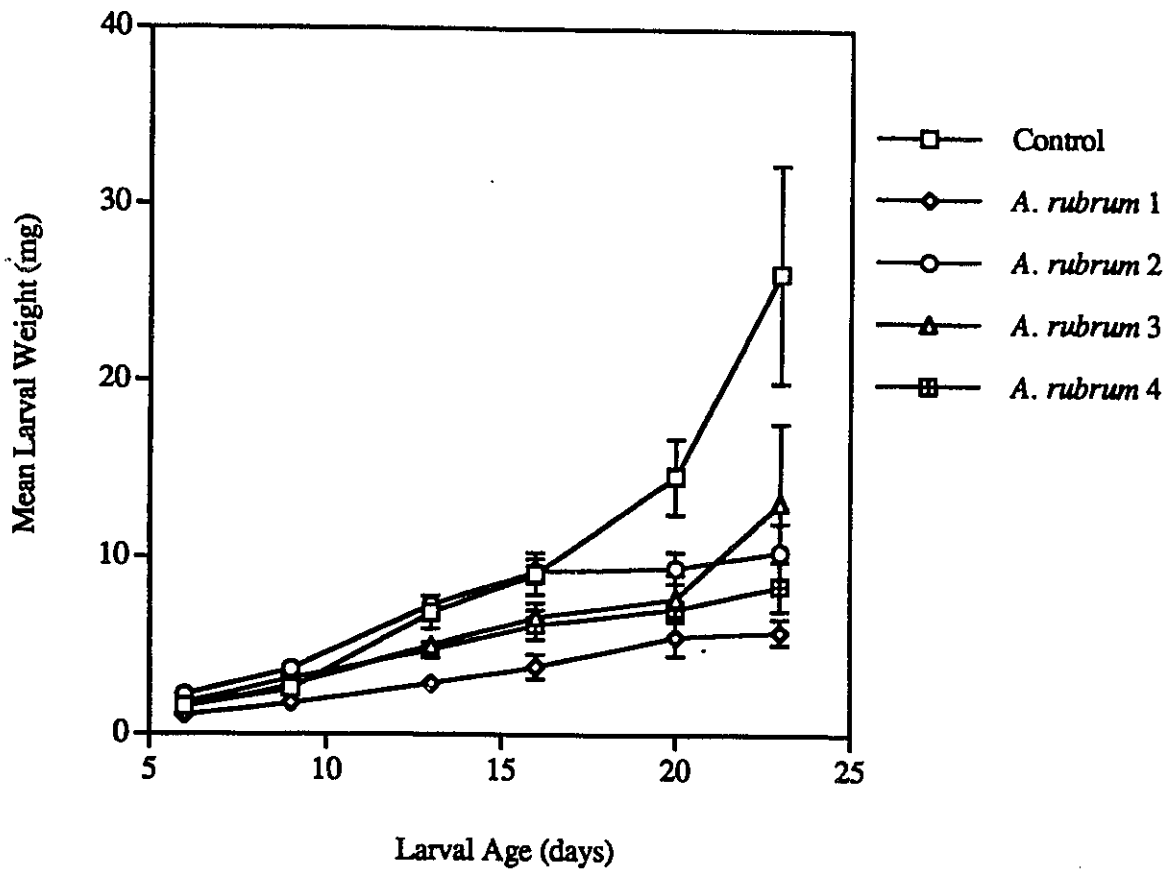
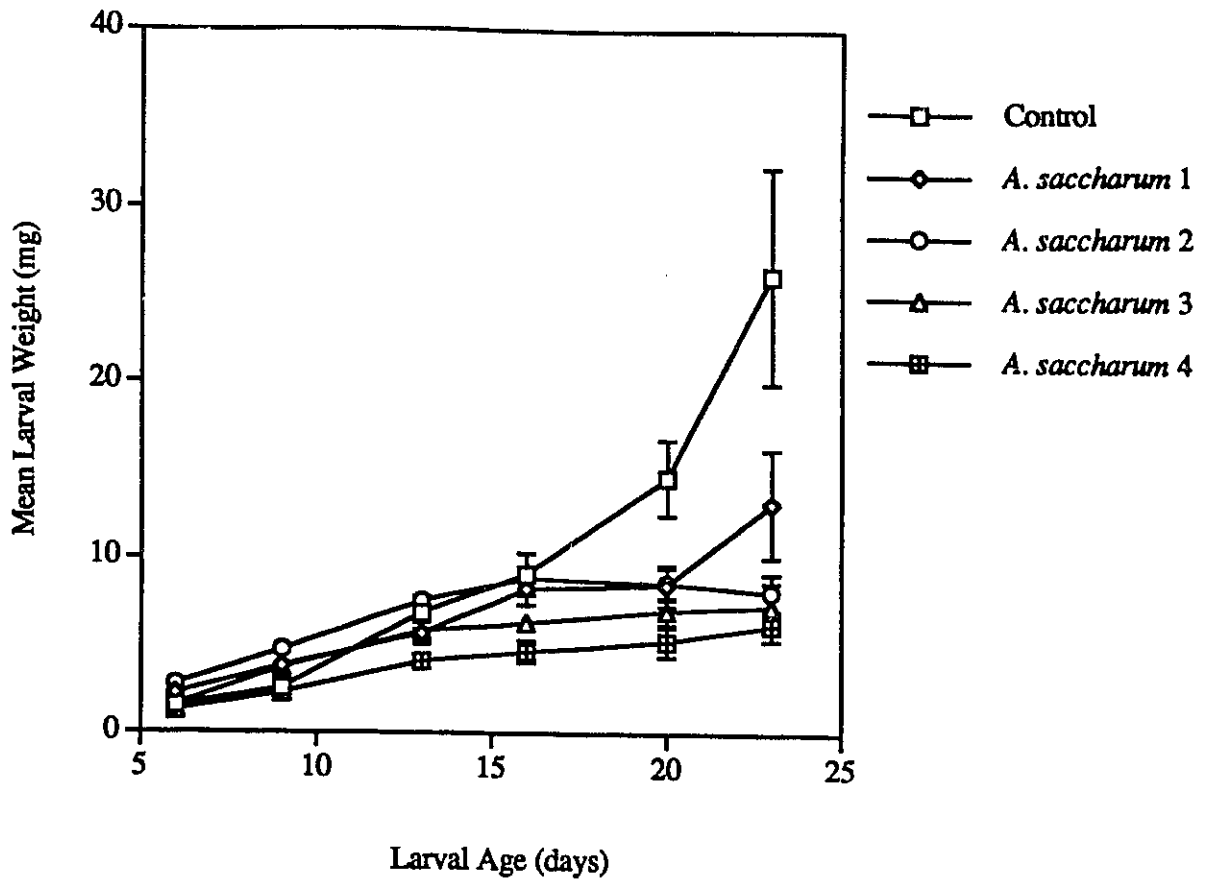
[†]80% ethanol and 20% double distilled water.

[‡]70% acetone and 30% double distilled water.

Table 4.2: The matrix of p values for the comparison of FTC growth responses to the maple extracts and extraction procedures.

| Species | Solvent | Tree Number | P Value | | | |
|---------------------|-------------------|-------------|---------|---------|---------|------|
| | | | 1 | 2 | 3 | 4 |
| <i>A. saccharum</i> | Ethanol and Water | 1 | 1.00 | | | |
| | | 2 | 0.02 | 1.00 | | |
| | | 3 | 0.98 | 0.06 | 1.00 | |
| | | 4 | 0.77 | 0.21 | 0.94 | 1.00 |
| <i>A. rubrum</i> | Ethanol and Water | 1 | 1.00 | | | |
| | | 2 | 0.80 | 1.00 | | |
| | | 3 | 0.99 | 0.88 | 1.00 | |
| <i>A. saccharum</i> | Acetone and Water | 1 | 1.00 | | | |
| | | 2 | 0.38 | 1.00 | | |
| | | 3 | 0.08 | <0.0005 | 1.00 | |
| | | 4 | <0.0005 | <0.0005 | <0.0005 | 1.00 |
| <i>A. rubrum</i> | Acetone and Water | 1 | 1.00 | | | |
| | | 2 | <0.0005 | 1.00 | | |
| | | 3 | <0.0005 | 0.01 | 1.00 | |
| | | 4 | <0.0005 | <0.0005 | 0.91 | 1.00 |

Figures 4.3-4.4: Intraspecific variation of the activity of the maple extracts against the larvae of *M. disstria*. Growth dynamics of the larvae after feeding on artificial diet containing acetone extracts from either individual sugar maple trees (top), or individual red maple trees (bottom). The extracts were incorporated into the diet at a concentration of 100 $\mu\text{g/g}$, with the control diet containing acetone and water only. Error bars are \pm standard error.



both species created variable larval responses (Table 4.1), with it being probable that the FTC grew at a similar rate in two of the tree treatments as compared to Tree 1 for the sugar maple acetone extract (SMAE), and one of the tree treatments as compared to Tree 3 for the red maple acetone extract (RMAE), (Table 4.2).

All maple trees and both extraction solvents caused growth inhibiting effects in the same manner as the original life cycle bioassays, although because of the aforementioned problem with the microsporidia, the growth and development was not as rapid. Figures 4.1 to 4.4 present data up until day 23 of the FTC life cycle, instead of until the time of first pupation. If the culture had not become infected, it would be expected that the control larvae's and the treatment larvae's growth curves would approximate those of the control larvae and treatment larvae at 100 $\mu\text{g/g}$, in the earlier bioassays. Although there is some variability in the secondary activity of individual trees against *M. disstria*, it cannot be stated that there is enough variation to support the idea that some individual trees have greatly reduced defenses, and that this reduction would allow for preferential FTC defoliation, thereby allowing the insect to escape the growth and developmental problems associated with consuming sugar maple leaves.

4.3.3 Comparison of Activity of both Extraction Procedures

There is no significant difference (Table 4.3) in the amount of weight gained by the FTC (Table 4.4) between the two solvent combinations used for either species. Therefore, at the concentration tested (100 $\mu\text{g/g}$) one extraction procedure does not create greater larval weight reductions in *M. disstria* than the other.

4.3.4 Geographic Variation

Geographic location seems to influence to a small degree the toxicity of the maple extracts when one compares average *M. disstria* growth reduction. The Sault Ste. Marie extracts reduced larval growth to a greater extent than the extracts made from trees in Ottawa (Figure 4.5). There seems to be a small difference in secondary defensive activity between the two locations, however because of the per cent comparison, it is not known if

Table 4.3: Values for the two-way ANOVA completed on the effect of the two extraction procedures on log larval weight gain.

| Treatment (Source of Variation) | Degrees of Freedom | Mean Square | F Value | P Value |
|--|-----------------------|-------------|---------|---------|
| <i>A. saccharum</i> (ethanol vs. acetone) | 1, 75 | 73.38 | 0.40 | 0.53 |
| <i>A. rubrum</i> (ethanol vs. acetone) | 1, 62 | 27.91 | 0.23 | 0.64 |

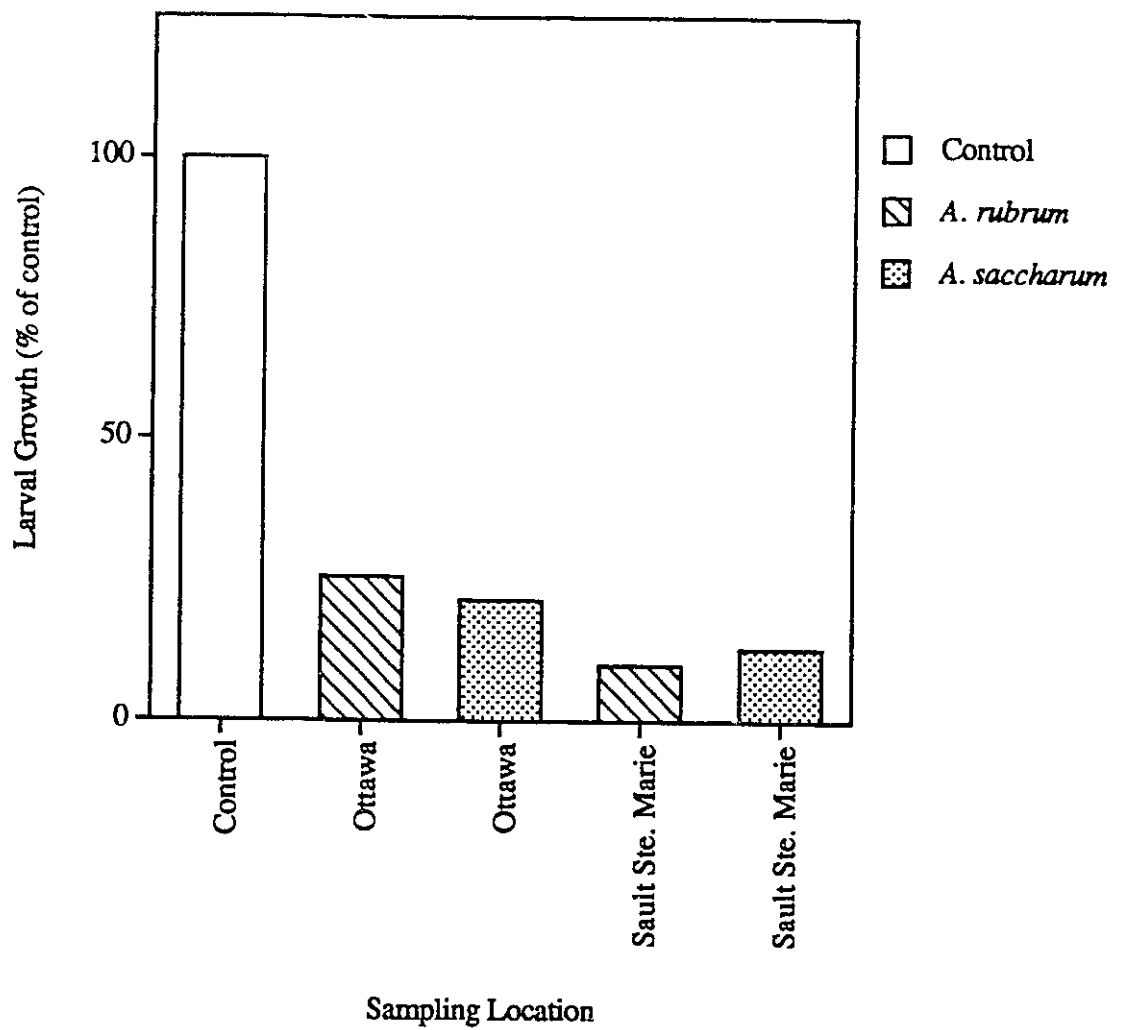
Table 4.4: *M. disstria* larval weight gain after 20 days of being fed an ethanol or acetone extract of either sugar maple or red maple.

| Species | Solvent System | Weight Gain (mg) [†] |
|---------------------|-------------------|----------------------------------|
| <i>A. saccharum</i> | Ethanol and Water | 8.69±2.03 [‡] |
| | Acetone and Water | 10.65±2.27 |
| <i>A. rubrum</i> | Ethanol and Water | 9.28±2.16 |
| | Acetone and Water | 7.87±1.78 |

[†]No significant difference in weight gain ($P > 0.05$ in Table 4.3).

[‡]Values are \pm standard error.

Figure 4.5: A comparison of the larval growth reducing activity of red maple and sugar maple ethanol extracts made from leaves collected in two areas, Sault Ste. Marie and Ottawa. The larval weight gain after 23 days was compared by expressing all values as the per cent amount larvae in the maple extract treatments gained in relation to those larvae in control.



this is significantly different. Because all maple extracts from both geographic areas still reduced larval growth as compared to control, one conclusion that can be drawn is that there is not enough variation in phytochemical activity (between Sault Ste. Marie and Ottawa) that would create a situation whereby the insect is known as a defoliator of *A. saccharum* in only one of these areas.

4.4 Discussion

4.4.1 Intraspecific Variation in Secondary Chemicals in Deciduous Trees

Lindroth and Bloomer (1991) observed that trembling aspen is not uniformly susceptible to FTC defoliation, and that “In aspen forests experiencing generally heavy defoliation, individual trees exhibiting only light defoliation are commonly observed (Lindroth pers. obs.)”. This preferential aspen defoliation by the FTC could be a function of various levels of defensive phytochemicals because in an earlier study (Lindroth et al. 1987) the researchers investigated variation in the levels of two principal trembling aspen phenolic glycosides (tremulacin and salicortin), among five individual aspen trees. These levels varied greatly; ranging from approximately 2 mg/g to 59 mg/g dry weight for tremulacin and from approximately 6 mg/g to 69.5 mg/g dry weight for salicortin in the five tree replicates sampled, at the time in the growing season that corresponded to the larval stage of *M. disstria*. These compounds are probably defensive as they were later shown to cause increased mortality and the development time spent within instars for the FTC (Lindroth and Bloomer 1991). Other researchers have found *A. saccharum* to have relatively little variation in total phenolics. Schultz et al. (1982) and Baldwin et al. (1987) described no significant differences in total phenolics between individual sugar maple trees as determined by a Folin-Denis assay, sampled in different states and in different years, but did find differences in yellow birch (*Betula allegheniensis* Britt.). This finding coupled with the interspecific results may be further proof that the phenolic compounds of sugar maple are the main defensive constituents of this species. That is, it is now known that the

crude sugar maple extracts cause deleterious effects in *M. disstria* through internal toxicity, and that most of the compounds in the active fraction of this extract are phenolics (Table 3.1). It follows then that the low variability of the levels of phenolics in *A. saccharum* could translate into the consistent (among different tree samples) anti-FTC effects seen here. In conclusion, although there is some variation in the secondary activity for red and sugar maple, there is not enough to cause different growth inhibiting abilities between individuals, as all of the extracts from the separate replicates showed anti-FTC activity within the respective species.

4.4.2 Sugar Maple and Red Maple Defenses as a General Phenomenon

As one would expect, there does seem to be variability in insect response to the maples' defensive components between the two test sites. However, the difference between Sault Ste. Marie and Ottawa is not great enough to explain why the FTC eat the foliage of *A. saccharum* when this species clearly causes deleterious effects on the *M. disstria*'s life cycle. It was thought that the Sault Ste. Marie growing location may be conducive to different levels of defensive compounds and that this increase in secondary chemicals would cause the negative growth effects seen in the bioassays done with the intact foliage or with the extracts from the Sault Ste. Marie trees. The discordance between field observations of *M. disstria* defoliating sugar maple trees and the laboratory results could then have been related to geography (i.e. the field reports were from different parts of eastern North America where the sugar maple trees could have had a relatively lower level of defensive compounds). This idea can be rejected. Although it was not possible to statistically compare the average weight gain of the larvae between the two locations, it is apparent that trees in both areas possess the ability to reduce larval fitness of *M. disstria*. It is acknowledged that these results cannot be a definitive answer to the issue of maple defensive variability due to the limitations of replicate numbers for the interspecific data and the choice of only two sampling sites for the geographic data.

Both *A. rubrum* and *A. saccharum* grow over a wide range of eastern North America. In the United States, red maple grows as far south as Florida and sugar maple reaches the northern border of Georgia and Alabama, and both species are not found west of Minnesota (Division of Timber Management Research 1965, Elias 1980). Variability in effectiveness of the defensive phytochemicals in these two species could be related to geography, but it is suggested here that the phytochemical defenses of *A. rubrum* and *A. saccharum* against the FTC are a general (and prevailing) phenomenon. Reports of FTC infestations from different forest regions in the U.S. and Canada are in agreement regarding sugar maple defoliation. Hodson (1941) reports defoliation of this tree in northern Minnesota, Gross (1991) studied an FTC infestation in sugar maple stands in southwestern Ontario (in an area from Lake Huron to Georgian Bay) and quantified reduced tree growth due to the defoliation. There is also a report of sugar maple being defoliated in New Brunswick by *M. disstria* (Magasi 1995), and the larvae were observed to consume sugar maple leaves during the last outbreak in the early 1990's in Sault Ste. Marie, Ontario (B. Helson pers. comm.). Likewise, there are references to the antifeedant nature of *A. rubrum* from different areas. Oliver (1964) states that red maple does not suffer FTC infestation in Louisiana, and again Hodson (1941) reports that this species is consistently avoided by the FTC in Minnesota.

As the experimental evidence does not support the idea of highly variable defensive activity either geographically or intraspecifically, and taking into account that sugar maple defoliation by *M. disstria* occurs over a wide area of eastern North America, it is concluded that the observations that this insect defoliates sugar maple in the field is not "at odds" with the negative developmental effects seen in the preceding experiments. It has been observed in the field and in the laboratory that the FTC will consume sugar maple leaves. It has now been demonstrated experimentally that if the insect eats the foliage or foliage extracts, it will suffer reduced growth, higher rates of mortality, reduced pupal weights and lower numbers of adult moths. Sugar maple is an FTC food host, but it is now known that this species

contains anti-FTC phytochemicals. This seemingly contradictory phenomenon is probably due to the lack of experimental data on FTC/sugar maple interactions. Although many field observations are reported, no comprehensive research has been completed on the growth and development effects *A. saccharum* causes in this insect. The only other research found in this area was published in another Master's thesis. Lorenzetti (1993) demonstrated that when fed sugar maple foliage, *M. disstria* experienced lower pupal weights and increased developmental time over those larvae fed trembling aspen leaves.

FTC defoliation of *A. saccharum* in the field could occur because, as suggested earlier, the larvae eat this foliage only when more suitable food sources have been exhausted. There is still the question however, of why the larvae would choose the maple species that causes relatively greater harm than the species that the larvae have consistently (in the laboratory and in the field) refused to eat. The refusal to eat *A. rubrum* could be related to qualitative or quantitative differences in the phytochemicals present in the two trees, as there are compounds present in red maple that are not present, or present at different concentrations, in sugar maple (Table 3.1). There may be a concentration threshold for the phytochemicals that are antifeedant (i.e. phenolic compounds), whereby at this threshold level the chemosensillae feedback system in the insect causes the "do not eat" behavioural response. However, there may be other factors in the red maple antifeedant equation. One such factor could be nutritional components in both species' leaves, as upon ingestion, defensive phytochemicals do not operate in isolation from other foliar components (Slansky 1992). It is known that some of the main nutritional elements in *P. tremuloides*, *A. saccharum* and *A. rubrum* follow a decreasing trend that matches the FTC food preferences of these three trees. For example, foliar nitrogen and water are most abundant in aspen followed by sugar maple and then red maple at the time in the season when the larvae would be feeding (Rickles and Matthew 1982, Schultz et al. 1982, Lindroth et al. 1987). The implications of the nutritional aspects of the foliage are addressed in the following chapter.

CHAPTER V:
WHY DO THE FTC EAT THE LEAVES OF SUGAR MAPLE BUT NOT
RED MAPLE?-GENERAL DISCUSSION AND FUTURE DIRECTIONS:

5.1 Introduction

The research presented in this thesis demonstrates that both red and sugar maple possess phytochemical defenses that are active against a generalist forest herbivore. Analysis of the active fraction of the extracts and bioassays with isolated compounds showed that these compounds are phenolics. Red maple had primarily an antifeedant action. When fed to the insect at levels lower than would be found in the intact leaf, the extract of red maple was found to be chronically toxic and detrimental effects accompanied the ingestion of the phytochemicals. Sugar maple was not antifeedant even though it was also chronically toxic and created greater growth and development effects in *M. disstria*. This concluding chapter will summarily address the two modes of action (antifeedancy and toxicity) of *A. rubrum* and *A. saccharum*. As both of these modes of action do not operate in the field in the absence of other foliar chemicals, the activity of the secondary compounds are discussed within the framework of the primary constituents. It is suggested that the ultimate decision of *M. disstria* to either accept or reject these two maples as a food source may be influenced by the nutritional, as well as the defensive, components of the leaf.

5.2 Antifeedant Phytochemicals in *A. rubrum*

The dramatic antifeedant nature of the intact red maple foliage was not seen in the artificial diet bioassays, because the concentrations used were below ecological levels. However, tests with RME in artificial diet still exhibited some antifeedant activity. It was hypothesized that as the concentration of RME in the diet approached those concentrations found in the intact leaf, the antifeedant activity between leaf and diet would become equal. There are some problems associated with testing for antifeedancy in artificial diet, and it

was decided not to attempt to use the diet bioassays to test for this because of a number of reasons. Firstly, the modified Addy's diet contains more sugar on a dry weight basis as would normally be present in the foliage of *P. tremuloides* at the correct phenological time (Lindroth et al. 1987, Lorenzetti 1993). This could influence the consumption of food by FTC in the presence of compounds that would usually cause antifeedant results within the leaf (as in Adams and Bernays 1978). There could be other issues associated with the use of artificial diet. Tests completed at Carleton University indicate that artificial diet is sometimes not a suitable substrate for the testing of antifeedancy as some components of the diet can "plug" the insect's chemosensillae (T. Arnason pers. comm.). Neither of the above would affect the internal toxicity of phytochemicals and therefore, all the results presented on larval growth inhibition, mortality and developmental effects are valid.

The experimental work on the antifeedant activity was completed at CFS by applying dissolved red maple compounds on the surface of trembling aspen leaves. This method would avoid the two aforementioned problems and hence give a more accurate description of the antifeedant nature of the phytochemicals in *A. rubrum*. Using an antifeedant index to quantify aspen leaf disk consumption, Dr. B. Helson at CFS categorized aspen extract applied on aspen disks to be "slightly stimulatory". SME was characterized as "neutral" with respect to FTC consumption of these disks. The results of the RME applied to aspen disks are more in concordance with the unaltered foliage results (RME on aspen disks caused reduced consumption), again suggesting that although the artificial diet bioassays may be useful in terms of finding the chronic effects or acute toxicological concentrations over the entire insects' life cycle, they may not adequately simulate the natural situation, possibly due to the discordance between the level of nutrients, or other factors, in the leaves and in the artificial diet.

Of the isolated *A. rubrum* compounds tested in the nutritional bioassay, two phenolics in particular were found to have the greatest antifeedant activity: gallic acid and methyl gallate (B. Helson pers. comm.). These are the compounds that probably confer

the resistance to FTC damage as compared to *A. saccharum*, as these compounds are not present, or present to a lesser, degree in the species that does not enjoy escape from FTC defoliation. As mentioned earlier, it would be interesting to test mixtures of the most antifeedant phytochemicals for synergistic or potentiating antifeedancy.

5.3 Chronic Toxicity of *A. rubrum* and *A. saccharum*

Phenolic compounds present in sugar and red maple are also responsible for the chronic toxicity seen here. Phenolics as a class of secondary chemicals are ubiquitous in woody plants and their parent compounds, the tannins, are found in all of the species in the Aceraceae (Bate-Smith 1977). The presence of these compounds in the foliage of trees may be a general anti-herbivore defensive mechanism. Levels of phenolics have been demonstrated to rise accordingly in leaves that have been defoliated by Lepidopteran larvae, accompanied by increases in phenolic levels in adjacent leaves (Hartley and Firm 1989) and these phytochemicals are known to cause detrimental growth effects in lepidopteran larvae (Chan et al. 1978, Mahoney et al. 1989, Scriber et al. 1989).

From the experimental data accumulated in this thesis on red maple toxicity, a hydrolysis product of the ellagitannins (ellagic acid) was found to be the most toxic chemical tested. As not all of the phytochemicals of *A. rubrum*, and none of the *A. saccharum* phytochemicals were tested, generalizations about the exact relationship between activity and chemical class cannot be made. Although the observation that the most antifeedant compounds in *A. rubrum* are products of the gallotannins and the most toxic is a product of the ellagitannins may represent a trend. This trend could further explain the relative greater chronic toxicity seen in *A. saccharum*. Ellagitannins and hydrolytic products (phenolics) have previously been isolated from this species (Hatano et al. 1990) and these products could be represented in sugar maple by the unidentified phenolic acids. This would mean that qualitative differences between the two maples species' accounts for the difference in chronic toxicity, with different phenolics originating from ellagitannins being responsible for the relative greater activity of sugar maple. It is without doubt that

both maples have phytochemical defenses that are active against *M. disstria*. However, the question of why the FTC eat the foliage of the species that has more potent chemical defenses still remains.

5.4 Nutritional Factors in *M. disstria* Feeding Preferences

The other major driving force behind insect feeding behaviour may influence the FTC's acceptance/rejection behaviour when presented with the foliage of both maples. This force is the primary, or nutritional component of the foliage.

It has been previously demonstrated that insects sometimes choose food sources based on nutritional aspects (Waldbauer and Friedman 1991, Slansky 1992) and differences in nutritional composition can also affect consumption rates (Slansky and Wheeler 1989). For example, Albert (1991) showed that the lepidopteran forest insect *Choristoneura fumiferana* Clem. (spruce budworm) responded to increasing dietary sucrose levels in a gaussian manner, whereby the highest sucrose level caused the budworm to consume about 16% as much as those larvae that were presented with a food source containing the median sucrose concentration. Adams and Bernays (1978) demonstrated that the balance between deterrent compounds and phagostimulants influence the behavioural response of the insect (i.e. to eat or not to eat). In this final discussion, data collected in the course of this thesis on one nutritional aspect, water, is addressed by relating water levels within the three species to leaf consumption by *M. disstria*. The nutritional aspects of *P. tremuloides*, *A. saccharum* and *A. rubrum* are also reviewed from previously published results and the merit of drawing final conclusions using this preliminary data is debated.

5.4.1 Foliar Water in *P. tremuloides*, *A. saccharum* and *A. rubrum*

Briefly, the per cent water present in the leaves used in the original leaf disk bioassay (section 2.2.2) was calculated by subtracting the final dry weight from the initial fresh weight and then dividing this value by the fresh weight. The average amount of foliar

Table 5.1: The water content of trembling aspen, sugar maple and red maple leaves collected at the seasonal time that corresponds to the larval stage of *M. disstria*

| Tree Species | Collection Date | Foliar Water (%) |
|-----------------------|-----------------|---------------------------------------|
| <i>P. tremuloides</i> | June 2, 1994 | 72.1±0.78 [†] a [‡] |
| <i>A. saccharum</i> | June 2, 1994 | 70.4±0.25 b |
| <i>A. rubrum</i> | June 2, 1994 | 67.7±0.21 c |

[†] Values are ± standard error.

[‡] Means followed by the same letter do not differ significantly in a Student-Neuman-Keuls (SNK) test at P<0.05.

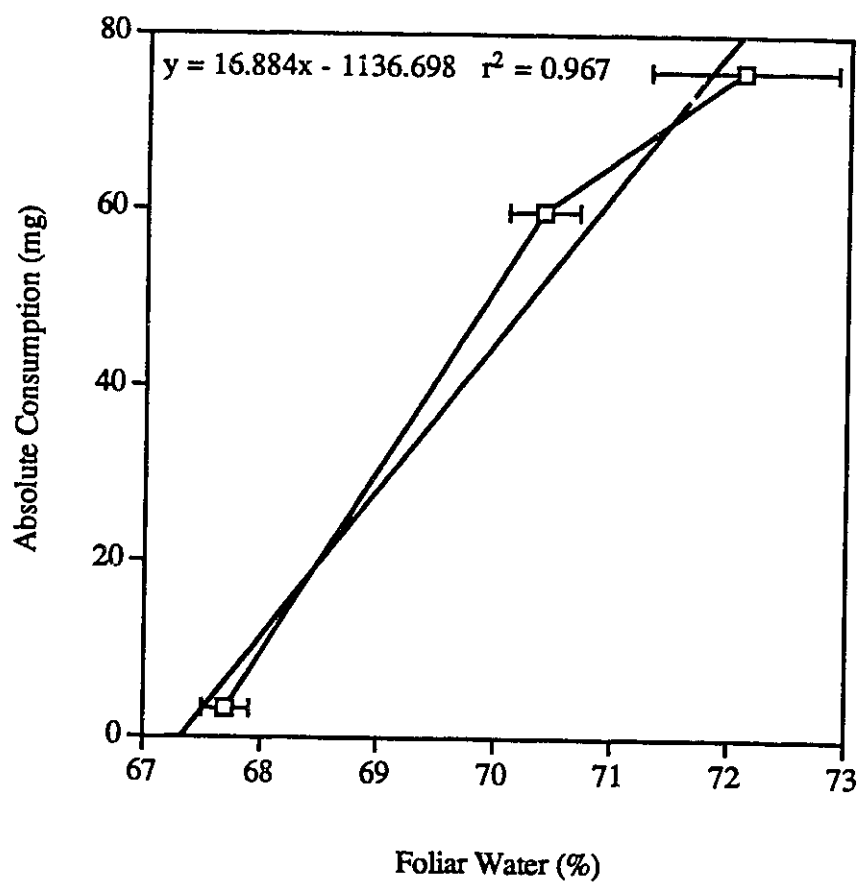
water present in the three species (Table 5.1) was separated using a multiple comparisons test (SNK) on SAS® (SAS 1982) and then graphed with the absolute amount of leaf eaten by the FTC. A correlation between the amount of foliar water and amount of leaf consumed was performed using on CA-Cricket® Graph III™ (Computer Associates International 1992).

The levels of foliar water were significantly different between the three species (Table 5.1). The greatest amount of foliar water was recorded in *P. tremuloides*, followed by *A. saccharum* and then *A. rubrum*. This decreasing trend in per cent water follows the food preference of FTC. When the absolute amount of leaf tissue eaten by the FTC in the nutritional indices bioassay was plotted against the water content of the leaves, a high correlation ($r^2=0.967$) was found (Figure 5.1).

5.4.2 Foliar Water and Lepidopteran Performance

The correlation between water content and consumption of the leaf by the FTC is compelling. This could indicate that foliar nutrients may play a role in the antifeedancy of *A. rubrum*. It is known that the amount of water present in an insect's food is a good predictor of, and strongly correlated to, upper performance levels (maximum RGR) of that insect (Scriber and Slansky 1981, Slansky and Scriber 1985). This is probably due to influence water has upon *in vivo* metabolism. The per cent water present in various food sources has been previously shown to affect metabolic efficiency in lepidopteran larvae. Scriber demonstrated that leaves possessing a relatively lower water content represented a greater metabolic cost for the consuming larvae (Scriber 1977). Plant sources with reduced water content also negatively affect internal efficiencies and growth rates of lepidopteran larvae (Reese and Beck 1978, Scriber 1979). Scriber went on to describe that tree feeders were more sensitive to low food source water than forb feeders and hypothesized that the reduced internal efficiencies associated with reduced water may occur because the larvae could use metabolic water from respiration to supplement low leaf water content (Scriber 1979). Reduced levels of water in the food source then, can create concomitant reductions

Figure 5.1: The levels of foliar water present in *P.tremuloides*, *A. saccharum* and *A. rubrum* as correlated to FTC consumption. Error bars are \pm standard error of the mean.



in larval performance, but there is not much evidence of other lepidopteran species having strong avoidance behaviours to food with simply lower levels of water. Albert (1991) found that older foliage avoided by spruce budworm was chemically suitable for the insect, and postulated that rejection was based on lower water content (50% in older needles compared to 80% in young needles), or leaf toughness.

5.4.3 Nutritional Chemicals and Secondary Chemicals

How then should one interpret the correlation of consumption and foliar water presented in Figure 5.1? The level of foliar nutrients is possibly related to the FTC response to *A. rubrum* in two ways: 1) the additive effects of poor nutrition and allelochemicals in *A. rubrum* cause the *M. disstria* larvae to reject this foliage; or 2) the nutrient levels do not cause, either separately or in conjunction with the allelochemicals, any behavioural response in the FTC, but are merely correlated with quantitative or qualitative levels of the phytochemicals of *A. rubrum*. Both of the preceding ideas should be regarded in the context of the question "Why do the FTC chose to eat the species that causes them relatively greater harm." The first hypothesis then, means that red maple is poorer nutritionally than sugar maple and this plus the allelochemicals drives the feeding behaviour in respect to the two species, whereas the second hypothesis means that the FTC choose sugar maple over red maple because the latter has different, or different concentrations of phytochemicals and the nutritional elements are simply correlated to these differences.

Ricklefs and Matthew (1982) demonstrated that nitrogen and water follow a decreasing trend in aspen, sugar maple and red maple, but it is not known if the FTC response would follow the same positive correlation to increasing levels of these nutrients as seen with water. The high r^2 value in Figure 5.1 may cause one to conclude that water does indeed influence FTC consumption, but before this conclusion can be made the possible correlation of foliar defensive components and nutritional components must first be addressed.

Lindroth et al. (1987) found that anti-FTC phenolic glycosides in aspen leaves were correlated to nitrogen but not to water. The wealth of data in Ricklefs and Matthew (1982), provides more evidence that defenses and nutrition could be correlated. These researchers analyzed nutritional, as well as defensive components of 34 deciduous trees growing in Ontario. For illustrative purposes, data from Rickles and Matthew (1982), on the foliar levels of two main nutritional elements (nitrogen and water) and a defensive component (polyphenols), in *P. tremuloides*, *A. saccharum* and *A. rubrum* were correlated. The figures in Appendix V demonstrate that at least one class of phytochemical seems to be correlated with leaf nutritional aspects. Again, this correlation follows the FTC food host preference, with trembling aspen having the highest amounts of nitrogen and water and the lowest amounts of polyphenols, followed by sugar maple and then red maple.

It would appear that the FTC host preference follows a trend of decreasing nutrients, but these nutrients in turn may be correlated to the defensive phytochemicals. The antifeedant nature of red maple foliage could be due to the (relatively) highest level of defensive compounds such as polyphenols which in turn are correlated to the nutritional factors, or the antifeedant phenomenon could be due to additive nutritional/allelochemical effects. However, there is currently not enough information to discern between the two ideas. Foliar nutrients could still play a primary role in red maple antifeedancy, especially other nutrients such as sugar. The examples of Adams and Bernays (1978) who suggested that high sucrose levels can sometimes "override" antifeedant effects and that compounds that exert antifeedant actions at low sugar concentrations do not always elicit the same response at higher sugar levels, and of Ma (1977) who showed that response to the antifeedant warbungal was greatly reduced when the compound was used in a sucrose and agar substrate rather than in the intact foliage, imply that antifeedant compounds are not always "universally" antifeedant. Preliminary research already completed at the University of Ottawa seems to indicate that a greater antifeedant action is exhibited in artificial diet that contained reduced sugar levels. However, this test was marred by high control mortality

and a general lack of larval fitness due to the microsporidia infection. A future goal could be to clarify how nutrients, defensive chemicals, and the interaction of the two influence *M. disstria* feeding behaviour. Multiple stepwise regressions on the main nutritional and defensive elements of several host and non-host tree species would go a long way to achieving this goal.

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APPENDICES

Appendix I

Nutritional Indices Calculations

$$\text{RCR} = \frac{\text{weight}^\dagger \text{ of food ingested}}{\text{average larval weight} \times \text{days}}$$

$$\text{RGR} = \frac{\text{larval weight gained}}{\text{average larval weight} \times \text{days}}$$

$$\text{AD} = \frac{\text{weight of food ingested} - \text{weight of frass}}{\text{weight of food ingested}}$$

$$\text{ECI} = \frac{\text{weight gained}}{\text{weight of food ingested}}$$

$$\text{ECD} = \frac{\text{larval weight gained}}{\text{weight of food ingested} - \text{weight of frass}}$$

[†]All weights recorded after drying at approximately 60°C for 72 hours. Index values are multiplied by 100 to obtain a percentage.

Appendix II

The Addy Diet (modified from Addy 1969)

-ingredients for a 2500 ml "batch" of diet.

| Ingredient | Amount |
|--------------------------|---------|
| • vitamin free casein | 98.0 g |
| • dextrose | 98.0 g |
| • Wesson's salt mix | 28.0 g |
| • cholesterol | 5.0 g |
| • sorbic acid | 3.4 g |
| • methyl paraben | 1.7 g |
| • choline chloride | 2.8 g |
| • Alphacel | 50.0 g |
| • raw linseed oil | 9.2 ml |
| • sodium alginate | 14.0 g |
| • ascorbic acid | 14.0 g |
| • Vanderzant vitamin mix | 40.0 g |
| • aureomycin | 10.0 g |
| • whole wheat flour | 140.0 g |
| • agar | 42.0 g |

- 1) add agar to 1600 ml cold water and heat to approximately 85°C
- 2) mix the ingredients in order except the whole wheat flour with 500 ml water in a blender
- 3) add half of the heated agar to the blender and mix
- 4) add whole wheat flour to the blender and mix
- 5) add balance of the heated agar to the blender and mix for 2-3 minutes
- 6) dispense to appropriate containers

Appendix III

Yield of Extracts from Maple Foliage

Ottawa Ethanol Extracts.

-a combined total of 187.7 g of fresh *A. saccharum* foliage, from three samples, was extracted in 95% ethanol and double distilled water and yielded 26.2 g of extract (14.0%).

-a combined total of 265.6 g of fresh *A. rubrum* foliage, from three samples, was extracted in 95% ethanol and double distilled water and yielded 33.4 g of extract (12.6%).

Ottawa Acetone Extracts.

-a combined total of 606.4 g of fresh *A. saccharum* foliage, from three samples, was extracted in acetone and double distilled water and yielded 69.6 g of extract (11.5%)

-a combined total of 751.9 g of fresh *A. rubrum* foliage, from three samples, was extracted in acetone and double distilled water and yielded 90.1 g of extract (12.0%).

Appendix IV

Glossary of Abbreviated Terms

AD: Approximate Digestibility

ANCOVA: Analysis of Co-Variance

ANOVA: Analysis of Variance

A. rubrum : *Acer rubrum* L., (red maple)

A. saccharum : *Acer saccharum* Marsh. (sugar maple)

CFS: Canadian Forest Service, Sault Ste. Marie, Ontario

ECD: Efficiency of Conversion of Digested food

ECI: Efficiency of Conversion of Ingested food

FTC: Forest Tent Caterpillar

GST: Glutathione Transferase

P. tremuloides: *Populus tremuloides* Michx. (trembling aspen)

RCR: Relative Consumption Rate

RGR: Relative Growth Rate

RMAE: Red Maple Acetone Extract

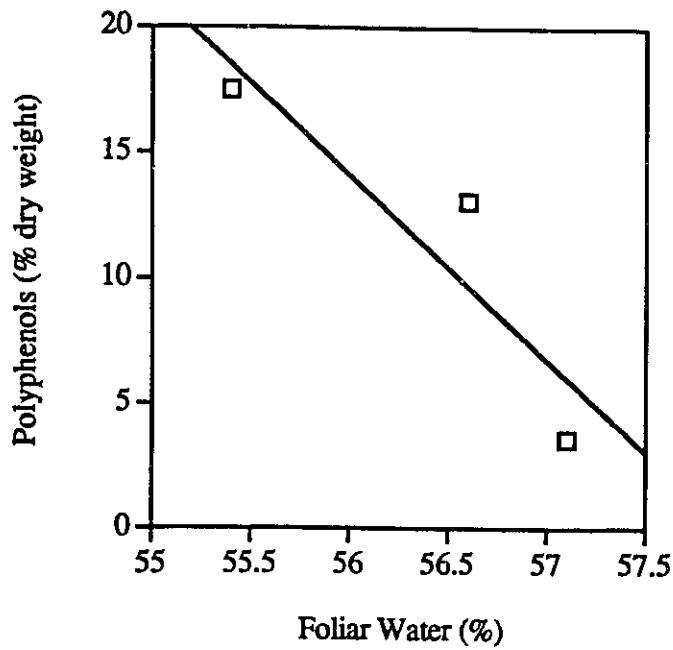
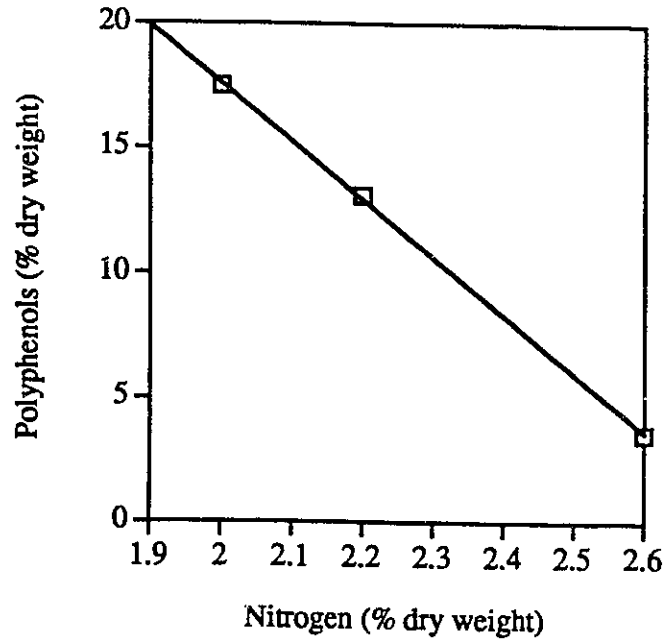
RME: Red Maple Extract

SMAE: Sugar Maple Acetone Extract

SME: Sugar Maple Extract

SNK: Student-Neuman-Keuls

Appendix V



The correlation of defensive compounds (polyphenols) to foliar nitrogen (top) and foliar water (bottom). Data obtained from Ricklefs and Matthew (1982).