UNIVERSITY OF ALBERTA

FOREST TENT CATERPILLAR - TACHINID PARASITOID INTERACTIONS ON DIFFERENT HOST PLANT SPECIES

BY

EDWARD BRIAN MONDOR

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

DEPARTMENT OF ZOOLOGY

EDMONTON, ALBERTA

FALL 1996

ISBN 0-612-18300-9

University of Alberta

Library Release Form

Name of Author: Edward Brian Mondor

Title of Thesis: Forest Tent Caterpillar - Tachinid Parasitoid Interactions on Different Host Plant Species

Degree: Master of Science

Year this Degree Granted: 1996

Edward B. Mondor

729-15th Street
Brandon, Manitoba
Canada
R7A 4W8

June 12, 1996

Insects are said to be stimulus-bound and instinctively fixed -- little machines in a deep sleep . . . Do insects indeed lack higher categories of behavior or is it that being anatomically different from us they do not show, for example, emotion because their eyes are pupiless and immobile, because they do not cry, sweat, or sulk? . . . perhaps these insects are little machines in a deep sleep, but looking at their rigidly armored bodies, their staring eyes, and their mute performances, one cannot help at times wondering if there is anyone inside.

V. G. Dethier

University of Alberta

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled *Forest Tent Caterpillar - Tachinid Parasitoid Interactions on Different Host Plant Species* by *Edward Brian Mondor* in partial fulfillment of the requirements for the degree of *Master of Science*.

Dr. Jens Roland
(Supervisor)

Dr. Bev Mitchell
(Committee member)

Dr. Jan Murie
(Committee member)

ABSTRACT

Leschenaultia exul (Townsend) and *Patelloa pachypyga* (Aldrich & Webber) are leaf-ovipositing tachinid parasitoids of the forest tent caterpillar (FTC) *Malacosoma disstria* (Hübner). Despite being two significant members of the FTC parasitoid complex, the behaviour and ecology of these two fly species is relatively unknown. It was discovered that the flies respond differently when their host feeds on aspen poplar *Populus tremuloides* Michx. compared to balsam poplar *Populus balsamifera* L. *L. exul* locates its host, leaf-oviposits, and subsequently parasitizes its host when it feeds on both tree species. *P. pachypyga* locates its host primarily when feeding on aspen poplar trees, with leaf-oviposition and resulting parasitism being higher on this tree species compared to balsam poplar trees. Because *P. pachypyga* was the dominant parasitoid of the FTC, larval 'wandering' from aspen poplar to feed on other tree species results in a reduced parasitism risk by feeding in relatively enemy-free space.

ACKNOWLEDGMENTS

I gratefully acknowledge the assistance of my supervisor Jens Roland. His philosophy of "giving me enough rope" not only taught me a great deal about academic freedom and independence but hopefully also expanded my horizons as a scientist in general. His patience with my inane statistical questions went far beyond the call of duty and was always appreciated.

I would also like to thank the members of my advisory committee Bev Mitchell and Jan Murie. Their assistance ranged anywhere from the initial size of the project to discussions on syntax and semantics. Their help in focusing this project contributed a great deal to its underlying success. I also express thanks to Barry McCashin who gave me an extremely quick course on gas chromatography. His ability to teach me the basic principles of G.C. in such a short period of time just provides evidence of his excellent technical expertise.

I thank laboratory assistants Norrine Ambrose, Stacey Rasmussen, and Kirsty Ward for their help in conducting various parts of this project. A special thanks to my assistant Sherri Fownes who worked tirelessly and provided me with friendship and support as well as excellent technical assistance. I would be remiss if I did not thank my office mates (past and present) Shelley, Lynne, Hiroki, Becky, Yves, and Corey as well as all the other undergraduate and graduate students I have met along my scholastic journey, for intriguing and broadening discussions.

Last, but certainly not least, this project would not have been possible without generous financial contributions from the following sources: NSERC postgraduate scholarship to EM, Walter H. Johns Graduate Fellowship, Canadian Circumpolar Institute of the University of Alberta grant, Alberta Department of Environmental Protection, Fish & Wildlife Trust Fund, University of Alberta Department of Biological Sciences, J. Gordon Kaplan Graduate Student Award, Faculty of Graduate Studies and Research of the University of Alberta, the Vice-President (Research) of the University of Alberta, Central Research Fund grant from the University of Alberta, and an NSERC operating grant to JR.

TABLE OF CONTENTS

CHAPTER PAGE

1. Introduction 1
   - 1.1 Background 1
   - 1.2 Forest tent caterpillar - tachinid parasitoid dynamics 4
   - 1.3 Thesis objectives 5
   - 1.4 Literature cited 8

2. Parasitoid Attraction 12
   - 2.1 Introduction 12
   - 2.2 Materials and methods 14
   - 2.3 Results 19
   - 2.4 Discussion 21
   - 2.5 Literature cited 25

3. Parasitoid Oviposition Strategies 31
   - 3.1 Introduction 31
   - 3.2 Materials and methods 33
   - 3.3 Results 37
   - 3.4 Discussion 40
   - 3.5 Literature cited 44

4. Egg Distribution and Parasitism 52
   - 4.1 Introduction 52
   - 4.2 Materials and methods 54
   - 4.3 Results 59
   - 4.4 Discussion 63
   - 4.5 Literature cited 68

5. General Conclusion 78
   - 5.1 Summary 78
   - 5.2 Future research 80
   - 5.3 Literature cited 82

Appendix 1 83

LIST OF TABLES

TABLE PAGE

1.1 Tachinid parasitoids that attack important pest species..... 11

3.1 Number of eggs oviposited by individual *L. exul* on FTC larval damaged aspen poplar *Populus tremuloides*, balsam poplar *Populus balsamifera*, or construction paper leaf clusters in choice experiments..... 47

LIST OF FIGURES

FIGURE PAGE

2.1 Plexiglas wind tunnel used for *L. exul* paired choice tests between different insect/plant combinations 27

2.2 Number of *L. exul* aggregating on the left or right side of the wind tunnel for each pair of treatments 28

2.3 Number of *L. exul* moving upwind on the left or right side of the wind tunnel for each pair of treatments 29

2.4 Mean number (+/- SE) of *L. exul* and *P. pachypyga* flies entering the canopy of aspen poplar and balsam poplar trees during 15 minute field observations 30

3.1 Mean number (+ SE) of tarsal contacts with FTC damaged leaf edges per minute by female *L. exul* 48

3.2 Mean number (+ SE) of ovipositions per minute by female *L. exul* 48

3.3 Mean number of times female *L. exul* moved from one leaf to another within a leaf cluster 49

3.4 Mean times (+ SE) spent on leaf clusters by female *L. exul* 49

3.5 A kinematic graph of *L. exul* searching behaviours on aspen poplar, *Populus tremuloides* leaf clusters 50

3.6 A kinematic graph of *L. exul* searching behaviours on balsam poplar, *Populus balsamifera* leaf clusters 51

4.1 Mean height (+ SE) of trees used for leaf cluster sampling 70

4.2 Mean abundance (+ SE) of FTC larvae abundances on trees used for leaf cluster sampling 70

4.3 Mean defoliation (+ SE) of trees used for leaf cluster sampling 71

4.4 Mean number (+/- SE) of eggs laid by *L. exul* and *P. pachypyga* flies on aspen poplar and balsam poplar trees 72

4.5 Mean number (+/- SE) of eggs laid by *L. exul* and *P. pachypyga* flies on aspen poplar and balsam poplar leaf clusters 73

4.6 Number of *L. exul* eggs laid as a function of defoliation of aspen poplar leaf clusters 74

4.7 Number of *L. exul* eggs laid as a function of defoliation of balsam poplar leaf clusters..... 74

4.8 Number of *P. pachypyga* eggs laid as a function of defoliation of aspen poplar leaf clusters..... 75

4.9 Number of *P. pachypyga* eggs laid as a function of defoliation of balsam poplar leaf clusters..... 75

4.10 Parasitism as a function of the number of eggs laid per tree on aspen poplar trees..... 76

4.11 Parasitism as a function of the number of eggs laid per tree on balsam poplar trees..... 76

4.12 Mean parasitism (+/- SE) of FTCs by *L. exul* and *P. pachypyga* on aspen poplar and balsam poplar trees..... 77

Chapter 1 Introduction

1.1 Background

Parasitoid ecology and behaviour have become popular fields of study in recent years, partly because of biological control programs and because parasitoids comprise about 25% of the world's total species (Price 1980). Research on parasitoids has focused on a limited number of species, primarily in the Order Hymenoptera (Vinson 1975, 1976). However, many species of Diptera are also parasitic. Researchers have attempted to study parasitic flies as 'honorary ichneumonids' but these insects are substantially different from hymenopterous parasitoids in both form and function and in their ecology and behaviour (Eggleton & Gaston 1992).

The Tachinidae are the most abundant group of non-hymenopterous insect parasitoids, comprising over 8 000 described species (Wood 1987; in Eggleton & Gaston 1992). It is one of the most diverse families of the true flies and for almost all species the larval stage is obligatorily endoparasitic in arthropods (O'Hara 1985). The majority of tachinids are lethal parasites making them excellent candidates for biological control agents for insect pests (Bryan et al. 1970). Tachinid parasitoids are important natural enemies of some very important pests (Table 1.1), attacking many groups of arthropods, especially lepidopteran larvae (Eggleton & Gaston 1992).

Tachinid parasitoids must first be able to locate their hosts, and are known to respond to a wide variety of host-seeking stimuli. The tachinid fly *Euphasiopteryx ochracea* locates its field cricket hosts by phonotaxis (Cade 1975). *Parasetigena silvestris* uses primarily visual stimuli to parasitize gypsy moth larvae (Odell & Godwin 1979). *Eucarcelia rutilla*

females are attracted by the sight of *Bupalus piniarius* moth larvae upon pine needles and even by objects that resemble host larvae (Herrebout & van der Veer 1969). Monteith (1955, 1958) found that *Bessa harveyi* and *Drino bohemica* were attracted primarily to the host insects and secondarily to their host's food plants. Host larval movement has also been shown to be attractive to *D. bohemica* (Monteith 1956).

Many studies of host-finding by tachinid parasitoids emphasize the importance of the larval food plants. Mesnil (1944) reported that *Staurochaeta albocingulata* was strongly attracted to *Juniperus* sp., the food plant of its host (in Herrebout et al 1969). *Drino bohemica* and *Bessa harveyi* were attracted to insect-damaged foliage particularly on unhealthy plants (Monteith 1964). The same two parasitoid species also preferred the odor of older foliage to that of new growth (Monteith 1966). *Lydella grisescens*, a parasite of the European corn borer *Ostrinia nubilalis* is attracted primarily to corn plants and secondarily to the host insect feeding on corn (Franklin & Holdaway 1966). Attack by *Eucelatoria bryani* is affected by variety, morphology, and phenology of plants fed on by its host *Heliothis* spp. (Martin et al. 1990). The only studies conducted on host-finding by a leaf-ovipositing tachinid parasitoid, *Cyzenis albicans*, demonstrated that this fly is attracted to odours from oak trees damaged by the winter moth *Operophtera brumata* (Hassell 1968, Roland et al. 1989, Roland et al. 1995).

Once tachinids have located their host insects they must parasitize them, and they do so in one of several ways. O'Hara (1985) has classified oviposition strategies of the Tachinidae into four categories based on whether they oviposit or larviposit and the location of the oviposition/larviposition: (1) macrotype egg deposited on or in host, (2) microtype egg deposited on foliage, (3) larvae deposited near, on, or in host, and (4) larvae deposited on soil or foliage. The type of stimuli releasing oviposition/larviposition activity depends strongly on the method of attack.

Voria ruralis is stimulated to lay macrotype eggs on hosts when one or both front tarsi touch the host insect (Elsey & Rabb 1970). Microtype egg depositors oviposit in response to a contact oviposition cue coming from damaged host plant leaves. Because laying eggs on host-browsed foliage is a relatively risky host-finding strategy, females typically produce thousands of eggs (O'Hara 1985). Hassell (1968) reported that exudate from damaged oak leaves stimulate *Cyzenis albicans* to oviposit. Dowden (1934) observed that the gypsy moth parasitoid *Zenillia libatrix* oviposits on almost any type of leaf whether or not they were fed upon by insects or otherwise damaged.

Tachinids that larviposit on or near the host are strongly influenced by host produced compounds. Chemicals present in the feces, haemolymph, and whole bodies of larvae of *Heliothis virescens* stimulate females of the tachinid *Archytas marmoratus* to deposit maggots in the vicinity of the host (Nettles & Burks 1975, Gross & Young 1984). A kairomone in the frass and vomitus of cutworm larvae *Agrotis ipsilon* triggers larviposition activity in the parasitoid *Bonnetia comta* (Clement et al. 1986). The European corn borer, *Ostrinia nubilalis,* parasite, *Lydella grisescens*, is attracted to frass and this leads to larviposition (Hsiao et al. 1966). *Lixophaga diatraeae* deposits its maggots on or near frass of its host, as the host's frass is the final releasing stimulus for larviposition (Bennett 1969, in Thompson et al. 1983; Roth et al. 1978). For tachinids larvipositing inside their hosts, the parasitoids must physically contact the host before larviposition occurs (Weseloh 1980). For *Eucelatoria* sp., a cuticular extract is the stimulus leading to oviposition (Burks & Nettles 1978).

Despite knowing how these tachinid parasitoids locate and attack host larvae, it is the resulting parasitism rates that determine the effectiveness of these flies as natural control agents. Tachinid parasitoids can greatly reduce host populations, but studies to date have not attempted to integrate behavioural studies with resulting parasitism rates observed

under natural situations. There has been little attempt to integrate detailed behaviour of tachinid parasitoids with the population consequences for their hosts. This is a mistake; if we know why individual insects behave as they do, we may then have better insight into why insect populations behave as they do. We are ignorant of the behaviours exhibited by most tachinid parasitoid species leading to parasitism. The sum of individuals' behaviours produce the overall actions of a species and therefore drive insect-parasitoid dynamics.

1.2 Forest tent caterpillar-tachinid parasitoid dynamics

The forest tent caterpillar (FTC) *Malacosoma disstria* Hübner (Lepidoptera:Lasiocampidae) is a significant defoliator of forest trees in North America (Hodson 1941). Local populations of larvae defoliate trembling aspen *Populus tremuloides* Michx. (Batzer 1955) and, at high densities, other tree and shrub species such as cottonwood *Populus deltoides*, Manitoba maple *Acer negundo*, birch *Betula* spp., ash *Fraxinus* spp., oak *Quercus* spp., elm *Ulmus* spp., basswood *Tilia americana* L., and balsam poplar *Populus balsamifera* L. (Deitz 1976). These defoliators undergo cyclic outbreaks, occurring approximately every 10 years (Hodson 1941, Sippell 1962). Decline of FTC populations is thought to result, in part, from mortality induced by its natural enemies such as sarcophagid fly parasitoids, tachinid fly parasitoids, and viral pathogens (Witter & Kulman 1972, 1979, Myers 1993, Roland 1993, Parry 1995).

In Canada, the predominant late-larval parasitoids attacking the caterpillars are the leaf-ovipositing tachinid flies *Leschenaultia exul* (Townsend) and *Patelloa pachypyga* (Aldrich & Webber). Our knowledge of these two parasitoids is limited. Bess (1936) provided the only description of the behaviour and ecology of *L. exul*. Virtually nothing

is known about the behaviour and ecology of *P. pachypyga*. As both of these parasitoids are leaf-ovipositors, olfactory cues given off by larval-damaged tree leaves are believed to have a major influence on how flies locate their hosts on different tree species.

Recent ideas regarding the factors that affect FTC outbreaks have included the effect of habitat heterogeneity on population processes. As habitats become increasingly fragmented, the duration of FTC outbreaks also increases (Roland 1993). Fragmentation is, however, only one aspect of habitat heterogeneity. Other kinds of heterogeneity may affect the duration of FTC outbreaks. For example, parasitism rates of FTCs on different tree species (host plant heterogeneity) has not been evaluated. Host plant heterogeneity may have a large impact on FTC parasitism rates, especially for tachinid parasitoids that locate their hosts by cueing in on volatile organic compounds emanating from damaged tree leaves. If only certain species of damaged leaves are attractive to the parasitoids then caterpillars feeding on non-attractive tree species would avoid parasitism by feeding in enemy-free space. Caterpillars feeding on non-attractive tree species may help to prolong an outbreak.

Thus far, the influence of different tree species on parasitoid attraction, oviposition, and parasitism in the FTC - tachinid parasitoid system has not been investigated. Only through experimental and observational bioassays will we be able to obtain answers to these questions.

1.3 Thesis objectives

I attempted to determine whether the two primary tree species fed on by FTCs in Alberta (aspen poplar and balsam poplar) influence the rate at which the caterpillars are

parasitized by the two late-larval tachinid parasitoids *L. exul* and *P. pachypyga*. In order to determine what affect host feeding on the 2 tree species has on parasitoid behaviour, we must examine all four stages of the parasitoid-host interaction (Vinson 1976): (1) host habitat location, (2) host location, (3) host acceptance, and (4) host suitability (parasitism).

In Chapter 2, I attempted to identify the stimuli attracting *L. exul* and *P. pachypyga* to their hosts' habitat, using a combination of wind tunnel experiments and observational bioassays. I attempted to determine whether or not the parasitoids are attracted to FTCs directly or to the plant on which larvae are feeding. Wind tunnel experiments were conducted using only *L. exul* due to the difficulty in rearing and maintaining *P. pachypyga* in the laboratory. Field observational bioassays, however, were conducted on both fly species.

In Chapter 3, I examine the oviposition and searching strategies exhibited by gravid female *L. exul*. Gravid female flies were allowed to oviposit on different plant species to determine if the flies have a preference for ovipositing on a particular plant species. A sequential analysis of the behaviours leading to oviposition was then conducted to determine if these flies randomly lay eggs on trees or if they have 'strategies' when laying eggs. I then determine if this 'strategy' differs depending on host plant species.

In Chapter 4, I examine the spatial distribution of eggs and rates of parasitism by *L. exul* and *P. pachypyga* on each host plant species under natural conditions. In this way, I assessed how the detailed behavioural patterns observed in the laboratory are translated into patterns of oviposition and parasitism in the field. These patterns indicate the importance of host plant heterogeneity on parasitism, and the potential importance of enemy-free space on host-parasitoid dynamics.

In Chapter 5, I present the conclusions on the above experiments and observational bioassays and will conclude with some general insights into the behaviour of these two parasitoids. Directions for future research will then be discussed.

1.4 Literature cited

Batzer, H. O. 1955. Some effects of defoliation of aspen, *Populus tremuloides* Michx., stands in northern Minnesota by the forest tent caterpillar, *Malacosoma disstria* Hbn., with notes on parasitism of cocoons by *Sarcophaga aldrichi* Park. and cocooning habits of the host. M.Sc. Thesis, University of Minnesota, St. Paul. 66 pp.

Bess, H. A. 1936. The biology of *Leschenaultia exul* Townsend, a tachinid parasite of *Malacosoma americana* Fabricius and *Malacosoma disstria* Hübner. Annals of the Entomological Society of America 29:593-613.

Bryan, D. E., Jackson, C. G. and Patana, R. 1970. Biological comparison of two species of *Eucelatoria* parasitic in *Heliothis* spp. Journal of Economic Entomology 63:1469-1472.

Burks, M. L. and Nettles Jr., W. C. 1978. *Eucelatoria* sp.: Effects of cuticular extracts from *Heliothis virescens* and other factors on oviposition. Environmental Entomology 7:897-900.

Cade, W. 1975. Acoustically orienting parasitoids: Fly phonotaxis to cricket song. Science 190:1312-1313.

Clement, S. L., Rubinik, W. L. and McCartney, D. A. 1986. Larviposition response of *Bonnetia comta* (Dipt.:Tachinidae) to a kairomone of *Agrotis ipsilon* (Lep.:Noctuidae). Entomophaga 31:277-284.

Deitz, J. 1976. Forest Tent Caterpillars. Manitoba Department of Agriculture pamphlet. 6 pp.

Dowden, P. B. 1934. *Zenillia libatrix* Panzer, a tachinid parasite of the gypsy moth and the brown-tail moth. Journal of Agriculture Research 48:97-114.

Eggleton, P. and Gaston, K. J. 1992. Tachinid host ranges: a reappraisal (Diptera:Tachinidae). Entomologist's Gazette 43:139-143.

Elsey, K. D. and Rabb, R. L. 1970. Biology of *Voria ruralis* (Diptera:Tachinidae). Annals of the Entomological Society of America 63:216-222.

Franklin, R. T. and Holdaway, F. G. 1966. A relationship of the plant to parasitism of European corn borer by the tachinid parasite *Lydella grisescens*. Journal of Economic Entomology 59:440-441.

Gross Jr., H. R. and Young, O. P. 1984. *Archytas marmoratus* (Diptera:Tachinidae): Screened-cage evaluations of selected densities of adults against larval populations of *Heliothis zea* and *Spodoptera frugiperda* (Lepidoptera:Noctuidae) on whorl and tassel stage corn. Environmental Entomology 13:157-161.

Hassell, M. P. 1968. The behavioural response of a tachinid fly (*Cyzenis albicans* (Fall.)) to its host, the winter moth (*Operophtera brumata* (L.)). Journal of Animal Ecology 37:627-639.

Herrebout, W. M. and van der Veer, J. 1969. Habitat selection in *Eucarcelia rutilla* Vill. III. Preliminary results of olfactometer experiments with females of known age. Zeitschrift fur Angewandte Entomologie 64:55-61.

Herrebout, W. M., Tates, A. D. and van der Veer, J. 1969. Habitat selection in *Eucarcelia rutilla* Vill. IV. Experiments with gravid females. Zeitschrift fur Angewandte Entomologie 64:218-232.

Hodson, A. C. 1941. An ecological study of the forest tent caterpillar, *Malacosoma disstria* Hbn., in Northern Minnesota. Minnesota Agriculture Experiment Station Technical Bulletin 148:55 pp.

Hsiao, T. H., Holdaway, F. G. and Chiang, H. C. 1966. Ecological and physiological adaptations in insect parasitism. Entomologia Experimentalis et Applicata. 9:113-123.

Kelleher, J. S. 1960. Life history and ecology of *Doryphorophaga doryphorae* (Riley), a tachinid parasite of the Colorado potato beetle. Ph.D. Thesis, University of Minnesota, Minneapolis. 70pp.

Martin Jr., W. R., Nordlund, D. A. and Nettles Jr., W. C. 1990. Response of parasitoid *Eucelatoria bryani* to selected plant material in an olfactometer. Journal of Chemical Ecology 16:499-508.

Monteith, L. G. 1955. Host preferences of *Drino bohemica* Mesn. (Diptera:Tachinidae), with particular reference to olfactory responses. The Canadian Entomologist 87:509-530.

Monteith, L. G. 1956. Influence of host movement on selection of hosts by *Drino bohemica* Mesn. (Diptera:Tachinidae) as determined in an olfactometer. The Canadian Entomologist 88:583-586.

Monteith, L. G. 1958. Influence of host and its food plant on host-finding by *Drino bohemica* Mesn. (Diptera:Tachinidae) and interaction of other factors. Proceedings of the Tenth International Congress of Entomology 2:603-606.

Monteith, L. G. 1964. Influence of the health of the food plant of the host on host-finding by tachinid parasites. The Canadian Entomologist 96:1477-1482.

Monteith, L. G. 1966. Influence of new growth on the food plant of the host on host-finding by *Drino bohemica* Mesnil (Diptera:Tachinidae). The Canadian Entomologist 98:1205-1207.

Myers, J. A. 1993. Population outbreaks in forest Lepidoptera. American Scientist 81:240-251.

Nettles Jr., W. C. and Burks, M. L. 1975. A substance from *Heliothis virescens* larvae stimulating larviposition by females of the tachinid, *Archytas marmoratus*. Journal of Insect Physiology 21:965-978.

Odell, T. M. and Godwin, P. A. 1979. Attack behaviour of *Parasetigena silvestris* in relation to host density and behaviour. Annals of the Entomological Society of America 72:281-286.

O'Hara, J. E. 1985. Oviposition strategies in the Tachinidae family of beneficial parasitic flies. Agriculture and Forestry Bulletin 8:31-34.

Parry, D. 1995. Larval and pupal parasitism of the forest tent caterpillar, *Malacosoma disstria* Hübner (Lepidoptera:Lasiocampidae), in Alberta, Canada. The Canadian Entomologist 127:877-893.

Price, P. W. 1980. Evolutionary Biology of Parasites. Princeton, New Jersey: Princeton University Press.

Roland, J. 1993. Large-scale forest fragmentation increases the duration of tent caterpillar outbreak. Oecologia 93:25-30.

Roland, J., Evans, W. G. and Myers, J. H. 1989. Manipulation of oviposition patterns of the parasitoid *Cyzenis albicans* (Tachinidae) in the field using plant extracts. Journal of Insect Behaviour 2:487-503.

Roland, J., Denford, K. E., and Jimenez, L. 1995. Borneol as an attractant for *Cyzenis albicans*, a tachinid parasitoid of the winter moth, *Operophtera brumata* L. (Lepidoptera:Geometridae). The Canadian Entomologist 127:413-421.

Roth, J. P., King, E. G. and Thompson, A. C. 1978. Host location behaviour by the tachinid, *Lixophaga diatraeae*. Environmental Entomology 7:794-798.

Sippell, W. L. 1962. Outbreaks of the forest tent caterpillar, *Malacosoma disstria* Hbn., a periodic defoliator of broad leafed trees in Ontario. The Canadian Entomologist 94:408-416.

Thompson, A. C., Roth, J. P. and King, E. G. 1983. Larviposition kairomone of the tachinid *Lixophaga diatraeae*. Environmental Entomology 12:1312-1314.

Vinson, S. B. 1975. Biochemical evolution between parasitoids and their hosts. In: Price, P. W. (ed.) Evolutionary Strategies of Parasitic Insects and Mites. New York: Plenum Press.

Vinson, S. B. 1976. Host selection by insect parasitoids. Annual Review of Entomology 21:109-133.

Weseloh, R. M. 1980. Host recognition behaviour of the tachinid parasitoid, *Compsilura concinnata*. Annals of the Entomological Society of America 73:593-601.

Witter, J. A. and Kulman, H. M. 1972. A review of the parasites and predators of tent caterpillars (*Malacosoma* spp.) in North America. Minnesota Agriculture Experiment Station Technical Bulletin 289:48 pp.

Witter, J. A. and Kulman, H. M. 1979. The parasite complex of the forest tent caterpillar in northern Minnesota. Environmental Entomology 8:723-731.

Table 1.1 Tachinid parasitoids that attack important pest species.

Tachinid Parasitoid	Host insect	Source(s)
Archtas marmoratus	corn insects; *Heliothis zea* & *Spodoptera frugiperda*	Sabrosky 1978
Bessa harveyi	larch sawfly *Pristophora erichsonii*	Turnock 1978
Blepharipa pratensis	gypsy moth *Lymantria dispar*	Shields 1976
Compsilura concinnata	gypsy moth *Lymantria dispar*	Weseloh 1980
Cyzenis albicans	winter moth, *Operophtera brumata*	Embree & Sisojevic 1965
Drino bohemica	Numerous sawflies	Monteith 1963
Eucarcelia rutilla	*Bupalus piniarius*	Herrebout et al. 1969
Eucelatoria spp.	bollworm, *Heliothis zea* tobacco worm, *Heliothis virescens*	Jackson et al. 1969, Nettles Jr. 1981
Eumasicera sp.	butterfly, *Cissia confusa*	DeVries 1984
Euphasiopteryx ochracea	cricket, *Gryllus integer*	Cade 1975
Leschenaultia adusta	salt marsh caterpillar, *Estigmene acrea*	Taylor 1954
Leschenaultia exul	Tent caterpillars; *Malacosoma americanum, M. disstria, M. pluviale*	Bess 1936, Sippell 1957
Lixophaga diatraeae	sugarcane borer, *Diatraeae saccharalis*	Roth et al. 1978
Myiopharus doryphorae	Colorado potato beetle, *Leptinotarsa decemlineata*	Kelleher 1960
Omotoma fumiferanae	spruce budworm, *Choristoneura fumiferana*	Coppell & Smith 1957
Parasitigena silvestris	gypsy moth, *Lymantria dispar*	Weseloh 1976
Patelloa pachpyga	Forest tent caterpillar, *Malacosoma disstria*	Sippell 1957
Sturmiopsis inferens	sugarcane shoot borer, *Chilo infuscatellus*	David et al. 1981
Voria ruralis	cabbage looper, *Trichoplusia ni*	Elsey & Rabb 1970
Winthemia fumiferanae	spruce budworm, *Choristoneura fumiferana*	Hebert et al. 1990
Zenillia libatrix	gypsy moth, *Lymantria dispar*	Dowden 1934

Chapter 2 Parasitoid Attraction

2.1 Introduction

The forest tent caterpillar (FTC) *Malacosoma disstria* (Hübner) (Lepidoptera:Lasiocampidae) is a significant defoliator of forest trees in North America (Hodson 1941). The principal host tree is aspen poplar, *Populus tremuloides* Michx., but at high densities the caterpillars will also feed on other tree and shrub species such as other poplars, elm, green ash, mayday, bur oak, and birch (Cerezke 1991). In the aspen parkland region of central Alberta the caterpillars defoliate primarily aspen poplar and balsam poplar, *Populus balsamifera* L., in decreasing order of abundance (Roland & Taylor 1995). Outbreaks of this insect are in part suppressed by natural enemies such as sarcophagid fly parasitoids, tachinid fly parasitoids, viral pathogens, and various predators (Witter & Kulman 1972, 1979).

At least 52 species of Diptera are known to parasitize tent caterpillar larvae and pupae in North America; 41 of these species are tachinids (Witter & Kulman 1972). In Canada, the predominant tachinid parasitoids attacking late-instar FTC larvae are *Leschenaultia exul* (Townsend) and *Patelloa pachypyga* (Aldrich & Webber) (Sippell 1957, Parry 1994). Both of these tachinids are univoltine and lay microtype eggs on foliage on which the host caterpillars feed. *L. exul* is widely distributed throughout North America (Bess 1936, Brooks 1946). *P. pachypyga* has a more restricted geographic range existing primarily in northeastern North America (Thompson 1953, Arnaud 1978).

L. exul is a specialist parasitoid, being restricted to only three species of lepidopteran larvae: *Malacosoma americanum*, *M. disstria*, and *M. pluviale* (Sippell 1957, Arnaud

1978, Parry 1994). *P. pachypyga* has an even more restricted host range, normally attacking only *M. disstria* (Sippell 1957, Witter & Kulman 1972, Arnaud 1978, Parry 1994). Both of these tachinids commonly parasitize up to 25% of late instar larvae in aspen feeding populations (Sippell 1957, Witter & Kulman 1979, Parry 1995, Roland & Taylor 1995). However, these percentages can vary greatly among years and locations (Bess 1936).

To be successful, these parasitoids must first locate the host's habitat (Vinson 1976). The flies do not attack the host caterpillars directly but, instead, lay their eggs on damaged foliage near feeding host larvae. Eggs are ingested by the host and hatch inside the host's gut. Neonate larvae move to the salivary glands, and then migrate to the haemocoel where they complete development (Bess 1936, O'Hara 1985). Maggots emerge from the host, drop to the ground, and pupate directly beneath the host tree where adult flies emerge the following spring. Habitat selection may therefore seem superfluous since these parasitoids pupate and subsequently emerge from beneath the host tree. However, parasitoids commonly travel to other locations to nectar feed, to mate, and to mature eggs (Herrebout 1967), and their hosts move from one host plant species to another.

Odours from both the host and the hosts' food plants are important host-finding cues used by insect parasitoids (Vet & Dicke 1992). Because *L. exul* and *P. pachypyga* are leaf ovipositors, different host plant species may influence the herbivore-parasitoid interaction from initial attraction of the parasitoids to parasitism of the host. Bess (1936) observed *L. exul* females ovipositing on wild cherry trees near feeding tent caterpillar larvae. Sippell (1957) noted that wherever large numbers of caterpillars could be found, *L. exul* could be found on the leaves of aspen poplar trees. Despite the importance of the leaf ovipositing fly *P. pachypyga*, its behaviour and chemical ecology are completely unknown.

It is unknown whether the parasitoids are attracted to the host caterpillar or the host's primary food plant (aspen poplar). It is also unknown whether the parasitoids are attracted to host-feeding only on the principal host plant (aspen poplar) or on any plant species. The only studies conducted on host-finding by a leaf-ovipositing tachinid parasitoid, *Cyzenis albicans* (Fall.) demonstrated that this fly is attracted to odours from oak trees damaged by the winter moth *Operophtera brumata* but not apple trees (Roland et al. 1989, Roland et al. 1995).

I hypothesize that adult *L. exul* and *P. pachypyga* locate FTCs by detecting a volatile chemical cue emanating from larval-damaged aspen poplar leaves (the principal host plant of the herbivorous host larvae). By using this chemical stimulus, flies could determine that actively feeding caterpillars are nearby and that foliage is available on which to oviposit. Responses of the parasitoids to host habitat stimuli were evaluated using two bioassays: (1) wind tunnel choice experiments, so that all sources of both visual and olfactory stimuli could be experimentally controlled and (2) field bioassays of fly attraction to host trees with varying levels of FTC larvae and host plant defoliation, so that parasitoid behaviour could be evaluated under natural conditions where a complex array of stimuli are present.

2.2 Materials and methods

2.2.1 Wind tunnel bioassay

Adult *L. exul* were collected on 10 May 1994 from aspen forests in the Ministik Hills, approximately 30 km east of the city of Edmonton, Alberta, Canada. Forest at this site consists mainly of aspen poplar, *Populus tremuloides* interspersed with, in descending

order of abundance, balsam poplar, *Populus balsamifera*, paper birch *Betula papyrifera*, and white spruce *Picea glauca* (Roland & Taylor 1995). Trees at this site showed a range of FTC defoliation from light to extremely heavy.

Flies were collected when they were either mating on exposed substrates such as fallen logs, roads, tall grass blades in clearings, or while nectar feeding on buffaloberry (*Shepherdia canadensis*). This shrub is unique in that it flowers before it leafs out, thereby providing an abundant source of nectar in the aspen poplar understory in springtime when there are virtually no other nectar sources available (Noble 1985).

Flies were maintained for approximately two weeks (16 days) in a controlled environment chamber at 23 °C during the day and 13 °C at night, with a corresponding photoperiod of L16:D8, and a relative humidity of 60%. Flies were fed fresh honey, powdered milk, sugar, and water *ad libitum*. Males and females were kept together in the cages to ensure that females were mated and gravid.

Caterpillars were collected in the field from aspen poplar foliage in the Ministik Hills area. FTCs were maintained in the laboratory and supplied with fresh aspen foliage daily. Aspen poplar and balsam poplar foliage used in the bioassays were collected every second day and were kept in water to keep them fresh.

The flies' responses to test items were evaluated in a variable-speed wind tunnel. The tunnel was 50 cm x 50 cm in section, and 200 cm in length and was made of clear Plexiglas. Air was drawn through the tunnel at a very slow rate of speed (0.3 m/s), simply to waft the volatiles towards the flies and not to create wind, *per se*. The floor of the tunnel was lined with medium brown paper held down with large stones. The paper and stones were used to provide the flies with reference points while flying towards test

items. All wind tunnel testing was performed between 08:00 and 17:00 h to avoid testing flies at times when they would not normally be active (Bess 1936).

Test items were located on separate sides of the wind tunnel, in the farthest upwind section. For each test, one fly was introduced through a hole in the top of the downwind section of the tunnel. Each fly was then permitted 25 minutes to begin searching. At 1, 2, 5, 10, 15, 20, and 25 minutes after the fly had entered the wind tunnel, its position was recorded by the number of the quadrant in which it was located (Figure 2.1). At the end of each 25 minute test, the fly was given the opportunity to rest and the wind tunnel was allowed to exhaust residue odours for approximately 5 minutes while the next pair of test items were placed inside the tunnel. The same fly was then exposed to the next pair of test items. This procedure continued until each fly was exposed to all pairs of test items. All choices were presented to the flies in a random order and the positions of the similar items were alternated between sides of the tunnel between tests.

Nineteen flies were selected at random and presented with each of the following paired choices:

(a) Undamaged aspen leaves vs. Undamaged balsam leaves -- Even though both tree species are fed on by caterpillars, aspen is the preferred host plant. Flies should not be attracted to leaves without feeding damage, as this would be an inefficient host-finding strategy. I predicted that the flies would not prefer undamaged aspen compared to undamaged balsam leaves in choice tests.

(b) Aspen leaves with FTC damage vs. Aspen leaves cut with scissors (mechanical damage) -- Herbivore damaged plants emit specific chemical cues indicating the presence of larval feeding damage, while mechanical damage releases non-specific chemical cues

(Turlings et al. 1993). Both types of damage are visually similar, so this pairing estimates only olfactory attraction. I predicted that flies would prefer the side of the wind tunnel containing caterpillar damaged leaves as compared to mechanically damaged leaves, as the former would be more indicative of feeding larvae and would result in a more efficient host-finding strategy.

(c) FTCs eating aspen leaves vs. FTCs eating balsam leaves -- Aspen is the preferred host plant of the FTC. Therefore, *L. exul* should be attracted to volatile cues emanating from larval-damaged aspen leaves. By having the same number of caterpillars on the two tree species I measured the parasitoids attraction to only volatile olfactory stimuli from the two plant-herbivore complexes, as larval stimuli alone did not differ between the two choices. I predicted that larval-damaged aspen poplar leaves would be more attractive than larval-damaged balsam poplar leaves.

(d) FTCs only vs. Model FTCs -- Olfactory cues from host insects are potentially attractive to tachinid flies. I compared real caterpillars to model caterpillars. Model caterpillars were manufactured from white construction paper rolled into tubes 4 cm long x 1 cm in diameter. The white paper tubes were then colored with pencil crayons to mimic the coloration patterns of FTCs. The visual stimuli between the real and model caterpillars were the same but the olfactory cues were different. I predicted that real caterpillars would be more attractive than the model caterpillars.

(e) FTC frass vs. Wet sand -- Caterpillar frass contains modified host plant compounds and could be a powerful olfactory cue for attracting *L. exul*. I compared the attractiveness of frass to wet sand. Since the two items were almost visually identical, the only difference between them was olfactory cues. I predicted that caterpillar frass would be more attractive than wet sand.

For each set of paired choices, plant specimens were placed in water-filled 500 ml glass jars (covered with brown paper to eliminate any possible visual attraction to water) and then placed in the wind tunnel. Real FTCs, model FTCs, frass, and sand were all placed on the bottom of petri dishes 10 cm in diameter and then placed on the top of the glass jars in the upwind section of the wind tunnel.

Statistical analyses. Two sets of analyses were performed: (1) to determine which side of the wind tunnel flies aggregated on, even if the flies did not move upwind, and (2) to determine which side of the wind tunnel flies were attracted to upwind. The proportion of sample points that each fly was observed searching on each side of the wind tunnel was compared using the nonparametric Sign test. A fly having more sample points on the side of the wind tunnel containing treatment 'x' was assigned a '+', while a fly having more sample points on the side of the tunnel containing treatment 'y' was assigned a '-'.

2.2.2 Field bioassay

Fly behaviour was observed in the canopies of small trees with varying levels of defoliation and host caterpillar abundance. Aspen poplar and balsam poplar trees, 2 - 5 m tall, were chosen at random for the experiment from an area 200 m x 320 m on the corner of Range Road 212 and Ministik Estate Road at Ministik Hills. Small trees were used to facilitate observations of insects throughout the tree canopy. For each tree the number of FTCs was recorded along with the approximate percent defoliation. During the following 15 minute interval, the number of *L. exul* and *P. pachypyga* entering the tree canopy was recorded. *L. exul* were easily identified due to their large size and characteristic zig-zag flight pattern. *P. pachypyga* were difficult to distinguish from *Arachnidomyia aldrichi* (Diptera:Sarcophagidae) except that *P. pachypyga* have much darker eyes than do *A. aldrichi*. These two species were easily distinguished once they had landed on the tree.

Fifteen minute samples were made repeatedly, alternating between aspen poplar and balsam poplar trees. No tree was observed more than once. Observations were made on 4 different days between 30 May and 8 June 1995 at various times (however, all between the times of 08:00 and 17:00 hours) and at various temperature and light conditions.

Statistical analyses. A total of 48 aspen poplar and 44 balsam poplar trees were selected for this field bioassay. The number of flies entering the tree canopies was compared using a 2-factor analysis of variance, with the main factors being tree species and fly species. Stepwise multiple regression was then conducted on the data to determine how well factors such as date of observation, fly species, tree species, defoliation (arcsin transformed), and FTC larval abundance accounted for the variation in fly attraction. The overall regression was then further collapsed to determine how much each of the factors influenced attraction of different fly species on the two different host plant species.

2.3 Results

2.3.1 Wind tunnel bioassay

No significant difference was found in the number [illegible] *l* on the side of the wind tunnel containing undamaged aspen poplar leaves versus undamaged balsam poplar leaves (P=0.36) (Figure 2.2). No difference was found in the number of flies on the side with caterpillar damaged aspen poplar leaves compared to mechanically damaged aspen poplar leaves (P=1.00) (Figure 2.2). Significantly more flies were on the side of the wind tunnel with caterpillars actively feeding on aspen poplar leaves versus caterpillars eating balsam poplar leaves (P=0.019) (Figure 2.2). No preference was shown for caterpillars only versus model caterpillars (P=1.00) (Figure 2.2). The flies preferred the side of the

wind tunnel containing caterpillar frass (from feeding on aspen) versus wet sand (P=0.019) (Figure 2.2).

Among *L. exul* that moved upwind, there was no significant difference between undamaged aspen poplar leaves and undamaged balsam poplar leaves (P=0.55) (Figure 2.3). No difference was found between caterpillar damaged aspen poplar leaves and mechanically damaged aspen poplar leaves (P=1.00) (Figure 2.3). Significantly more flies moved upwind towards caterpillars actively feeding on aspen poplar leaves compared to caterpillars feeding on balsam poplar leaves (P=0.012) (Figure 2.3). No difference was found between FTCs only versus model FTCs (P=1.00) (Figure 2.3), nor was there any difference between caterpillar frass (from aspen poplar) versus wet sand (P=0.22) (Figure 2.3).

2.3.2 Field bioassay

More *L. exul* and *P. pachypyga* entered the canopies of aspen poplar trees compared to the canopies of balsam poplar trees (F=5.87, df=1, P=0.016). A significantly greater number of *P. pachypyga* flies were present in the field than *L. exul* flies (F=37.45, df=1, P=0.0001), but there was no significant interaction between tree species and fly species (F=2.4, df=1, P=0.12) (Figure 2.4). Both fly species preferred aspen poplar.

Stepwise multiple regression included the effects of date, defoliation and larval abundance in addition to tree species on tachinid abundance. The overall regression identified 2 significant factors; date (coefficient=-0.16, P=0.036) as the number of flies observed decreased slightly from the first observation period to the final observation period, and fly species (coefficient=1.45, P=0.0001) indicating more *P. pachypyga* than *L. exul* in the canopies (overall model; F=13.13, P=0.0001, r^2=0.23).

For *L. exul*, the only significant factor was larval abundance (coefficient=0.00273, P=0.0072) as the number of flies entering the tree canopies increased slightly when the number of larvae increased irrespective of the tree species they were feeding upon, (overall model; F=5.6, P=0.0015, r^2=0.16). This may reflect the correlation between larval abundance and the level of recent defoliation rather than total defoliation. It is unlikely that parasitoids were visually attracted to the caterpillars because the majority of the larvae were clustered on the tree trunks and in many cases were hidden by the canopy leaves of the tree. These larvae would not have been visible to the parasitoids until the flies had already entered the tree canopies. For *P. pachypyga*, tree species was the only significant factor (coefficient=-0.92, P=0.0445) with aspen trees attracting more flies than balsam poplar trees irrespective of defoliation level or host caterpillar abundance (overall model; F=3.82, P=0.026, r^2=0.08).

2.4 Discussion

It has been proposed that host specialists are more likely to have an innate response to stimuli that directly indicate the presence of the host or the host's habitat than are generalists (Vet et al. 1983, Waage 1979). From the available evidence it appears that *L. exul* and *P. pachypyga* are specialists that respond to cues from the hosts habitat, although each species responds to slightly different cues.

In the wind tunnel experiment *L. exul* oriented to and flew upwind towards caterpillars eating aspen poplar leaves preferentially over FTCs eating balsam poplar leaves, indicating plant volatiles or the herbivore-aspen complex as the attractive stimuli. The flies also aggregated in an airstream containing frass volatiles, once again implicating plant compounds or the host insect-plant complex as attractive stimuli. From field

observations, however, it can be inferred that this fly species is attracted to recent leaf damage or the herbivore-host plant complex irrespective of the tree species upon which it occurred. This is not the first time that discrepancies between laboratory and field studies have been described for a tachinid parasitoid. Roth et al. (1978) found that sugarcane plants were attractive to the tachinid parasitoid *Lixophaga diatraeae* in field cage tests but not in olfactometer tests. Discrepancies such as these emphasize the need to corroborate laboratory studies with field studies. If a parasite is exposed to individual stimuli, its responses may differ from that induced by two or more stimuli presented simultaneously (Monteith 1958). The species of food plant of the host, the food plants of alternate host caterpillars, and the close proximity of non-food or alternate food plants have all been found to influence host-searching by tachinid parasitoids (Monteith 1960).

There are several potential explanations for the different results obtained in the wind tunnel experiments and the field observational studies. First, aspen poplar and balsam poplar branches that had been cut off of trees (and hence suffered some damage) were used for attraction studies in the wind tunnel. Cut branches may differ in both the quality and quantity of volatile organic compounds released, as opposed to intact plant specimens (Roth et al. 1978). Secondly, the number of caterpillars on the aspen and balsam specimens in the wind tunnel did not differ, but there may have been more defoliation on the aspen poplar leaves because it is the preferred host plant of the caterpillars (Hildahl 1977). This again implicates volatiles arising from recent defoliation or the herbivore-host plant complex as the attractive stimuli. Therefore, both the wind tunnel experiments and observational bioassays can be interpreted as indicating that *L. exul* is attracted to recent plant defoliation or the herbivore-host plant complex irrespective of the tree species upon which it occurs.

P. pachypyga showed a more clear cut pattern. This species uses aspen poplar trees as an initial host-finding cue regardless of level of defoliation or larval abundance. Some parasitoids are thought to seek out a particular habitat before they detect the presence of hosts (Cushman 1926, Salt 1935). Nettles (1980) gave one of the first definitive reports of a tachinid (*Eucelatoria* sp.) that is more strongly attracted to the food plant cotton vs. non-host okra plants, than it is to the host caterpillars (*Heliothis virescens*, *Spodoptera eridania*, and *Estigmene acrea*).

By responding to recent host plant damage and not limiting its host searching to only one plant species, *L. exul* would still be able to find host larvae dispersing from aspen poplar trees to other host plants such as balsam poplar trees. Because it attacks at least 3 species of *Malacosoma* sp., recent larval damage on different tree species may be an effective host-finding strategy for *L. exul*. Responding to leaf damage irrespective of tree species may at least partially account for the flies' success in different geographic areas where the caterpillars feed on different species of food plants, e.g. wild cherry trees in New England states (Bess 1936).

In contrast, by being attracted to only one plant species, *P. pachypyga* focuses its host searching efforts on only the principal host plant of the FTC, aspen poplar. Responding to larval damage on only one tree species may be an effective host-finding strategy for *P. pachypyga* as it normally attacks only *M. disstria* (see introduction). This would be an effective host-finding strategy in an area dominated by aspen poplar trees; however, the host-finding strategy would tend to serve less well in areas where alternative FTC food sources are more abundant. Whether these attractive stimuli result in different oviposition and parasitism rates by *L. exul* and *P. pachypyga* on aspen and balsam poplar trees has yet to be determined and will be the topics of chapters 3 and 4.

2.4.1 Conclusion

L. exul and *P. pachypyga* locate their host caterpillars using different cues. *L. exul* appears to locate FTCs by using recent defoliation or the herbivore-host plant complex as an initial host-finding mechanism. In contrast, *P. pachypyga* cues in on aspen trees to find FTC larvae. Even though I have attempted to answer a few of the questions regarding how the tachinids *L. exul* and *P. pachypyga* locate their host caterpillars, many more questions remain to be answered. The FTC, tachinid parasitoid, poplar tree tritrophic interaction is complex. Further behavioural studies of both host insects and parasitoids will enable us to discern how tachinid parasitoids contribute to the natural suppression of FTC outbreaks and the parasitoids' role in endemic populations.

2.5 Literature cited

Arnaud Jr., P. H. 1978. A host-parasite catalog of North American Tachinidae (Diptera). USDA Miscellaneous Publications 1319:860 pp.

Bess, H. A. 1936. The biology of *Leschenaultia exul* Townsend, a tachinid parasite of *Malacosoma americana* Fabricius and *Malacosoma disstria* Hübner. Annals of the Entomological Society of America 29:593-613.

Brooks, A. R. 1946. A revision of the North American species of *Leschenaultia* Sens. Lat. (Diptera:Larvaevoridae). The Canadian Entomologist 78:169-182.

Cerezke, H. F. 1991. Forest Tent Caterpillar. Forestry Canada, Northwest Region, Northern Forestry Centre, Edmonton, Alberta. Forestry Leaflet Number 10.

Cushman, R. A. 1926. Location of individual hosts versus systematic relation of host species as a determining factor in parasitic attack. Proceedings of the Entomological Society of Washington 28:5-6.

Herrebout, W. M. 1967. Habitat selection in *Eucarcelia rutilla* Vill. (Diptera:Tachinidae) I. Observations on the occurrence during the season. Zeitschrift fur Angewandte Entomologie 60:219-229.

Hildahl, V. 1977. Forest Tent Caterpillar. Northern Forest Research Centre, Edmonton, Alberta. 4 pp.

Hodson, A. C. 1941. An ecological study of the forest tent caterpillar, *Malacosoma disstria* Hbn., in Northern Minnesota. Minnesota Agriculture Experiment Station Technical Bulletin 148:55 pp.

Monteith, L. G. 1958. Influence of food plant of host on attractiveness of the host to tachinid parasites with notes on preimaginal conditioning. The Canadian Entomologist 90:478-482.

Monteith, L. G. 1960. Influence of host plants other than the food plants of their host on host-finding by tachinid parasites. The Canadian Entomologist 92:641-652.

Nettles Jr., W. C. 1980. Adult *Eucelatoria* sp.: Response to volatiles from cotton and okra plants and from larvae of *Heliothis virescens*, *Spodoptera eridania*, and *Estigmene acrea*. Environmental Entomology 9:759-763.

Noble, W. 1985. *Shepherdia canadensis*: its ecology, distribution, and utilization by the grizzly bear. Unpublished manuscript on file at US Department of Agriculture/Forest Service, Intermountain Research Station, Fires Sciences Laboratory, Missoula, Montana, 29 pp.

O'Hara, J. E. 1985. Oviposition strategies in the Tachinidae, a family of beneficial parasitic flies. Agriculture and Forestry Bulletin 8:31-34.

Parry, D. 1994. The impact of predators and parasitoids on natural and experimentally created populations of forest tent caterpillar, *Malacosoma disstria* Hübner (Lepidoptera:Lasiocampidae). M.Sc. Thesis. University of Alberta, Edmonton, Alberta, Canada. 91 pp.

Parry, D. 1995. Larval and pupal parasitism of the forest tent caterpillar, *Malacosoma disstria* Hübner (Lepidoptera:Lasiocampidae), in Alberta, Canada. The Canadian Entomologist 127:877-893.

Roland, J., Evans, W. G. and Myers, J. H. 1989. Manipulation of oviposition patterns of the parasitoid *Cyzenis albicans* (Tachinidae) in the field using plant extracts. Journal of Insect Behaviour 2:487-503.

Roland, J., Denford, K. E., and Jimenez, L. 1995. Borneol as an attractant for *Cyzenis albicans*, a tachinid parasitoid of the winter moth, *Operophtera brumata* L. (Lepidoptera:Geometridae). The Canadian Entomologist 127:413-421.

Roland, J. and Taylor, P. D. 1995. Herbivore - natural enemy interactions in fragmented and continuous forests. In: Cappuccino, N. and Price, P. W. (eds.). Population Dynamics: New Approaches and Synthesis. San Diego, California: Academic Press Inc.

Roth, J. P., King, E. G. and Thompson, A. C. 1978. Host location behaviour by the tachinid, *Lixophaga diatraeae*. Environmental Entomology 7:794-798.

Salt, G. 1935. Experimental studies in insect parasitism. III. Host selection. Proceedings of the Royal Society of London (B). 117:413-435.

Sippell, W. L. 1957. A study of the forest tent caterpillar *Malacosoma disstria* Hbn. and its parasite complex in Ontario. Ph.D. Thesis. University of Michigan, Ann Arbor, Mich. 147 pp.

Thompson, W. R. 1953. The tachinid parasites of *Archips cerasivorana* Fitch. (2) *Eusisyropa blanda* O. S. (Diptera). The Canadian Entomologist 85:393-404.

Turlings, T. C. J., McCall, P. J., Alborn, H. T., and Tumlinson, J. H. 1993. An elicitor in caterpillar oral secretions that induces corn seedlings to emit chemical signals attractive to parasitic wasps. Journal of Chemical Ecology 19:411-425.

Vet, L. E. M. and Dicke, M. 1992. Ecology of infochemical use by natural enemies in a tritrophic context. Annual Review of Entomology 37:141-172.

Vet, L. E. M., van Lenteren, J. C. V., Heymans, M. and Meelis, E. 1983. An airflow olfactometer for measuring olfactory responses of hymenopterous parasitoids and other small insects. Physiological Entomology 8:97-106.

Vinson, S. B. 1976. Host selection by insect parasitoids. Annual Review of Entomology 21:109-133.

Waage, J. K. 1979. Foraging for patchily-distributed hosts by the parasitoid *Nemeritis canescens*. Journal of Animal Ecology 48:353-371.

Witter, J. A. and Kulman, H. M. 1972. A review of the parasites and predators of tent caterpillars (*Malacosoma* spp.) in North America. Minnesota Agriculture Experiment Station Technical Bulletin 289:48 pp.

Witter, J. A. and Kulman, H. M. 1979. The parasite complex of the forest tent caterpillar in northern Minnesota. Environmental Entomology 8:723-731.

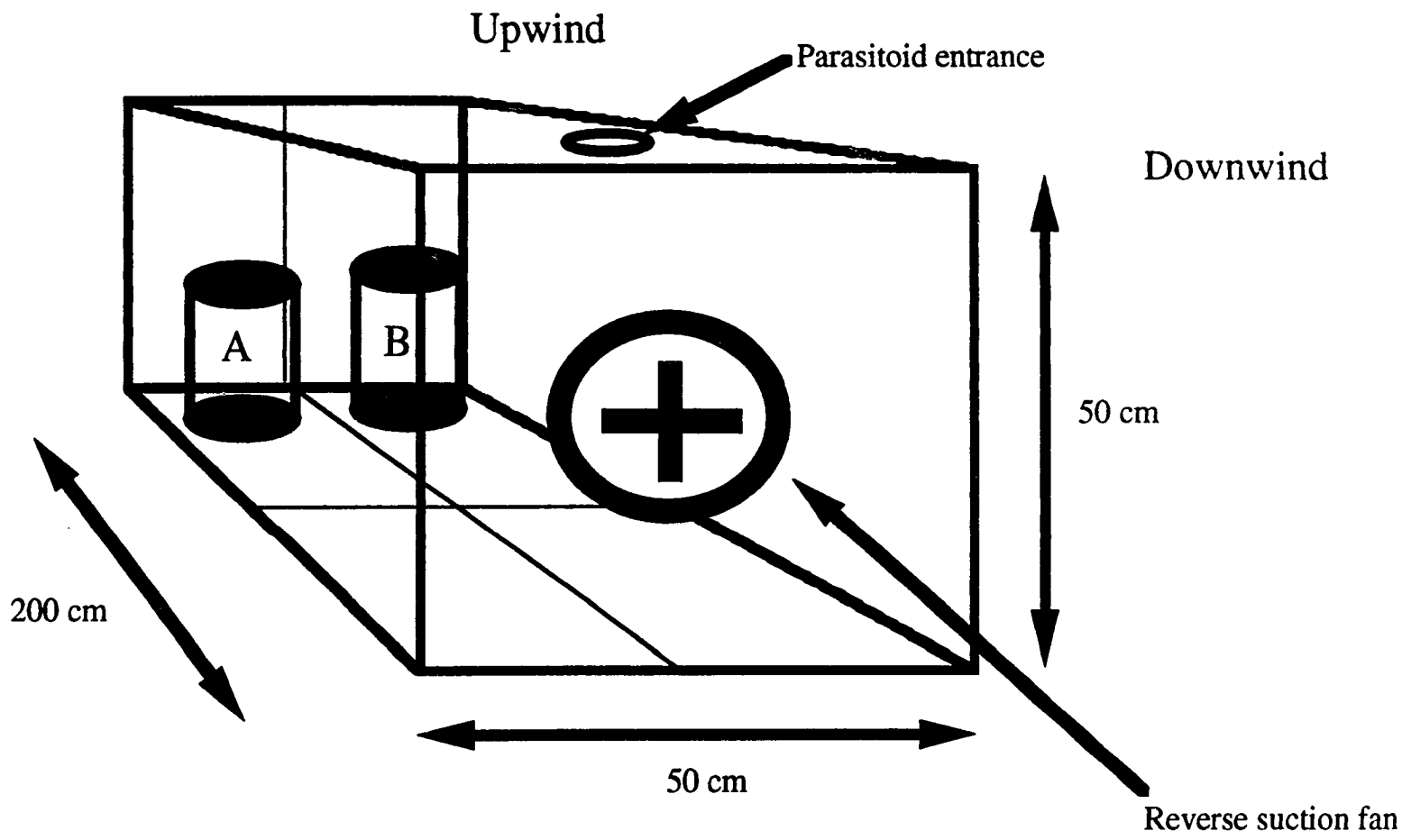

Figure 2.1. Plexiglas wind tunnel used for *L. exul* paired choice tests between different insect/plant combinations. 'A' and 'B' are the positions of the two items during a paired choice test. Lines on the floor of the wind tunnel delineate left/right and upwind/downwind quadrants.

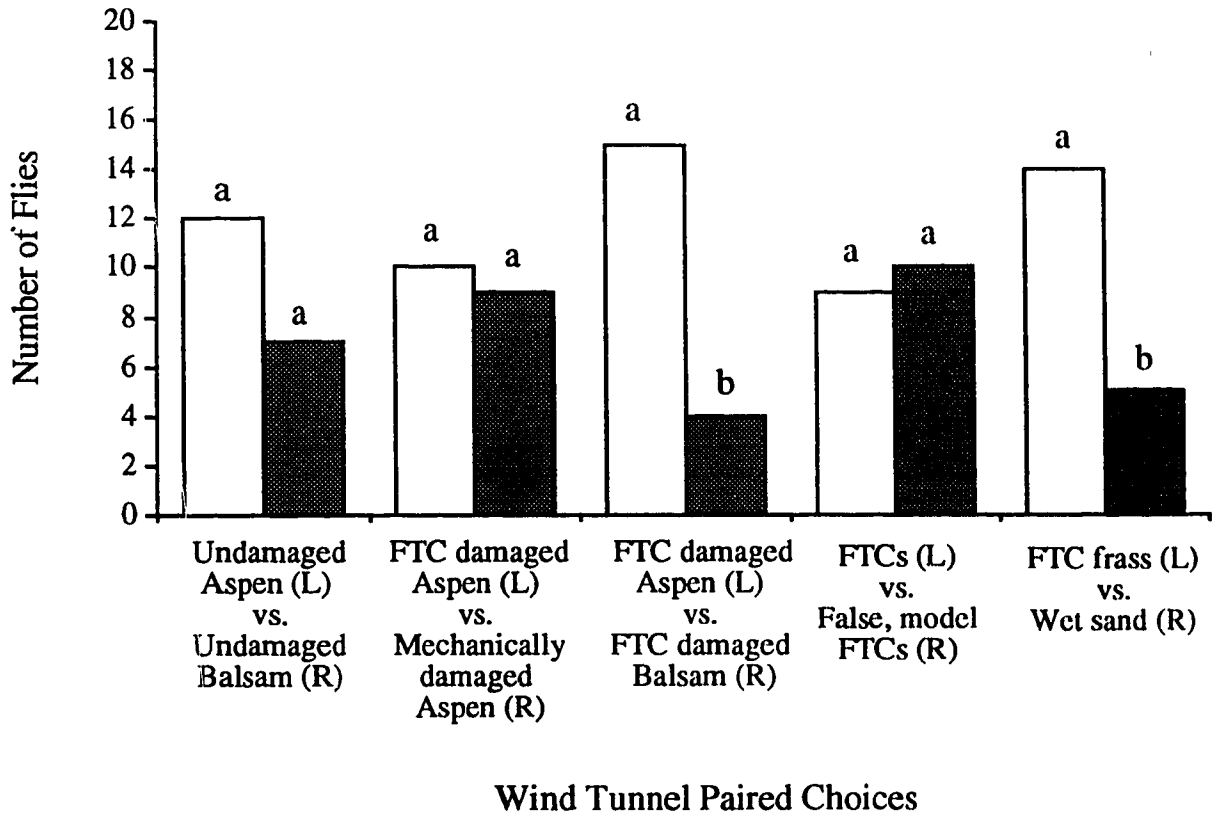

Figure 2.2. Number of *L. exul* aggregating on the left (L) or right (R) side of the wind tunnel for each pair of treatments. Within each choice test, columns followed by different letters are significantly different from each other (Sign test $P<0.05$).

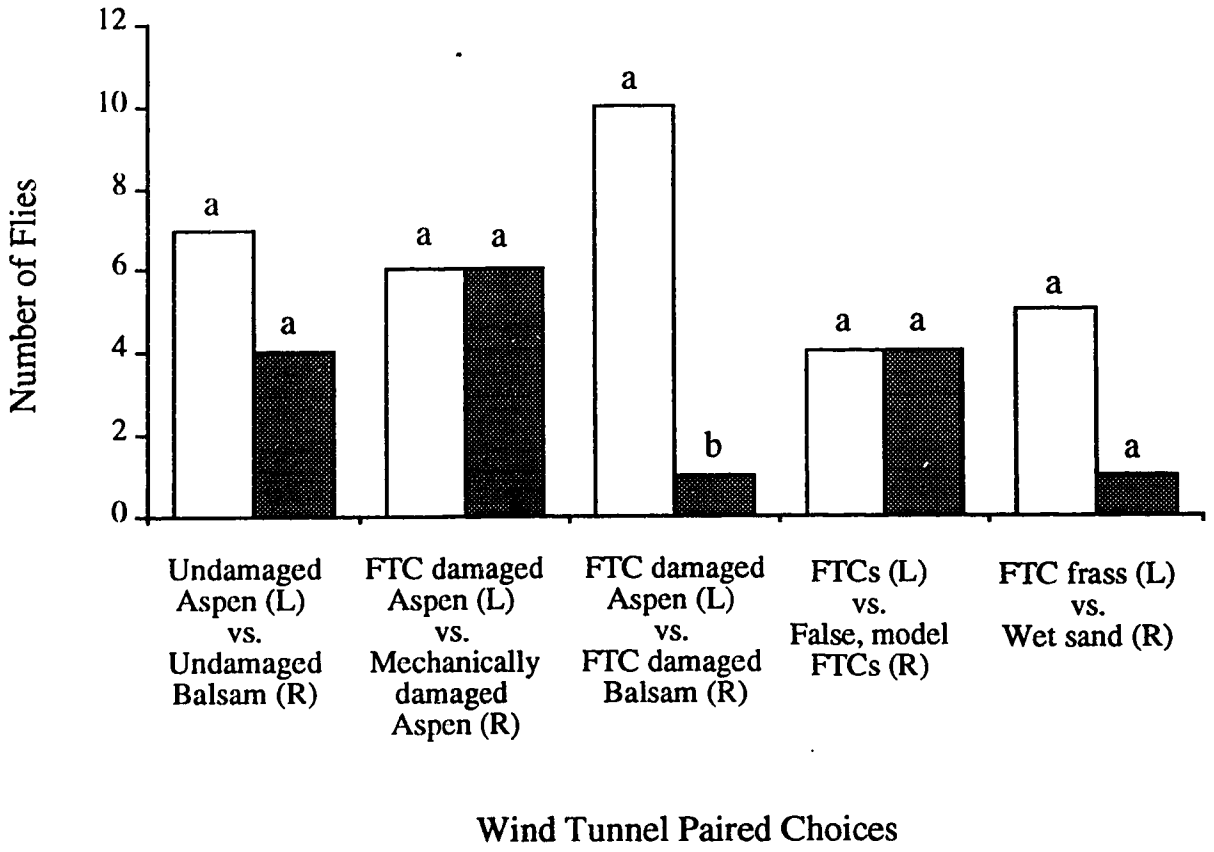

Figure 2.3. Number of *L. exul* moving upwind on the left (L) or right (R) side of the wind tunnel for each pair of treatments. Within each choice test, columns followed by different letters are significantly different from each other (Sign test $P<0.05$).

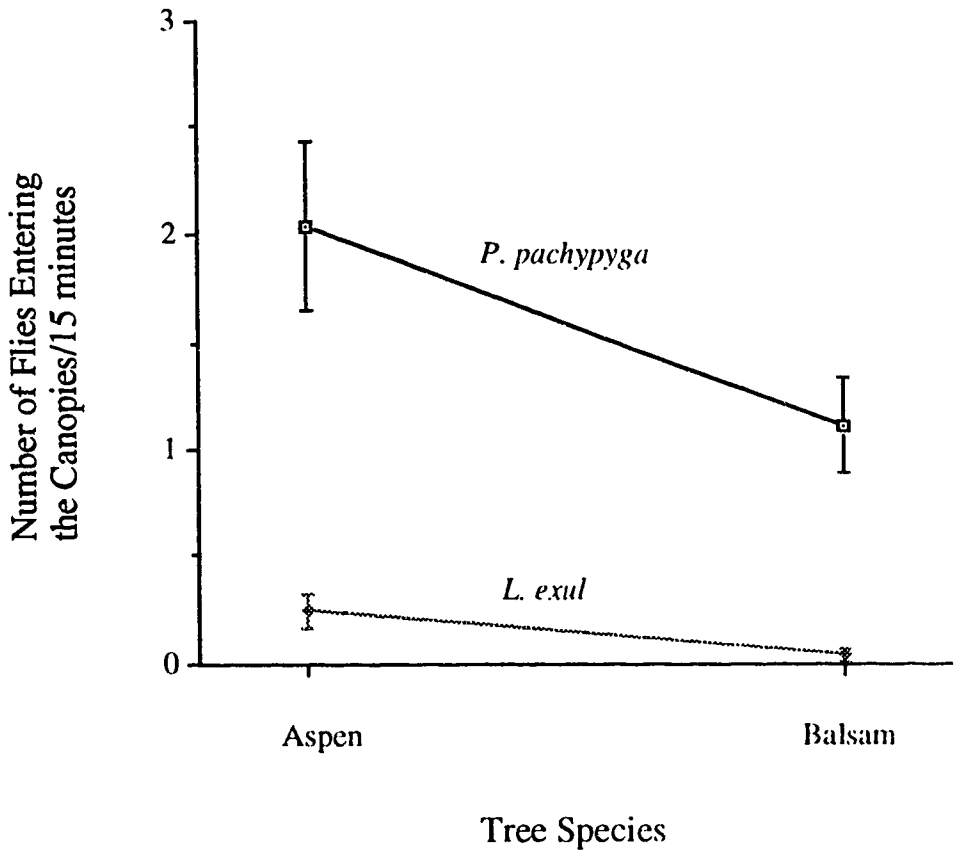

Figure 2.4. Mean number (+/- SE) of *L. exul* and *P. pachypyga* flies entering the canopy of aspen poplar and balsam poplar trees during 15 minute field observations. There is no significant interaction between fly species and tree species (F=2.4, df=1, P=0.12).

Chapter 3 Parasitoid Oviposition Strategies

3.1 Introduction

Dipteran parasites, particularly tachinids, are important natural enemies of some of our most important insect pests such as the tobacco worm *Heliothis virescens* (Nettles 1982) and the sugarcane borer *Diatraea saccharalis* (Roth et al. 1982). Many studies have examined the process of parasitoid attraction but we know little about subsequent behaviours. Four stages of host location (Vinson 1976) are recognized: (1) host habitat location, (2) host location, (3) host acceptance, and (4) host suitability (parasitism). Parasitoid search is difficult to study in the field, and most studies focus on the outcome of searching behaviour, the spatial distribution of parasitism (e.g. Morrison & Strong 1980). Detailed behavioural observation is necessary however to elucidate the mechanisms producing such patterns.

Forest tent caterpillar (FTC), *Malacosoma disstria* (Hübner) (Lepidoptera:Lasiocampidae), a significant defoliator of aspen poplar (*Populus tremuloides* Michx.) and other tree and shrub species such as balsam poplar (*Populus balsamifera* L.) (Hodson 1941, Cerezke 1991), is attacked by two leaf-ovipositing tachinid parasitoids *Leschenaultia exul* and *Patelloa pachypyga*. In Chapter 2, I demonstrated that the tachinid parasitoid *L. exul* is attracted to recent defoliation or the herbivore-host plant complex on both aspen poplar and balsam poplar trees. The oviposition preference of this fly and the searching behaviour leading to oviposition are not known. Bess (1936, 1937) conducted the only studies on the oviposition behaviour of *L. exul*. Flies reared from *M. americanum* larvae oviposited on foliage when the ends of cherry leaves were clipped or *M. americanum* larvae were placed within their cage. Once

oviposition began, it continued without further clipping of leaves or presentation of host larvae. Oviposition was attributed to the odor liberated by either the host or host plant.

Leaf-ovipositing tachinids with microtype eggs rely on contact cues from damaged host plants to induce oviposition. The gypsy moth parasitoid *Zenillia libatrix* oviposits on almost any type of leaf if the leaf edges have been fed upon or damaged (Dowden 1934). Leaf damage on oak trees and the resulting exudate stimulate oviposition by *Cyzenis albicans* (Hassell 1968). Oviposition by *C. albicans* in response to contact oviposition cues (sucrose and fructose) occurs on both apple foliage and oak foliage with leaf damage (Roland 1986).

I hypothesized that *L. exul* would oviposit on damaged leaves, irrespective of plant species. This hypothesis was tested by comparing oviposition rates on larval damaged leaf clusters from both aspen poplar and balsam poplar. Aspen poplar and balsam poplar leaves with similar levels of defoliation should be equally acceptable to the parasitoids for oviposition. A contact cue from damaged plants would result in the flies laying eggs only on the leaves of trees where damage has occurred, thereby increasing the chances of egg ingestion by host larvae and increasing subsequent parasitism of host larvae.

Based on the previous studies of leaf-ovipositing tachinid parasitoids mentioned above, I hypothesized that tarsal contact with leaf damage was the stimulus leading to oviposition. This was evaluated by conducting a sequential analysis of the behaviours involved in the search for oviposition sites by *L. exul*. Sequence analysis has been used to describe other animals' behaviour, including the organization of song in birds (Lemon & Chatfield 1971), courtship patterns in chalcid wasps (Leonard & Ringo 1978), communication in cockroaches (Fraser & Nelson 1984), grooming behaviour in mosquitoes (Walker & Archer 1988), and mating behaviour of tachinid parasitoids (Reitz & Adler 1991).

Sequence analysis has not been used to determine the sequence of searching behaviours leading to oviposition by parasitoids. Sequence analysis may help to determine similarities or differences in parasitoid searching sequences and oviposition on different host plant species.

3.2 Materials and methods

3.2.1 Host-plant oviposition experiment

Adult flies, caterpillars and plant specimens were all collected from the site described in Chapter 2. Flies were captured on 4 - 12 May 1995 and were maintained until testing in a controlled environment chamber cycling at 23 °C during the day and 13 °C at night, with a photoperiod of L16:D8, and a relative humidity of 60%. Flies were fed honey, powdered milk, sugar, and water *ad libitum*. Males and females were kept together in the cages to ensure that the female flies were gravid at the time of testing.

Forest tent caterpillars were placed on aspen poplar and balsam poplar branches in the laboratory to provide natural defoliation. Prior to fly testing, defoliated leaf clusters were removed from the branches and placed in small Erlenmeyer flasks filled with water. These flasks were then placed in 4 l cylindrical Plexiglas cages. Leaf clusters of approximately 5-7 leaves with 10-20% defoliation were used in the experiment.

Females were selected at random from the holding cages. Each of eight female flies were then placed for 24 hours in separate cages and exposed to all of the following experimental conditions in the observation chamber simultaneously: (a) an aspen poplar leaf cluster, (b) a balsam poplar leaf cluster, and (c) a cluster of 5 - 7 artificial aspen

poplar-shaped leaves made out of green construction paper. By using construction paper leaves I was able to assess, crudely, the visual impact that aspen leaves have on oviposition in the absence of olfactory or tactile stimuli of real leaves. Each female fly was tested only once.

At the end of the 24 hour interval flies were removed from the cages along with the leaf clusters. Cages were thoroughly washed between tests to eliminate any residual compounds from the fly or the plant samples. The number of eggs oviposited on each plant specimen was counted.

Statistical analyses. Flies not laying any eggs at the end of the 24 hour period were not included in the analyses. Due to heterogeneous variances in the number of eggs laid on the three substrates, counts were compared with a nonparametric one way Kruskal-Wallis analysis of variance. Paired comparisons between treatments were made using the Simultaneous Test Procedure.

3.2.2 Female search behaviour

Flies, foliage, and caterpillars were obtained from the site described in Chapter 2. Gravid female flies were placed individually in the Plexiglas cages with a single larval-damaged leaf cluster. Flies were allowed to accommodate to the cages for approximately 5 minutes. Preliminary observations indicated that this was an appropriate accommodation period; frantic flight activity ceased, and grooming behaviours began. Based on previous observation, the searching repertoire consisted of three distinct behaviours: (1) Tarsal contact with leaf damage - The fly contacted either the left or right anterior tarsal segment to recent leaf damage, (2) Oviposition - The female fly curved its abdomen down so that the tip of the abdomen contacted one of the leaves and

deposited an egg, (3) Changing leaves - The fly moved, either by short hopping flights or by walking, to another leaf in the cluster.

Recordings began from the time a fly was placed on the leaf cluster until the fly left the leaf cluster, or 10 minutes, whichever came first. This is similar to search duration used in studies of other parasitoids (Navasero & Elzen 1989). Each fly was tested sequentially up to 5 times in order to evaluate its typical searching behaviour. This procedure was then replicated 15 times with different female flies for both aspen poplar and balsam poplar leaf clusters. Behaviours were recorded on a voice activated tape-recorder and later transcribed. All observations were conducted in the laboratory at 20 °C between 09:00 and 17:00 hrs, times when the flies would normally be active in the field (Bess 1936).

Statistical Analyses. Two methods were used to analyze these data. First, search behaviours were transcribed into an ethogram (a checklist of behaviours that the flies exhibited), from which repeated measure analyses of variance 'k=2' (Zar 1984) were conducted with the main factor being tree species (aspen vs. balsam). A separate analysis of variance was conducted for; (a) number of tarsal contacts per minute, (b) number of ovipositions per minute, (c) number of leaves the fly moved to within the leaf cluster per minute, and (d) total time spent on the plant specimen. Secondly, subsamples of linear sequences of search behaviours were converted into a transitional matrix of preceding and succeeding behaviours (aspen poplar, n=35; 7 individuals, 5 replicates each and balsam poplar, n=10; 2 individuals, 5 replicates each). Behavioural transitions were then analyzed for zeroth order (behaviours occur at random, i.e. complete independence), first order (behaviour a leads to behaviour b, behaviour c leads to behaviour b, etc.), and second order (behaviour a and b combined lead to behaviour c, behaviour a and c combined lead to behaviour b, etc.) Markov chain dependence, using overall chi-square

statistics with Yates Correction for Continuity on the complete matrix (Slater 1973, Cane 1978). If the transition matrix was significant, the behavioural repetitions were tested for significance against a random model by collapsing the entire table into a 2x2 contingency table about the cell of interest. This allowed me to determine which transitions occurred significantly greater or significantly fewer times than would be expected from a random model (Appendix 1).

If at least one behaviour occurred as a repeated behaviour sequence the behavioural transitions were tested for significance using an abridged matrix eliminating repetitions of the same behaviour a (behaviour a leads to a, leads to a, etc.). Self-transitions (immediate repetitions of the same behaviour) can inflate the scores of behaviours that occur repeatedly. Eliminating self-transitions permits better estimates of behavioural transitions without being biased by behavioural repetitions (Slater 1973). The abridged matrix was then collapsed into a 2x2 contingency table about the cell of interest to determine which transitions occurred significantly greater or significantly fewer times than would be expected from the random model.

To visualize behavioural transitions, kinematic graphs of behavioural sequences were constructed from the transitional matrices by showing frequencies (that is, raw numbers) of transitions among different behavioural states. I interpreted searching sequences based upon the general structure of the kinematic graphs, as well as the significance of the various chi-square statistics.

3.3 Results

3.3.1 Host-plant oviposition experiment

The number of eggs laid by individual flies varied tremendously (Table 3.1). There were significant differences between the number of eggs laid by *L. exul* on the three leaf cluster types (H=6.32, df=2, P=0.043) with significantly more eggs laid on balsam poplar leaves than on construction paper leaves (U=25, P<0.05). All flies laid more eggs on balsam poplar than on aspen poplar leaves; however, there was no significant difference between the two tree species (U=6, P>0.05). No difference was found in the number of eggs laid on aspen poplar vs. paper leaves (U=15, P>0.05).

3.3.2 Female search behaviour

Tarsal contacts with FTC damaged leaf edges did not differ significantly between aspen poplar and balsam poplar leaf clusters (F=1.360, df=1, P=0.25) (Figure 3.1) nor did they change from early to late trials of the same individual (F=1.257, df=4, P=0.29). There was no interaction between tree species and early to late trials of the same individual (F=0.389, df=4, P=0.82).

The number of ovipositions per minute did not differ between the two tree species (F=0.014, df=1, P=0.91) (Figure 3.2). The rate of egg-laying did not differ significantly from early to late trials of the same individual (F=1.825, df=4, P=0.13), nor was there a significant interaction between tree species and time on the number of ovipositions per minute (F=0.396, df=4, P=0.81).

Gravid female flies did not change leaves at a significantly greater rate on either of the two plant species (F=1.895, df=1, P=0.18) (Figure 3.3), nor was there an effect of time on rate of changing (F=0.637, df=4, P=0.64), nor a time by tree species interaction (F=0.787, df=4, P=0.54). Gravid female flies did not spend different amounts of time on aspen or balsam poplar leaf clusters (F=0.078, df=1, P=0.78) (Figure 3.4). Flies did not differ significantly in the amount of time they spent on leaf clusters from early to late trials of the same individual (F=0.142, df=4, P=0.97). Likewise, there was no interaction between time and tree species (F=1.142, df=4, P=0.34) on the rate of moving between leaves.

3.3.3 Pattern of sequential search behaviour

The average number of behaviours per search interval was 11.00 (SE=2.86) on aspen poplar and 34.3 (SE=20.21) on balsam poplar. However, in this analysis it is not the number of behaviours we are interested in (see previous ANOVA) but rather the sequence of behaviours

3.3.3.1 First order transitions

For searching sequences on aspen poplar, the 3x3 transition matrix was analyzed as a contingency table and was found to be significant overall (χ^2=51.846, P=0.0001), thereby violating Markov zeroth order dependence (i.e. behaviours do not occur at random). This indicates the existence of at least first order dependence between pairs of behaviours. Analysis of this matrix indicated that tarsal contacts with leaf damage occurred repeatedly in sequence (χ^2=14.884, P=0.0001) as did changing leaves (χ^2=13.056, P=0.0003). Ovipositions, however, did not occur repeatedly (χ^2=3.175, P=0.075). Eliminating behavioural repetitions to better determine any pattern in

transitions indicated that tarsal contact with leaf damage preceded oviposition (χ^2=18.789, P=0.0001) more often than at random and oviposition preceded tarsal contact with leaf damage more often than at random (χ^2=9.854, P=0.0017). Interestingly, tarsal contact with leaf damage is inversely related to changing leaves (χ^2=9.674, P=0.0019), (i.e. if a fly contacts leaf damage it will either touch the leaf damage again or it will oviposit before moving to a new leaf). No other transitions were statistically significant (Figure 3.5).

The flies engage in an almost identical pattern of searching behaviour on balsam poplar leaf clusters as on aspen poplar leaf clusters. The 3x3 contingency table was found to be significant overall (χ^2=59.973, P=0.0001) indicating at least first order dependence. Tarsal contacts with leaf damage occurred as repeated sequences (χ^2=7.577, P=0.0059) as did changing leaves (χ^2=16.283, P=0.0001). Ovipositions did not occur repeatedly (χ^2=0.029, P=0.86). After eliminating behavioural repetitions, tarsal contact with leaf damage preceded oviposition (χ^2=33.609, P=0.0001) and oviposition preceded tarsal contact with leaf damage (χ^2=20.329, P=0.0001). Tarsal contact with leaf damage was once again inversely related to changing leaves (χ^2=10.479, P=0.0012). No other transitions were statistically significant (Figure 3.6).

3.3.3.2 Second order transitions

In order to evaluate the existence of Markov second order dependence, search behaviours on aspen poplar were transcribed into a 3x9 transition matrix and analyzed as a contingency table. Second order dependencies were evident (χ^2=96.9295, P=0.0001). The only behavioural repetitions that were significant involved tarsal contact with leaf damage: (1) oviposit and touch leading to touch (χ^2=15.3491, P=0.0001) and (2) change and touch leading to touch (χ^2=5.8049, P=0.016). Behavioural transitions were analyzed

only for those transitions that were non-zero in the collapsed frequency table (Zar 1984). Tarsal contact with leaf damage was once again important, with the only significant transitions being: (1) touch and touch leading to oviposition (χ^2=8.7102, P=0.0032) and (2) touch and oviposit leading to touch (χ^2=5.4806, P=0.019). These last two results again suggest the importance of repeated tarsal contact leading to oviposition and of tarsal contact with leaf damage and oviposition occurring in a cyclical relationship.

Fly behaviour on balsam poplar also showed second order dependence (χ^2=124.5094, P=0.0001). Touch and change lead to change (χ^2=6.7433, P=0.0094), oviposit and touch lead to touch (χ^2=8.5987, P=0.0034) and oviposit and change lead to change (χ^2=4.1381, P=0.042). As with aspen foliage, tarsal contact with leaf damage was important with: (1) touch and touch leading to oviposition (χ^2=18.1272, P=0.0001), and (2) touch and oviposition leading to touch (χ^2=11.3694, P=0.0007). These last two statistics again suggest that tarsal contact leads to oviposition, and that tarsal contact with leaf damage and oviposition occur in a cyclical relationship.

3.4 Discussion

3.4.1 Host-plant oviposition

Hymenopterous and dipterous parasitoids will search extensively for hosts on some plants while ignoring others (Martin et al. 1990). *L. exul* is attracted to both aspen poplar and balsam poplar larval-damaged foliage (Chapter 2) and apparently does not distinguish between aspen and balsam poplar foliage when ovipositing, confirming my first hypothesis. These results are similar to those for the gypsy moth parasitoid *Z. libatrix* , which oviposits on almost any type of leaf, if the leaf edges have been fed upon or cut

(Dowden 1934). Numbers of eggs laid by *C. albicans* on oak and apple trees do not differ significantly (Roland 1986).

There was no difference in the number of eggs laid on aspen poplar and balsam poplar leaf clusters in the choice experiment, but there was a large variance among females in the number of eggs laid. Both Odell & Godwin (1984) and Roland (1986) reported great variation in the number of eggs laid by individual females in laboratory experiments.

A trend towards higher numbers of eggs on balsam poplar leaf clusters may result from their being more resinous than aspen poplar leaves. In fact, balsam leaf clusters with no defoliation receive many fly eggs (personal observation). The chemical composition of this resin is unknown but plant sugars are known to lead tachinid flies to oviposit (Hassell 1968). A small number of eggs was found on the construction paper leaves. Laying eggs on non-host plants may be due to the stress of entering a new environment (Nair & McEwen 1976).

3.4.2 Female searching behaviour

L. exul females behave similarly on the two host plant species. Searching on aspen poplar and balsam poplar foliage consists of the following sequences: (1) Multiple tarsal contacts with leaf damage lead to a single oviposition, (2) After oviposition, the fly moves back to the region containing the leaf damage and touches leaf damage again, and (3) The fly occasionally changes leaves several times in a row and begins ovipositing when leaf damage is contacted. While I can not directly infer causation from an observational bioassay, there is a very strong correlation between tarsal contact with larval leaf damage and oviposition, confirming my second hypothesis that tarsal contact with leaf damage is the stimulus leading to oviposition.

The cabbage root fly *Erioischia brassicae* (Bouche) is stimulated to oviposit in soils upon contacting mustard-oil glucosides from host plants (Traynier 1965, 1967). The carrot fly *Psila rosae* is stimulated to oviposit on carrot leaves because of a contact oviposition cue, trans-methylisoeugenol (Beruter & Stadler 1971, Stadler 1972; as in Guerin et al. 1983). Many Lepidoptera are known to oviposit upon tarsal contact with leaf surfaces. For example, the cabbage butterfly *Pieris brassicae* (Ma & Schoonhoven 1973, Renwick & Radke 1987), the citrus-feeding swallowtail butterfly *Papilio xuthus* (Nishida et al. 1987), and the Aristolochiaceae-feeding swallowtail butterfly *Atrophaneura alcinous* (Nishida & Fukami 1989) all oviposit on host plant leaves in response to contact stimulants.

Odell & Godwin (1984) reported a search strategy similar to that of *L. exul* for the tachinid parasitoid, *Blepharipa pratensis*. Upon contact with a recently damaged plant edge (cut, torn, or larval-damaged) *B. pratensis* orients perpendicular to the edge and moves back and forth with the front tarsi grasping the damaged edge. Leaf exudates arrest the fly on the leaf and increase tarsal examination (searching). If the edge of an eaten leaf is contacted, oviposition usually follows. The adult cabbage maggot *Hylemya brassicae* (Diptera:Anthomyiidae) exhibits similar behaviour when searching for oviposition sites near host plants (Nair & McEwen 1976). A gravid female fly lands on a radish (an alternate host for the cabbage maggot), becomes increasingly active, extends its ovipositor occasionally, and walks down the plant stem to oviposit an egg in the soil close to the plant stem.

After gravid *L. exul* females had touched leaf damage and oviposited they occasionally changed leaves several times in succession and began ovipositing again once leaf damage was contacted. This behavioural pattern would result in the flies not ovipositing all of their eggs on one damaged leaf but, rather, distributing their eggs over a large area, thereby increasing the chance of parasitizing caterpillars on nearby leaves as well as the

leaf currently being fed upon. This behavioural pattern may help the flies parasitize several larvae in a colony instead of superparasitizing individual caterpillars, in which normally only one fly larvae can complete development (Sippell 1957).

3.4.3 Conclusion

Even though the tachinid parasitoid *L. exul* has a high reproductive capacity, with large females producing over 5000 eggs (Bess 1936), it does not broadcast its eggs randomly on host plants. Gravid females use a common host searching strategy on both aspen and balsam poplar trees which increases the chances of its eggs being ingested by actively feeding caterpillars. Once on a host plant, the fly uses FTC damaged plant edges as a releasing stimulus to oviposit. After ovipositing the fly does not leave the area immediately but searches nearby leaves to locate other feeding damage. Laboratory observations indicate this to be a very effective host finding strategy. How these behaviours translate to the spatial distribution of eggs and host parasitism in the field is the topic of Chapter 4.

3.5 Literature cited

Bess, H. A. 1936. The biology of *Leschenaultia exul* Townsend, a tachinid parasite of *Malacosoma americana* Fabricius and *Malacosoma disstria* Hübner. Annals of the Entomological Society of America 29:593-613.

Bess, H. A. 1937. Methods used in rearing *Leschenaultia exul*. in: Galtsoff, P. S., Lutz, F. E., Welch, P. S., and Needham, J. G. (chairmen). Culture Methods for Invertebrate Animals. New York, NY: Dover Publications Inc.

Cane, V. R. 1978. On fitting low-order Markov chains to behaviour sequences. Animal Behaviour 26:332-338.

Cerezke, H. F. 1991. Forest Tent Caterpillar. Forestry Canada, Northwest Region, Northern Forestry Centre, Edmonton, Alberta. Forestry Leaflet Number 10.

Dowden, P. B. 1934. *Zenillia libatrix* Panzer, a tachinid parasite of the gypsy moth and the brown-tail moth. Journal of Agriculture Research 48:97-114.

Fraser, J. and Nelson, M. C. 1984. Communication in the courtship of a madagascan hissing cockroach. I. Normal courtship. Animal Behaviour 32:194-203.

Guerin, P. M., Stadler, E. and Buser, H. R. 1983. Identification of host plant attractants for the carrot fly, *Psila rosae*. Journal of Chemical Ecology 9:843-861.

Hassell, M. P. 1968. The behavioural response of a tachinid fly (*Cyzenis albicans* (Fall.)) to its host, the winter moth (*Operophtera brumata* (L.)). Journal of Animal Ecology 37:627-639.

Hodson, A. C. 1941. An ecological study of the forest tent caterpillar, *Malacosoma disstria* Hbn., in Northern Minnesota. Minnesota Agriculture Experiment Station Technical Bulletin 148:55 pp.

Lemon, R. E. and Chatfield, C. 1971. Organization of song in cardinals. Animal Behaviour 19:1-17.

Leonard, S. H. and Ringo, J. M. 1978. Analysis of male courtship patterns and mating behaviour of *Brachyneria intermedia*. Annals of the Entomological Society of America 71:817-826.

Ma, W. C. and Schoonhoven, L. M. 1973. Tarsal contact chemosensory hairs of the large white butterfly, *Pieris brassicae* and their possible role in oviposition behaviour. Entomologia Experimentalis et Applicata 16:343-357.

Martin Jr., W. R., Nordlund, D. A. and Nettles Jr., W. C. 1990. Response of parasitoid *Eucelatoria bryani* to selected plant material in an olfactometer. Journal of Chemical Ecology 16:499-508.

Morrison, G. and Strong Jr., D. R. 1980. Spatial variations in host density and the intensity of parasitism: Some empirical examples. Environmental Entomology 9:149-152.

Nair, K. S. S. and McEwen, F. L. 1976. Host selection by the adult cabbage maggot, *Hylemya brassicae* (Diptera:Anthomyiidae): Effect of glucosinolates and common nutrients on oviposition. The Canadian Entomologist 108:1021-1030.

Navasero, R. C. and Elzen, G. W. 1989. Responses of *Microplitis croceipes* to host and nonhost plants of *Heliothis virescens* in a wind tunnel. Entomologia Experimentalis et Applicata 53:57-63.

Nettles Jr., W. C. 1982. Contact stimulants from *Heliothis virescens* that influence the behaviour of females of the tachinid, *Eucelatoria bryani*. Journal of Chemical Ecology 8:1183-1191.

Nishida, R. and Fukami, H. 1989. Oviposition stimulants of an Aristolochiaceae-feeding swallowtail butterfly, *Atrophaneura alcinous*. Journal of Chemical Ecology 15:2565-2575.

Nishida, R., Ohsugi, T., Kokubo, S. and Fukami, H. 1987. Oviposition stimulants of citrus-feeding swallowtail butterfly, *Papilio xuthus* L. Experientia 43:342-344.

Odell, T. M. and Godwin, P. A. 1984. Host selection by *Blepharipa pratensis* (Meigen), a tachinid parasite of the gypsy moth *Lymantria dispar* L. Journal of Chemical Ecology 10:311-320.

Reitz, S. R. and Adler, P. H. 1991. Courtship and mating behaviour of *Eucelatoria bryani* (Diptera:Tachinidae), a larval parasitoid of *Heliothis* species (Lepidoptera:Noctuidae). Annals of the Entomological Society of America 84:111-117.

Renwick, J. A. A. and Radke, C. D. 1987. Chemical stimulants and deterrents regulating acceptance or rejection of crucifers by cabbage butterflies. Journal of Chemical Ecology 13:1771-1775.

Roland, J. 1986. Parasitism of winter moth in British Columbia during build-up of its parasitoid *Cyzenis albicans*: attack rate on oak *v.* apple. Journal of Animal Ecology 55:215-234.

Roth, J. P., King, E. G. and Hensley, S. D. 1982. Plant, host, and parasite interactions in the host selection sequence of the tachinid *Lixophaga diatraeae*. Environmental Entomology 11:273-277.

Sippell, W. L. 1957. A study of the forest tent caterpillar *Malacosoma disstria* Hbn. and its parasite complex in Ontario. Ph.D. Thesis, University of Michigan, Ann Arbor, Michigan. 147 pp.

Slater, P. J. B. 1973. Describing sequences of behaviour. In: Bateson, P. P. G. and Klopfer, P. H. (eds.). Perspectives in Ethology. New York and London: Plenum Press.

Traynier, R. M. M. 1965. Chemostimulation of oviposition by the cabbage root fly *Erioischia brassicae* (Bouche). Nature 207:218-219.

Traynier, R. M. M. 1967. Stimulation of oviposition by the cabbage root fly *Erioischia brassicae*. Entomologia Experimentalis et Applicata 10:401-412.

Vinson, S. B. 1976. Host selection by insect parasitoids. Annual Review of Entomology 21:109-133.

Walker, E. D. and Archer, W. E. 1988. Sequential organization of grooming behaviours of the mosquito, *Aedes triseriatus*. Journal of Insect Behaviour 1:97-109.

Zar, J. H. 1984. Biostatistical Analysis - 2nd edition. Englewood Cliffs, New Jersey: Prentice Hall Publishers.

Table 3.1. Number of eggs oviposited by individual *L. exul* on FTC larval damaged aspen poplar *Populus tremuloides*, balsam poplar *Populus balsamifera*, or construction paper leaf clusters in choice experiments. (N = 5 replicates).

Aspen Poplar	Balsam Poplar	Construction Paper
0	25	1
33	128	2
0	4	0
7	12	1
13	98	3
Median = 7 Range = 0 - 33	Median = 25 Range = 4 - 128	Median = 1 Range = 0 - 3

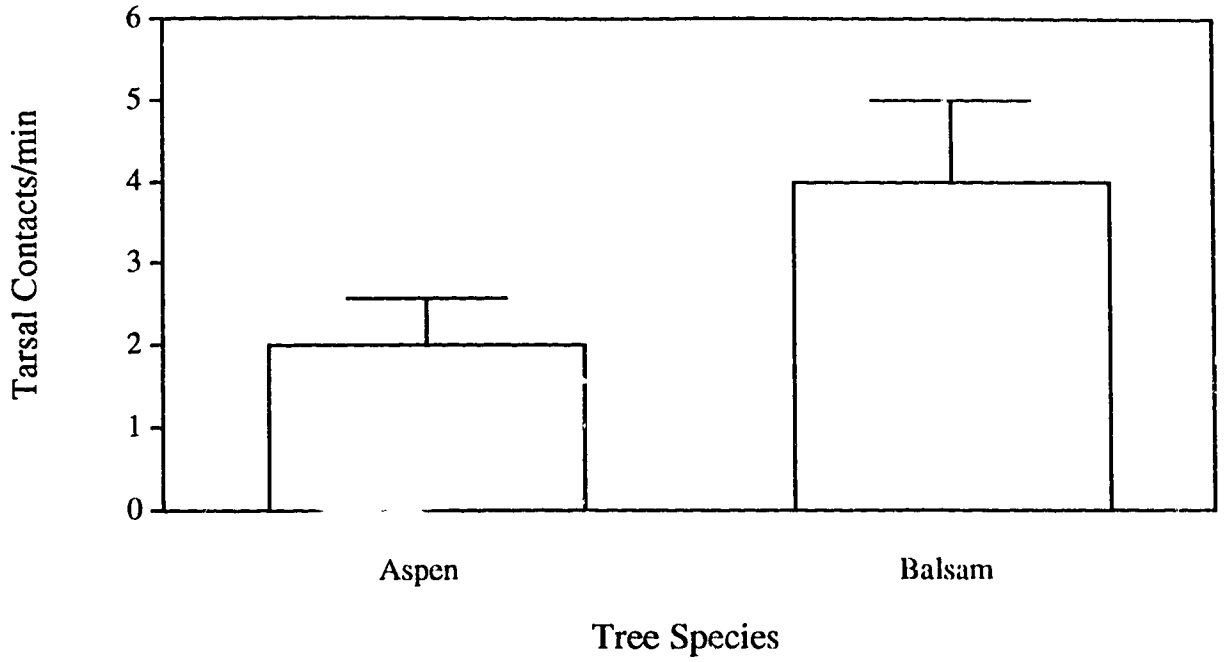

Figure 3.1. Mean number (+SE) of tarsal contacts with FTC-damaged leaf edges per minute by female *L. exul*.

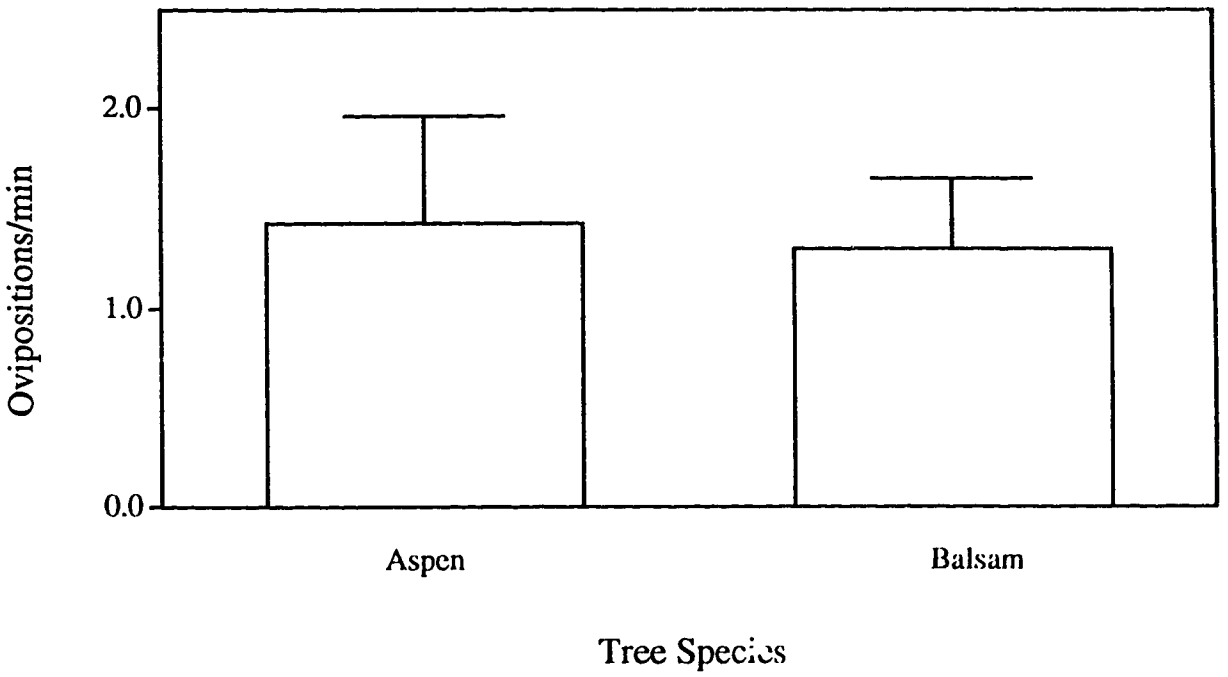

Figure 3.2. Mean number (+ SE) of ovipositions per minute by female *L. exul*.

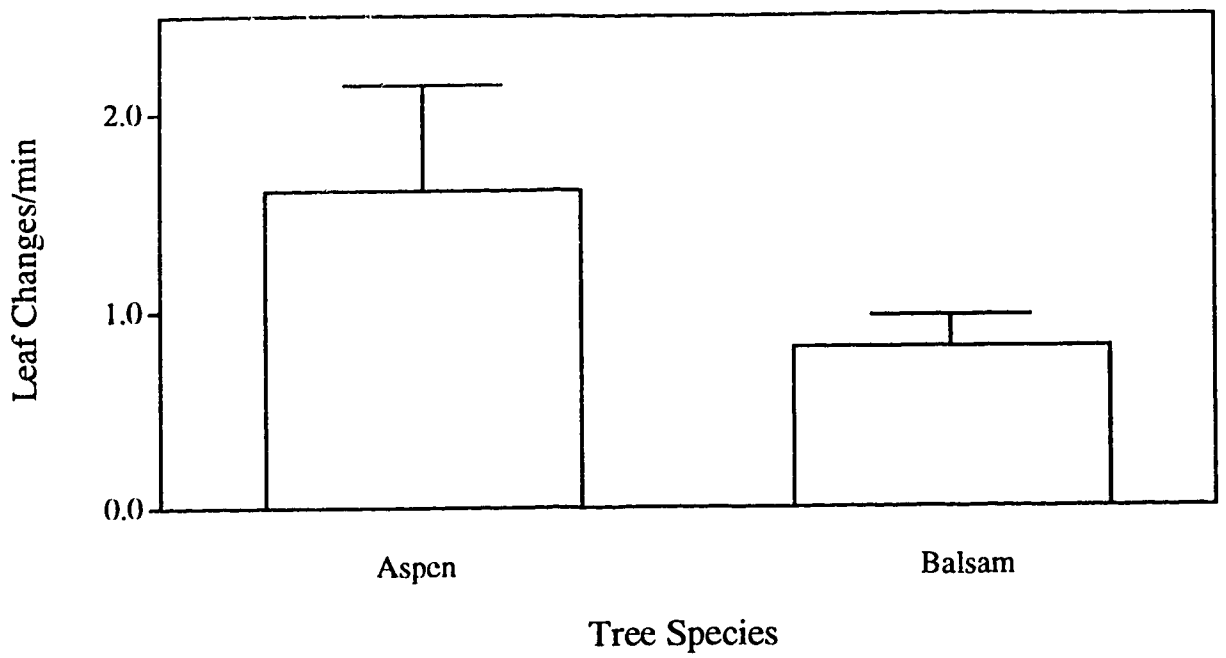

Figure 3.3. Mean number (+ SE) of times female *L. exul* moved from one leaf to another within a leaf cluster.

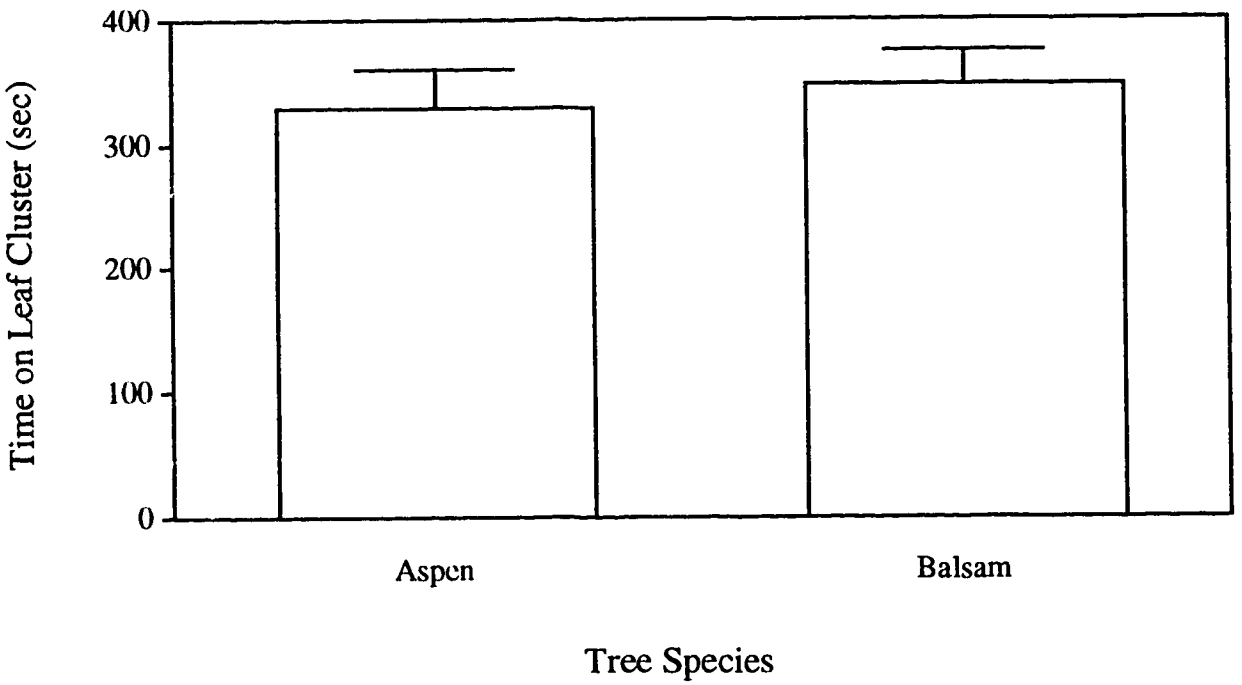

Figure 3.4. Mean times (+ SE) spent on leaf clusters by female *L. exul.*

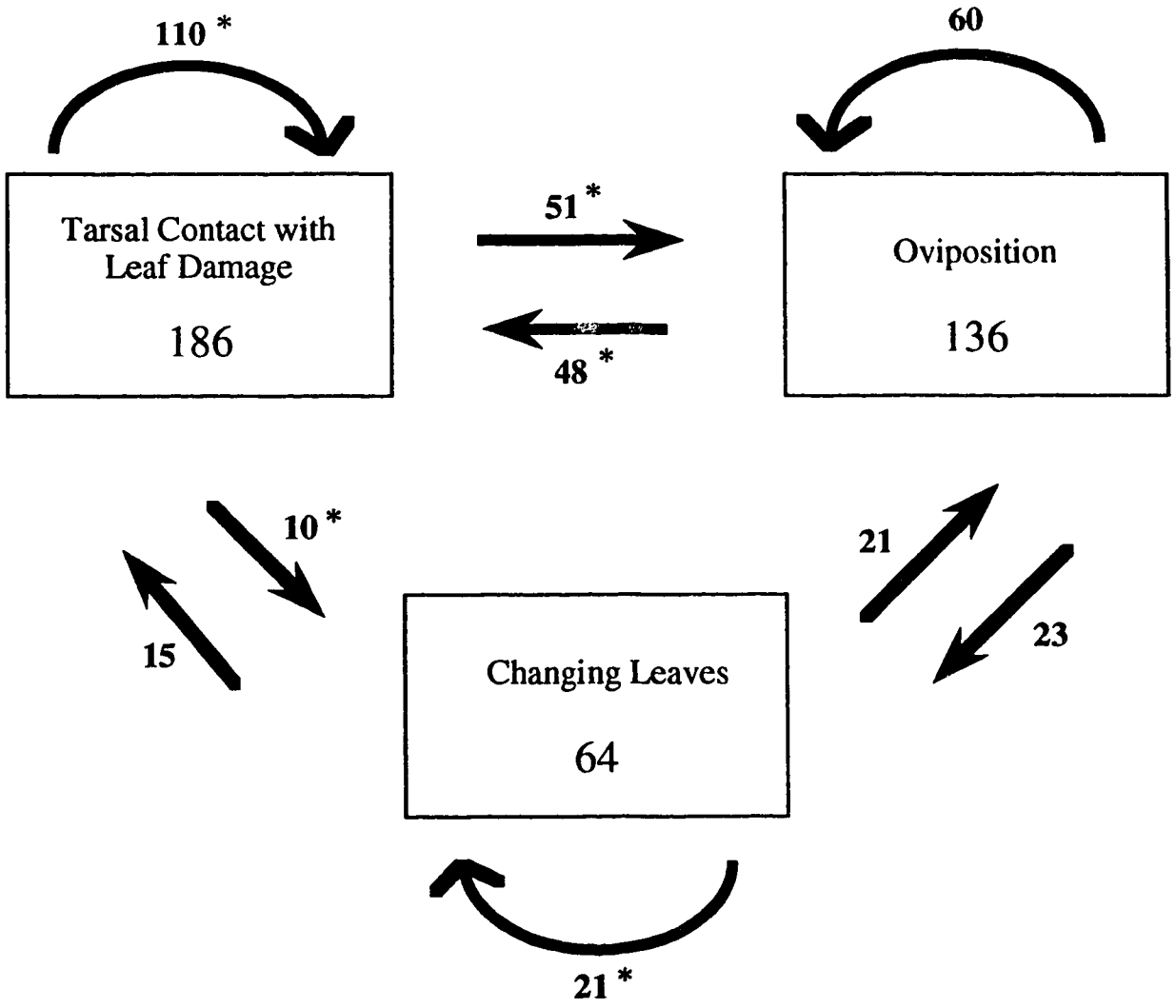

Figure 3.5. A kinematic graph of *L. exul* searching behaviours on aspen poplar, *Populus tremuloides* leaf clusters. Boxes represent behavioural acts; numbers inside the boxes are frequencies of behaviours. Arrows represent transitions between behaviours; numbers next to arrows are numbers of transitions, * indicate significant, non-random transitions.

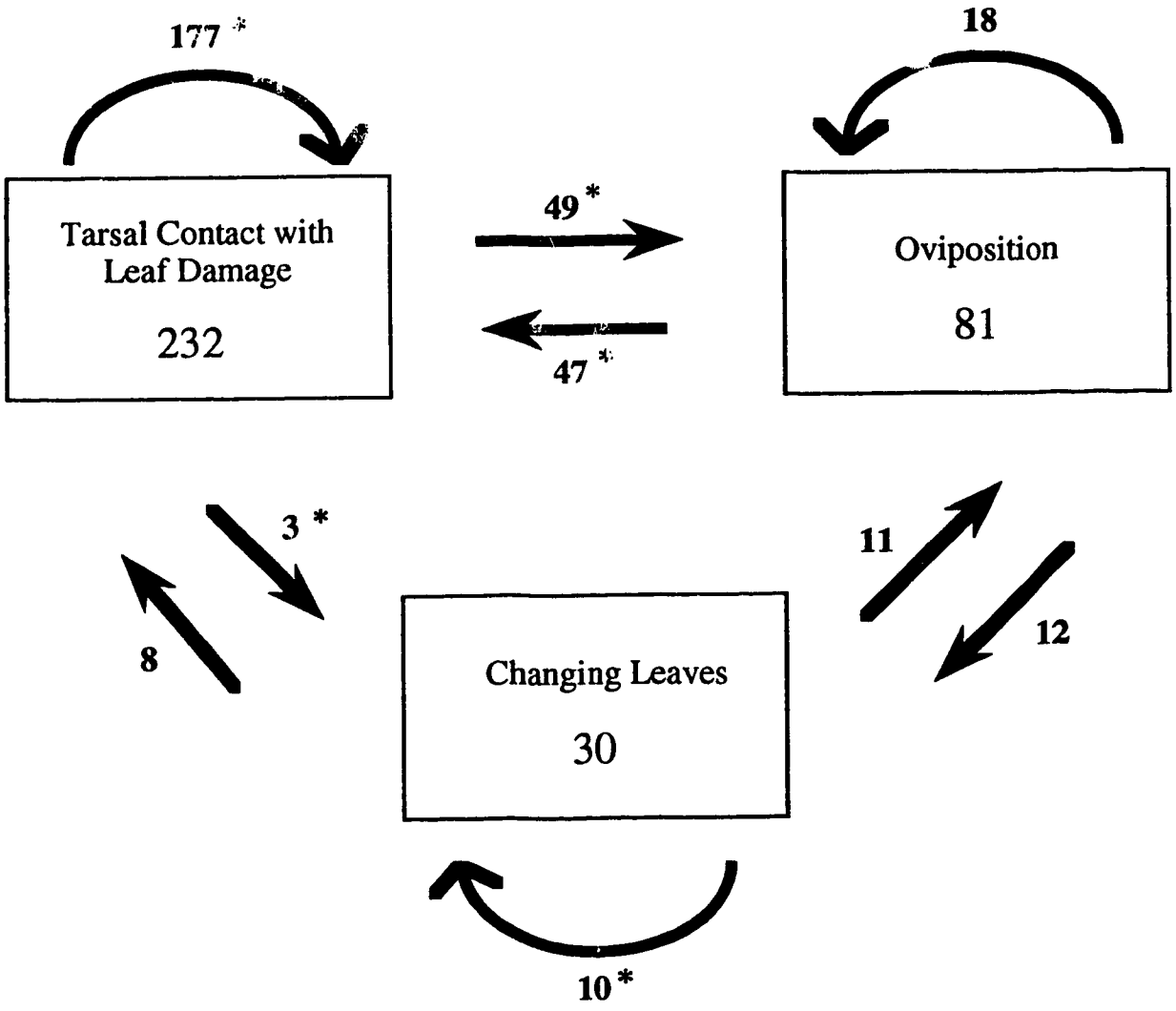

Figure 3.6. A kinematic graph of *L. exul* searching behaviours on balsam poplar, *Populus balsamifera* leaf clusters. Boxes represent behavioural acts; numbers inside the boxes are frequencies of behaviours. Arrows represent transitions between behaviours; numbers next to arrows are numbers of transitions, * indicate significant, non-random transitions.

Chapter 4 Egg Distribution and Parasitism

4.1 Introduction

Many tachinid parasitoids are known to exhibit habitat-specific parasitism of host larvae as a result of specific host-searching strategies. In forest habitats, factors such as height in the canopy (Weseloh 1981) and the presence of food sources for the adult parasitoids (Leius 1967) are factors that can affect parasitism rates. For example, parasitism by *Compsilura concinnata* of gypsy moth larvae, *Lymantria dispar*, is higher when the host is in groups of trees than when on isolated trees (Schwenke 1958), and higher in the lower canopy of trees than in the upper canopies (Tigner et al. 1974, Barbosa et al. 1975).

Rates of parasitism can also be affected by the plant species on which the host is found (Simmons et al. 1975, Price et al. 1980). In 1914, Picard and Ribaud were the first to report the effect of host habitat on parasitism (in Nettles 1980). Insect parasitoids are attracted to volatiles from damaged host plants and use olfactory cues to find host larvae (Vet & Dicke 1992). Parasitoids usually respond, preferentially, to olfactory cues arising from principal host plant species of the herbivore (Price et al. 1980), as these cues provide the parasitoids with the most reliable host-finding stimuli (Vet & Dicke 1992). Hence, if a host insect can feed on a plant species that is not attractive to parasitoids, it may exist in comparatively enemy-free space and experience reduced parasitism.

The forest tent caterpillar's (FTC) *Malacosoma disstria* (Hübner) (Lepidoptera:Lasiocampidae) principal host tree is aspen poplar *Populus tremuloides* Michx.; however, larvae can disperse to feed on a variety of other tree species including balsam poplar *Populus balsamifera* L. (Cerezke 1991). As the decline of FTC

populations is believed to be strongly associated with increased suppression by its natural enemies such as the leaf-ovipositing tachinid parasitoids *Leschenaultia exul* and *Patelloa pachypyga* (Roland 1993), it is important to know if these two fly species produce different rates of parasitism on different plant species. If the flies differentially oviposit on, and subsequently parasitize FTCs on different tree species, larvae emigrating from the principal host tree species, aspen poplar, to feed on other tree species such as balsam poplar may be 'wandering' into enemy-free space and subsequently experience reduced parasitism. The result of caterpillars feeding in enemy-free space may be a prolonging of a FTC outbreak in an area.

In this chapter, I examine the spatial pattern of oviposition and parasitism on aspen poplar and balsam poplar trees by the two leaf-ovipositing tachinid parasitoids *L. exul* and *P. pachypyga* in response to FTC feeding damage. I hypothesized that the spatial pattern of oviposition and parasitism by *L. exul* and *P. pachypyga* should differ when the larvae are feeding on different host plant species. *L. exul* is attracted to FTC leaf damage on both aspen poplar and balsam poplar trees (Chapter 2) and laboratory experiments indicate that both tree species are equally acceptable for oviposition by gravid females (Chapter 3). Therefore, *L. exul* would be expected to oviposit similar numbers of eggs on aspen poplar and balsam poplar tree leaves in the field and cause similar levels of parasitism on the two tree species. In contrast, *P. pachypyga* is differentially attracted to aspen poplar trees (Chapter 2). This fly species should preferentially enter the canopies of aspen poplar and then use contact cues for oviposition on FTC larval-damaged leaves. Since *P. pachypyga* is not initially attracted to balsam poplar trees (Chapter 2) there is no opportunity to respond to level of damage as a contact cue. The result would be fewer eggs laid on balsam poplar tree leaves. Parasitism rates should reflect this dichotomy, with higher parasitism rates by *P. pachypyga* on aspen poplar trees than on balsam poplar trees. In addition, for both tree species more eggs should be laid by both species of

parasitoid on more defoliated trees than on less defoliated trees, and rates of parasitism should reflect this.

4.2 Materials and methods

4.2.1 Spatial distribution of tachinid eggs

Spatial patterns of oviposition by *L. exul* and *P. pachypyga* were evaluated among trees at a Ministik Hills field site, on the corner of Range Road 212 and Ministik Estate Road. The stand composition consists mainly of aspen poplar interspersed with, in descending order of abundance, balsam poplar, paper birch, and white spruce (Roland & Taylor 1995). This site has both large mature trees (approximately 10 m in height) and younger trees (approximately 1- 6 m in height). In 1995, this area was undergoing a large FTC outbreak and had been generating significant numbers of both *L. exul* and *P. pachypyga* parasitoids for at least 2 years.

In 1995, *L. exul* adults were first observed mating on 6 May and *P. pachypyga* on 9 May. Preoviposition lasts approximately 2-3 weeks (depending on the temperature) and oviposition generally lasts for about 5-6 weeks (Bess 1936, 1937). Thus, if *L. exul* has a 2 week pre-oviposition period and continues laying eggs for approximately 5-6 weeks, oviposition would have begun on approximately 20 May 1995 and continued until, at the earliest, 24 June 1995. This time of oviposition was corroborated by the fact that field collected flies maintained in the lab did not start ovipositing until 20 May 1995. *P. pachypyga* is assumed to have a similar phenology to *L. exul* although *P. pachypyga* emerges slightly later in the spring. *P. pachypyga* then should have begun laying eggs on approximately 23 May 1995 and continued until 27 June 1995.

Leaf clusters (a group of approximately 5-7 leaves arising from a single bud) were gathered on 29 May 1995 and a few tachinid eggs were found. All leaf cluster sampling was subsequently conducted on 9 - 10 June 1995, approximately 3 weeks after *L. exul* had commenced ovipositing and approximately 2.5 weeks after *P. pachypyga* had commenced ovipositing.

Leaf clusters were collected from thirty trees (20 aspen poplar and 10 balsam poplar) ranging in height from 1.6 - 4.65 m. These trees did not contain any FTC egg masses but they did contain fourth/fifth instar caterpillars that had 'wandered' from larger, more mature trees. Leaf clusters were systematically collected from the entire canopy of small trees, thus reducing any bias due to microhabitat preferences of FTCs or of the parasitoids. One concern in conducting this part of the experiment is the variation in number of tachinid eggs that may occur from tree to tree (Roland 1986). All trees were within the same area (measuring approximately 75 x 100 m), thereby controlling for larger-scale variation in defoliation, host, and parasitoid abundance. Trees were separated by at least 2 m however, to reduce drifting volatiles from one plant to another (Sabelis & De Jong 1988); parasitoids may be attracted to a neighboring tree simply because it is downwind of the tree releasing the attractive volatiles.

Trees were marked with flagging tape and identified by number. The overall percent defoliation for the tree and larval abundance was recorded. Two leaf clusters were taken from each of 8 sectors: north top, north bottom, east top, east bottom, south top, south bottom, west top, and west bottom. A random number between one and ten was used to select the branch to be sampled within each quadrant. Another random number between one and ten was then selected to determine which leaf cluster would be sampled on the branch. Each cluster was counted individually from the tip of the branch to the trunk of the tree until the number selected from the table had been reached. If a branch did not

contain enough leaf clusters, counting continued on the same branch with the first leaf cluster being given the next number in series (e.g. if the number six was chosen and a branch had four leaf clusters, the second leaf cluster was sampled). A total of 480 leaf clusters from the 30 trees were collected.

Percent defoliation and number of larvae were also recorded for each leaf cluster. As an index of defoliation, the proportions of each of the leaves that had been eaten were summed and then divided by the total number of leaves on the cluster. Leaf clusters were transported back to the lab in an ice chest to prevent wilting, and kept refrigerated until they were examined for parasitoid eggs.

Eggs of *L. exul* and *P. pachypyga* were identified to species by comparing them to voucher specimens from each species. Eggs from the two species differ in colour and surface markings. *L. exul* eggs are silver to light brown with a very smooth surface. *P. pachypyga* eggs are dark brown to black with an intricate swirl pattern of deep, furrowed grooves forming circular patterns around the center of the egg's topmost surface. The number of eggs of each fly species was recorded for each leaf cluster.

Statistical analyses. To test whether trees selected for the study were equivalent, I compared their heights, their levels of defoliation, and caterpillar abundance using 2-tailed, non-paired t-tests.

The spatial distribution of eggs laid were compared at two different spatial scales. The first analysis was conducted at the whole tree level, comparing the number of eggs laid with fly species and tree species as main factors. Stepwise multiple regression then discerned whether whole tree defoliation and larval abundance had a significant effect on the number of eggs laid on the 30 trees. The regression was then further collapsed to

determine the effect that defoliation and larval abundance had on the number of eggs laid by each of the two species of parasitoids.

The second analysis examined the number of eggs laid by *L. exul* and *P. pachypyga* among leaf clusters. The effect of fly species, tree species, and sector were included in an analysis of variance on the number of eggs laid. This allowed me to discern whether there was a difference in the number of eggs laid by the two species of tachinid flies, whether the flies had a preference for laying eggs on either tree species, and whether the flies preferred laying eggs in one of the sectors of the tree canopy. Stepwise multiple regression then allowed me to determine whether factors such as defoliation and larval abundance had a significant effect on the number of eggs laid on each leaf cluster. The regression was then further collapsed to determine the effect that defoliation and larval abundance had on the number of eggs laid by each of the two species of parasitoids.

Conducting analyses at these two scales enables me to determine if the parasitoids respond more strongly to FTCs and damaged trees at the whole tree scale or at the finer scale of leaf clusters. That is, if the correlation coefficients are significantly greater at one scale it indicates that flies are responding more strongly to FTC damaged plants at that scale. Correlation coefficients were compared for the overall analysis and for each species of tachinid parasitoid, if the same factors were significant at both scales.

The distribution of *L. exul* and *P. pachypyga* eggs on both aspen poplar and balsam poplar foliage was analyzed to determine how well it followed a Poisson distribution. This analysis was conducted at both the whole tree level and the individual leaf cluster level to determine if eggs were laid randomly (the presence of one egg does not affect the probability of subsequent eggs being laid on the same tree or leaf cluster), uniformly (the presence of one egg reduces the probability of subsequent eggs being laid on the same

tree or leaf cluster), or contagiously (the presence of one egg increases the probability of subsequent eggs being laid on the same tree or leaf cluster) by each fly species. This analysis provides further information on the scale at which the parasitoids are responding to the hosts' habitat.

4.2.2 Parasitism rates of FTCs on host trees

The same aspen poplar and balsam poplar trees selected for tachinid egg counts were also used to sample caterpillars for estimates of parasitism. From each tree, a maximum of 50 fourth/fifth instar FTCs were sampled on 9-10 June 1995. If a tree did not contain 50 caterpillars then all of the caterpillars on the tree were collected. The caterpillars were removed from the trees with soft forceps and transported back to the laboratory in cloth bags.

Some of these caterpillars may have been previously parasitized as these caterpillars 'wandered' from large natal trees to the small experimental trees. Previous parasitism may be more of a problem for adequately assessing the *L. exul* parasitism rate on the experimental trees as oviposition and subsequent parasitism of FTCs occurs slightly earlier for *L. exul* than for *P. pachypyga* (Parry 1994). *L. exul* begins ovipositing and parasitizing larvae when the larvae are in the fourth instar but both fly species parasitize their hosts primarily during the fifth instar (Parry 1994). It was not possible to raise caterpillars on the experimental trees for rates of parasitism as neonate FTC larvae can not survive on resinous leaves, such as balsam poplar (Hodson 1941). Thus, using naturally 'wandering' caterpillars was the best method available to equalize prior differences between aspen poplar and balsam poplar FTC populations for estimates of parasitism.

In the laboratory, larvae from each tree were maintained in a separate large brown paper bag. Caterpillars were fed fresh aspen foliage every second day. Foliage was gathered from a region where there has not been an outbreak for at least 8 years, and therefore no tachinid parasitoid eggs. Caterpillars were fed until they either died from parasitism or pupated. Once maggots emerged, they were allowed to pupate inside the paper bag. Fly puparia were identified to species using a taxonomic key for FTC parasitoid puparia (Ross 1953).

Statistical analyses. To determine if the FTCs consumed parasitoid eggs and were parasitized on the experimental trees, as opposed to bringing the majority of the parasites with them from the larger natal trees, simple regressions were conducted to determine if any relationship existed between the number of tachinid eggs found on the trees and parasitism rates of hosts on those trees. I used a 2-factor analysis of variance to determine the effects of fly species and tree species, and their interaction on the number of eggs laid. Stepwise multiple regression was then conducted to determine whether defoliation and larval abundance affected the parasitism rate. The effect of defoliation and larval abundance on parasitism by each species of parasitoid was analyzed separately.

4.3 Results

4.3.1 Field tree comparisons

Aspen poplar and balsam poplar trees selected for the field experiment did not differ significantly in size (height) (t=-0 51, df=28, P=0.62) (Figure 4.1), nor in the number of FTCs on them (t=1.06, df=28, P=0.30) (Figure 4.2). Defoliation levels were higher on

aspen poplar trees compared to balsam poplar trees (t=4.33, df=28, P=0.000) (Figure 4.3).

4.3.2 Whole tree egg counts

More eggs were laid on aspen poplar trees than on balsam poplar trees (F=9.74, df=1, P=0.0028). Most of these eggs were laid by *P. pachypyga* (F=27.62, df=1, P=0.0001), and *P. pachypyga* showed the strongest difference between tree species as evidenced by the interaction (F=8.46, df=1, P=0.0052) (Figure 4.4).

Two factors were important in predicting the number of eggs oviposited. More eggs were laid on aspen poplar than on balsam poplar trees (coefficient=-2.7, P=0.0101), and there were more *P. pachypyga* eggs than *L. exul* eggs on the trees (coefficient=5.83, P=0.0001) (overall model; F=16.21, P=0.0001, r^2=0.46). Oviposition by *L. exul* was unaffected by either defoliation or larval abundance. This lack of significance may be partially attributed to low power (0.67), but more likely it is due to the low number of *L. exul* eggs found on the trees (*L. exul*; mean=0.3 eggs per tree vs. *P. pachypyga*; mean=5.23 eggs per tree). For *P. pachypyga*, tree species was the only significant factor (coefficient= -5.11, P=0.010) with more eggs of this species being laid on aspen poplar trees than on balsam poplar trees (overall model; F=5.95, P=0.0072, r^2=0.31).

4.3.3 Leaf cluster egg counts

There was a significant difference in the number of eggs laid on the two tree species, with 3 times as many eggs laid on aspen poplar leaf clusters (F=16.32, df=1, P=0.0001). Most of these eggs were laid by *P. pachypyga* (F=45.65, df=1, P=0.0001). The interaction between tree species and fly species was also significant (F=14.2, df=1, P=0.0002) with

nearly equal numbers of eggs being laid by *L. exul* on both tree species, but 3 times more eggs laid on aspen than on balsam leaf clusters by *P. pachypyga* (Figure 4.5). There was no significant difference in the number of tachinid eggs found on leaf clusters from the different sampling quadrants (F=0.96, df=7, P=0.46). There were no other significant first or second order interactions.

Based on stepwise multiple regression analysis, I found that more eggs were laid on aspen (coefficient=-0.19, P=0.0001) and more were laid by *P. pachypyga* (coefficient=0.37, P=0.0001) (overall model; F=44.06, P=0.0001, r^2=0.08). None of the factors included in the analyses significantly predicted the number of *L. exul* eggs found on leaf clusters. This lack of significance was not due to low sample size (power=0.87), but was probably due to the extremely low number of *L. exul* eggs found on the leaves (*L. exul*; mean=0.02 eggs per leaf cluster vs. *P. pachypyga*; mean=0.28 eggs per leaf cluster) (Figures 4.6 and 4.7). For *P. pachypyga*, tree type was the only significant factor (coefficient=-0.36, P=0.0001), with many more eggs being oviposited on aspen leaf clusters compared to balsam poplar leaf clusters irrespective of defoliation or larval abundance (overall model; F=15.82, P=0.0001, r^2=0.03) (Figures 4.8 and 4.9). Defoliation may not be a significant factor because above 45% defoliation, many of the eggs laid on the leaf clusters may have been consumed by the caterpillars.

4.3.4 Oviposition scale comparisons

The overall correlation coefficient for eggs laid on leaf clusters (r=0.28) was significantly different from the correlation coefficient for eggs found on whole trees (r=0.68, z=3.92, P<0.05). It was not possible to statistically compare the overall correlation coefficients for the number of eggs laid by *L. exul* as neither of the coefficients were significant. For *P. pachypyga*, both of the correlation coefficients were significant, and both scales

implicate tree type as the single most important variable. The correlation coefficient for whole tree analysis (r=0.56) was significantly different from the coefficient for leaf cluster analysis (r=0.17, z=3.324, P<0.05) indicating that flies respond to aspen trees on a per tree basis rather than at the leaf cluster level.

4.3.5 Egg distributions

Among trees, *L. exul* laid eggs randomly on aspen poplar (χ^2=5.70, df=2, P>0.05). It was not possible to calculate the distribution on balsam poplar trees because of only having two categories (0 and 1), thereby resulting in 0 degrees of freedom for the statistical procedure. *P. pachypyga* eggs were more strongly clumped on aspen trees (χ^2=661.32, df=20, P<0.001; s^2/x=3.84) than on balsam poplar trees (χ^2=15.62, df=6, P<0.001; s^2/x=2.52).

Among leaf clusters, *L. exul* eggs were laid in a somewhat clumped fashion on aspen poplar foliage (χ^2=8.69, df=1, P<0.005; s^2/x=1.0). As with whole trees, it was not possible to evaluate the distribution of *L. exul* eggs on balsam poplar foliage. *P. pachypyga* eggs were more strongly clumped on aspen poplar leaf clusters (χ^2=106.42, df=4, P<0.001; s^2/x=2.4) than on balsam poplar leaf clusters (χ^2=15.69, df=2, P<0.001; s^2/x=1.43).

4.3.6 Parasitism

Among aspen poplar trees there was a significant relationship between number of eggs laid and parasitism rates of hosts (F=5.00, df=1, P=0.038) (Figure 4.10). On balsam poplar trees, however, there was no relationship between the number of eggs laid and the resulting parasitism rates of caterpillars (F=3.25, df=1, P=0.11) (Figure 4.11).

Parasitism rates were generally higher on aspen poplar trees than on balsam poplar trees (F=23.44, df=1, P=0.0001), and was higher by *P. pachypyga* than by *L. exul* (F=7.12, df=1, P=0.0099). There was a significant interaction between tree species and fly species (F=5.02, df=1, P=0.029) with similar parasitism rates by *L. exul* on the two tree types but much higher parasitism rates by *P. pachypyga* on aspen poplar compared to balsam poplar trees (Figure 4.12).

Three factors were important in predicting parasitism rates; (1) tree species (coefficient= -7.3, P=0.019) with higher rates on aspen poplar than on balsam poplar trees, (2) fly species (coefficient=7.92, P=0.0007) with *P. pachypyga* causing higher parasitism rates than *L. exul*, and (3) defoliation (coefficient=0.36, P=0.0060) with higher parasitism at higher levels of defoliation (overall model, F=12.94, P=0.0001, r^2=0.41). Separating the analyses by fly species, the only significant factor predicting parasitism by *L. exul* was defoliation (coefficient=0.38, P=0.0061) irrespective of tree species (overall model; F=4.86, P=0.016, r^2=0.26). For *P. pachypyga*, tree type was the only significant factor (coefficient=-14.99, P=0.0002) with higher parasitism rates occurring on aspen poplar trees compared to balsam poplar trees (overall model; F=12.29, P=0.0002, r^2=0.48).

4.4 Discussion

The success with which a parasitoid discovers hosts is determined by characteristics of the parasitoid species and by the modification of these characteristics through feedback from the environment (Burnett 1958). The tachinid parasitoids *L. exul* and *P. pachypyga* have very different responses to FTCs when the host larvae feed on different tree species. The apparent oviposition strategies used by the tachinid parasitoids result in very

different risks of parasitism for FTCs when feeding on aspen poplar and balsam poplar trees.

4.4.1 Oviposition

While extremely low numbers of *L. exul* eggs were found on foliage, the number of eggs laid by *L. exul* on aspen poplar and balsam poplar trees were similar. The low number of *L. exul* eggs discovered on the leaf clusters of both aspen and balsam poplar trees indicate one of three possible scenarios. First, *L. exul* may have a well defined host location/oviposition strategy, placing eggs adjacent to feeding larvae where they will be ingested almost immediately. Parry (1994) reported that *L. exul* was observed selectively ovipositing eggs near feeding groups of larvae. Second, because the caterpillars had been defoliating these particular field trees for a few weeks and because *L. exul* tends to lay its eggs before *P. pachypyga* does, the majority of *L. exul* eggs may have already been ingested by the feeding caterpillars at the time of leaf cluster collection. Parasitism of FTCs begins slightly sooner for *L. exul* with 25% of the parasitism occurring during the FTC fourth instar, compared with less than 5% for *P. pachypyga* (Parry 1994). Third, the caterpillars may have consumed the majority of *L. exul* eggs while on the large, mature aspen trees bringing the parasites with them to the new trees thereby shuffling the parasites among the independent variables measured. However, as previously mentioned, while the caterpillars may have brought a small number of parasites with them to the new host trees the flies do not begin parasitizing FTCs until the larvae are in the fourth instar.

Large numbers of *P. pachypyga* eggs were found on aspen poplar foliage. This confirms my prediction that the number of eggs laid would be higher on aspen poplar foliage than on balsam poplar foliage. A small number of eggs were found on balsam poplar trees so oviposition is possible on this tree species even though it is not the preferred tree species.

The effect of tree species on oviposition was significant at both a whole tree basis and at the level of leaf clusters. However, oviposition by *P. pachypyga* in response to cues like defoliation was better predicted by analyzing data at the scale of whole trees rather than leaf clusters. Correlation coefficients and clumping of eggs were both stronger effects when data was analyzed on a per tree basis.

For the gypsy moth parasite *Blepharipa pratensis*, leaf area of clusters previously browsed appears to have little if any influence on the number of eggs laid (Odell & Godwin 1984). It could also not be shown that winter moth defoliation was correlated with *Cyzenis albicans* egg counts (Embree & Sisojevic 1965). *C. albicans* tends to lay eggs where leaf damage is greatest, although many eggs are laid on nearby undamaged leaves (Hassell 1968, Roland 1986).

4.4.2 Parasitism

As predicted, parasitism of FTCs by *L. exul* was similar on both tree species. This was anticipated as *L. exul* flies are attracted to FTC defoliation on both aspen and balsam poplar (Chapter 2) and lay similar numbers of eggs on both plant species (Chapter 3). Parasitism rates are slightly higher on aspen poplar trees, which corresponds to the slightly higher defoliation levels found on aspen poplar trees. Defoliation on aspen poplar trees was approximately 63% greater than that on balsam poplar trees, corresponding to a 68% higher parasitism rate on aspen poplar trees.

Rates of parasitism of FTCs by *P. pachypyga* is significantly higher on aspen poplar trees than on balsam poplar trees, reflecting the pattern of egg abundance on the two tree species. There were three times as many *P. pachypyga* eggs found on aspen poplar than on balsam poplar foliage, but this translated into only a twofold difference in parasitism.

The lack of a direct correspondence between eggs and parasitism probably results from the clumped distribution of eggs on leaf clusters, the ingestion of multiple eggs during a feeding bout, and redundant attacks on the same caterpillar.

Patterns of parasitism correspond closely to those of Stark & Harper (1982) who found that host density was not highly correlated with the degree of FTC parasitism. Despite the common assumption of parasitoid aggregation in areas of high host density, positive density-dependence only occurs in about 25-50% of cases studied (Stiling 1987, and references therein). Roland (1986) found that host density of the winter moth *Operophtera brumata* predicted the number of eggs laid by the tachinid parasitoid *C. albicans* on apple trees and resulting rates of parasitism of host larvae. In the case of *L. exul*, host density mainly effects parasitism through its effect on defoliation. Similarly, *P. pachypyga* appear not to use host density as a direct cue, but rather, focus on stimuli arising from aspen poplar trees. Being attracted to a single tree species allows this species of parasitoid to restrict its search activities and oviposit on a host plant which is the principal food source of the FTC. Because of these two different search strategies by the two parasitoid species, FTCs moving to host plants other than aspen poplar may not escape attack from *L. exul* but may be in relatively enemy-free space with respect to *P. pachypyga*.

Habitat choice by other species of tachinids are known to cause habitat-specific parasitism of their hosts. Habitat-specificity has been indicated for several tachinid stalk borer parasitoids (Felland 1990). The tachinid *Gymnochaeta ruficornis* is most likely to parasitize stalk borer larvae in giant ragweed. *Sympiesis viridula*, however, parasitizes larvae in potatoes and in common ragweed associated with potatoes but it has only been recovered once from a larva in giant ragweed. Monteith (1960) demonstrated that

parasitism of *Pristiphora erichsonii* on *Larix laricina* trees by the tachinid *Bessa harveyi* was highest (74-86%) when the host was in homogeneous *L. laricina* stands but only 35-46% when other tree species were present. This difference was attributed to odor from the other trees producing a masking effect. It was not the presence of the other tree that influenced host finding by *B. harveyi*, but rather, the species of the other tree. The odor of the tree species was not repellent because parasites frequently rested on it and some larvae were parasitized while feeding on its branches. Monteith (1960) further pointed out that the proportion of sawflies escaping attack could be important to maintenance of the overall population, especially in years when parasitism is high.

4.4.3 Conclusions

L. exul lays similar numbers of eggs on aspen poplar and balsam poplar foliage and parasitizes similar numbers of FTCs on both tree species. In contrast, *P. pachypyga* lays significantly more eggs on aspen poplar trees and parasitizes more host larvae on this tree species. As *P. pachypyga* is the dominant parasitoid in central Alberta, caterpillars 'wandering' from one host plant to another to feed in enemy-free space may provide reservoirs of pests to maintain endemic FTC populations. Interactions between FTCs, tachinid parasitoids, and different tree species should be taken into account whenever they are studied in an area that is not botanically homogeneous.

4.5 Literature cited

Barbosa, P., Capinera, J. L., and Harrington, E. A. 1975. The gypsy moth parasitoid complex in western Massachusetts: a study of parasitoids in areas of high and low density. Environmental Entomology 4:842-846.

Bess, H. A. 1936. The biology of *Leschenaultia exul* Townsend, a tachinid parasite of *Malacosoma americana* Fabricius and *Malacosoma disstria* Hübner. Annals of the Entomological Society of America 29:593-613.

Bess, H. A. 1937. Methods used in rearing *Leschenaultia exul*. In: Galtsoff, P. S., Lutz, F. E., Welch, P. S., and Needham, J. G. (chairmen) Culture Methods for Invertebrate Animals. New York, NY: Dover Publications Inc.

Burnett, T. 1958. Effect of host distribution on the reproduction of *Encarsia formosa* Gahan (Hymenoptera:Chalcidoidea). The Canadian Entomologist 90:179-191.

Cerezke, H. F. 1991. Forest Tent Caterpillar. Forestry Canada, Northwest Region, Northern Forestry Centre, Edmonton, Alberta. Forestry Leaflet Number 10.

Embree, D. G. and Sisojevic, P. 1965. The bionomics and population density of *Cyzenis albicans* (Fall.) (Tachinidae:Diptera) in Nova Scotia. The Canadian Entomologist 97:631-639.

Felland, C. M. 1990. Habitat-specific parasitism of the stalk borer (Lepidotera:Noctuidae) in northern Ohio. Environmental Entomology 19:162-166.

Hassell, M. P. 1968. The behavioural response of a tachinid fly (*Cyzenis albicans* (Fall.)) to its host, the winter moth (*Operophtera brumata* (L.)). Journal of Animal Ecology 37:627-639.

Hodson, A. C. 1941. An ecological study of the forest tent caterpillar, *Malacosoma disstria* Hbn., in northern Minnesota. University of Minnesota Agricultural Experiment Station Technical Bulletin 148:55pp.

Leius, K. 1967. Influence of wild flowers on parasitism of tent caterpillar and codling moth. The Canadian Entomologist 99:444-446.

Monteith, L. G. 1960. Influence of host plants other than the food plants of their host on host-finding by tachinid parasites. The Canadian Entomologist 92: 641-652.

Nettles Jr., W. C. 1980. Adult *Eucelatoria* sp.: Response to volatiles from cotton and okra plants and from larvae of *Heliothis virescens*, *Spodoptera eridania*, and *Estigmene acrea*. Environmental Entomology 9:759-763.

Odell, T. M. and Godwin, P. A. 1984. Host selection by *Blepharipa pratensis* (Meigen), a tachinid parasite of the gypsy moth *Lymantria dispar* L. Journal of Chemical Ecology 10:311-320.

Parry, D. 1994. The impact of predators and parasitoids on natural and experimentally created populations of forest tent caterpillar, *Malacosoma disstria* Hübner

(Lepidoptera:Lasiocampidae). M.Sc. Thesis. University of Alberta, Edmonton, Alberta, Canada. 91 pp.

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

Roland, J. 1986. Parasitism of winter moth in British Columbia during build-up of its parasitoid *Cyzenis albicans*: attack rate on oak *v.* apple. Journal of Animal Ecology 55:215-234.

Roland, J. 1993. Large-scale forest fragmentation increases the duration of tent caterpillar outbreak. Oecologia 93:25-30.

Roland, J. and Taylor, P. D. 1995. Herbivore - natural enemy interactions in fragmented and continuous forests. In: Cappuccino, N. and Price, P. W. (eds.). Population Dynamics: New Approaches and Synthesis. San Diego, California: Academic Press Inc.

Ross, D. A. 1953. Key to puparia of the dipterous parasites of *Malacosoma* spp., in British Columbia. Entomological Society of British Columbia, Proceedings 49:19-23.

Sabelis, M. W. and De Jong, M. C. M. 1988. Should all plants recruit bodyguards? Conditions for a polymorphic ESS of synomone production in plants. Oikos 53:247-252.

Schwenke, W. 1958. Local dependence of parasitic insects and its importance for biological control. Proceedings of the Tenth International Congress of Entomology 4:851-854.

Simmons, G. A. Leonard, D. E. and Chen, C. W. 1975. Influence of tree species density and composition on parasitism of the spruce budworm, *Choristoneura fumiferana* (Clem. Environmental Entomology 4:832-836.

Stark, E. J. and Harper, J. D. 1982. Pupal mortality in forest tent caterpillar (Lepidoptera:Lasiocampidae): Causes and impact on populations in southwestern Alabama. Environmental Entomology 11:1071-1077.

Stiling, P. D. 1987. The frequency of density dependence in insect host-parasitoid systems. Ecology 68:844-856.

Tigner, T. C., Palm, C. E. and Jackson, J. J. 1974. Gypsy moth parasitism under and outside burlap skirts at two heights. Applied Forestry Research Institute Report 21:34 pp.

Vet, L. E. M. and Dicke, M. 1992. Ecology of infochemical use by natural enemies in a tritrophic context. Annual Review of Entomology 37:141-172.

Weseloh, R. M. 1981. Relationship between colored sticky panel catches and reproductive behaviour of forest tachinid parasitoids. Environmental Entomology 10:131-135.

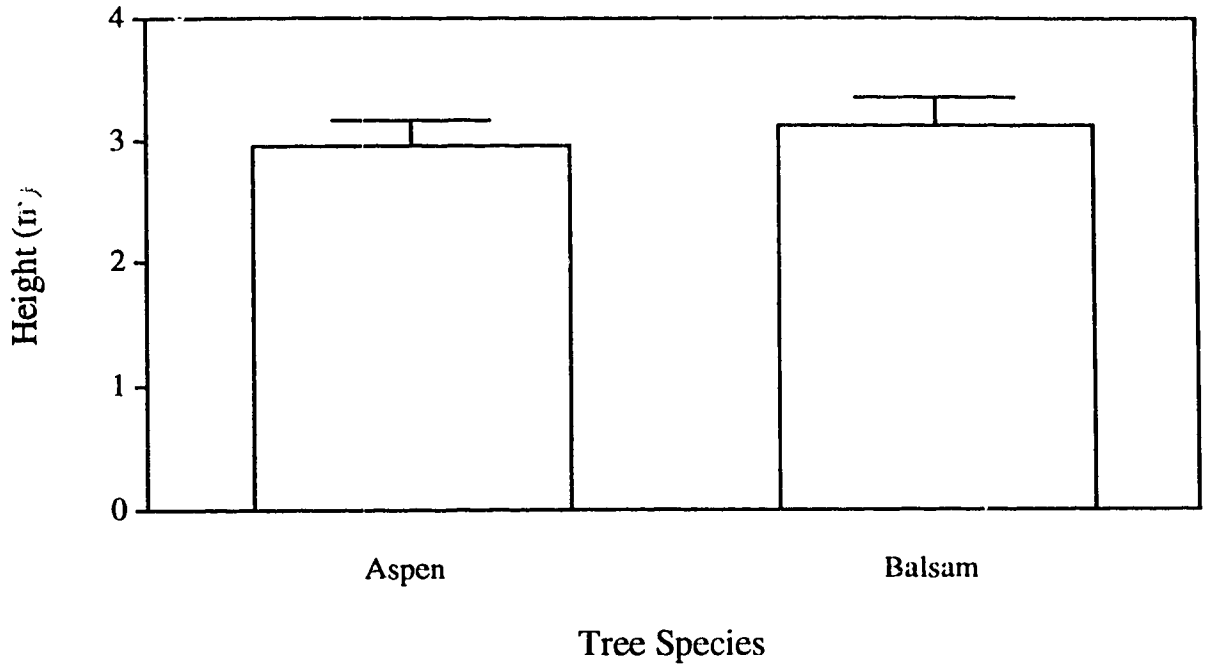

Figure 4.1. Mean height (+ SE) of trees used for leaf cluster sampling.

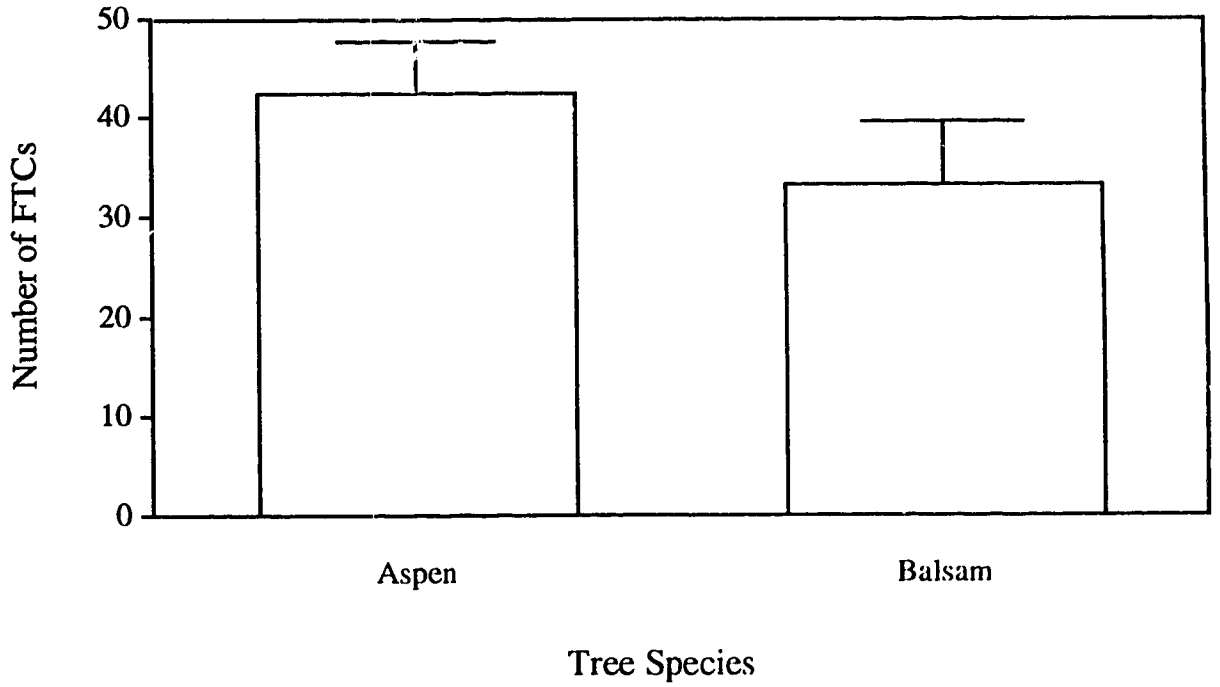

Figure 4.2. Mean abundance (+ SE) of FTC larvae on trees used for leaf cluster sampling.

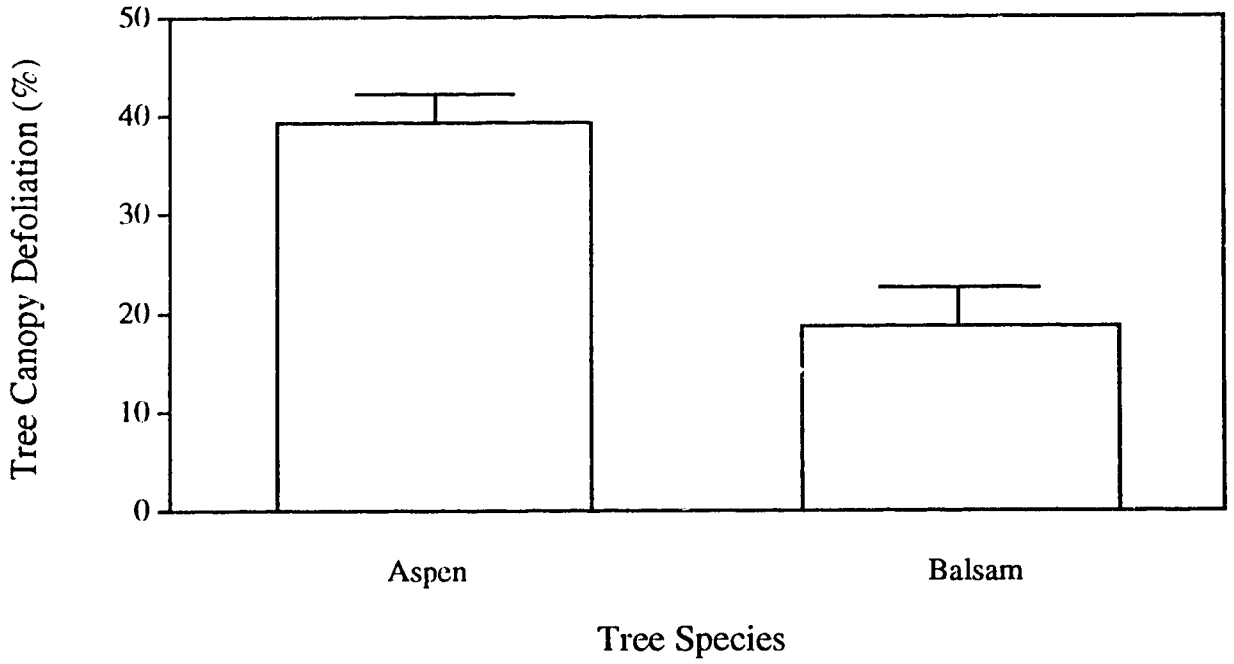

Figure 4.3. Mean defoliation (+ SE) of trees used for leaf cluster sampling.

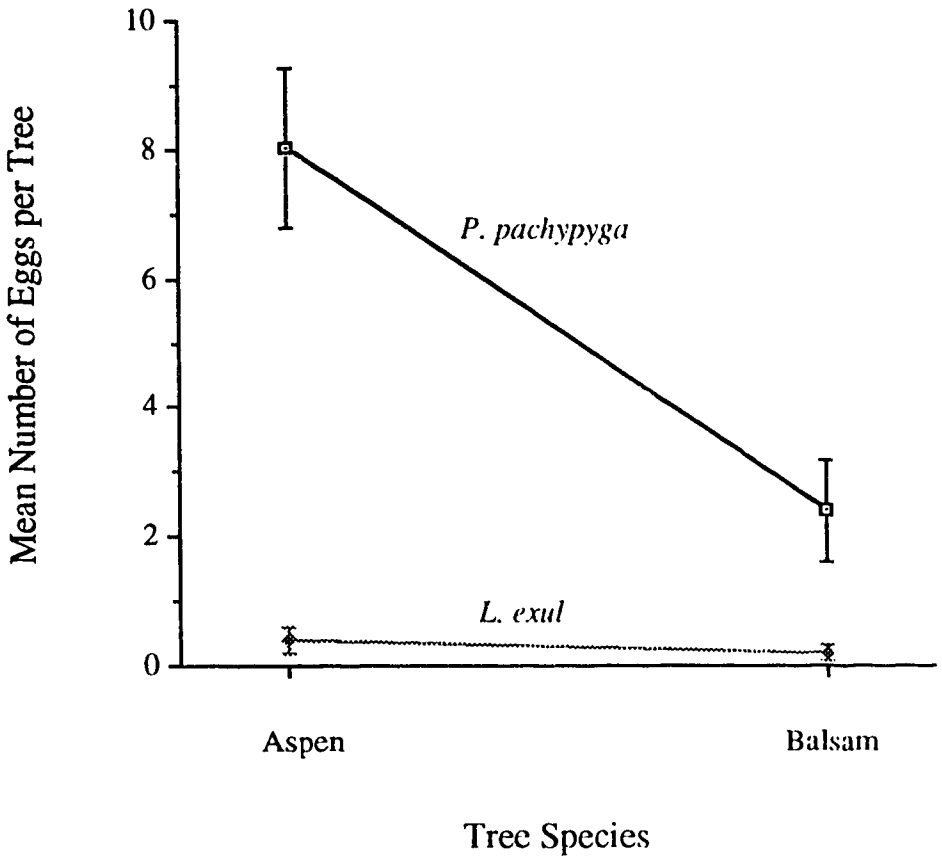

Figure 4.4. Mean number (+/- SE) of eggs laid by *L. exul* and *P. pachypyga* flies on aspen poplar and balsam poplar trees. There is a significant interaction between fly species and tree species (F=8.46, df=1, P=0.0052).

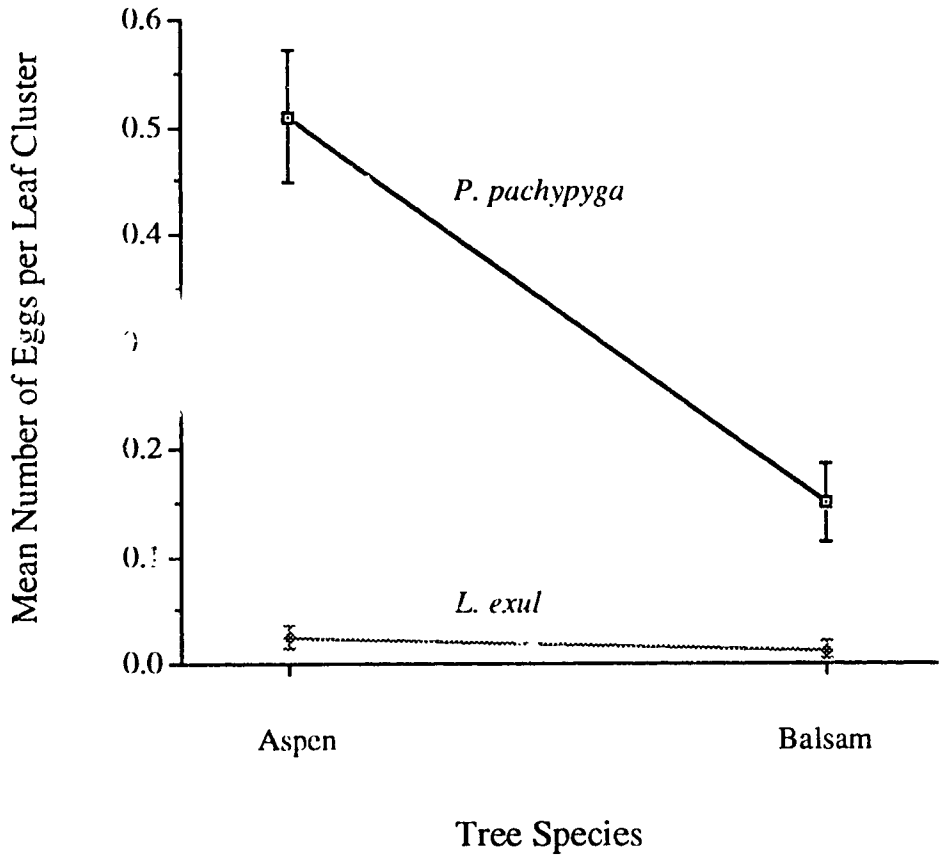

Figure 4.5. Mean number (+/- SE) of eggs laid by *L. exul* and *P. pachypyga* flies on aspen poplar and balsam poplar leaf clusters. There is a significant interaction between fly species and tree species (F=14.2, df=1, P=0.0002).

Figure 4.6. Number of *L. exul* eggs laid as a function of defoliation of aspen poplar leaf clusters.

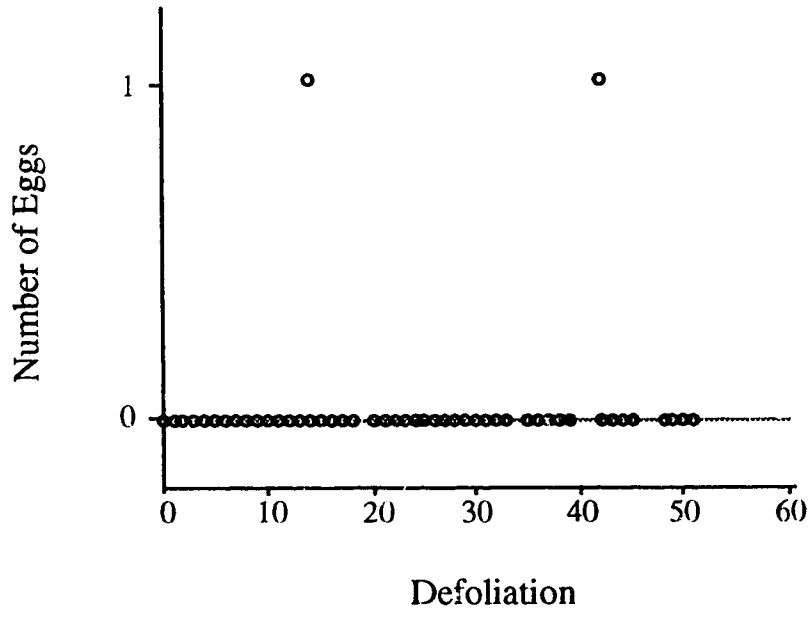

Figure 4.7. Number of *L. exul* eggs laid as a function of defoliation of balsam poplar leaf clusters.

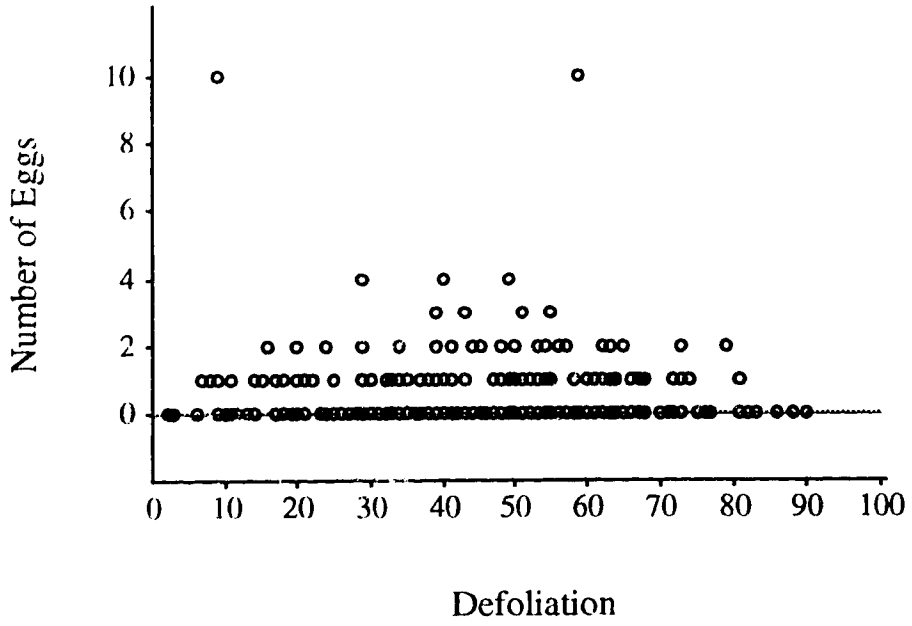

Figure 4.8. Number of *P. pachypyga* eggs laid as a function of defoliation of aspen poplar leaf clusters.

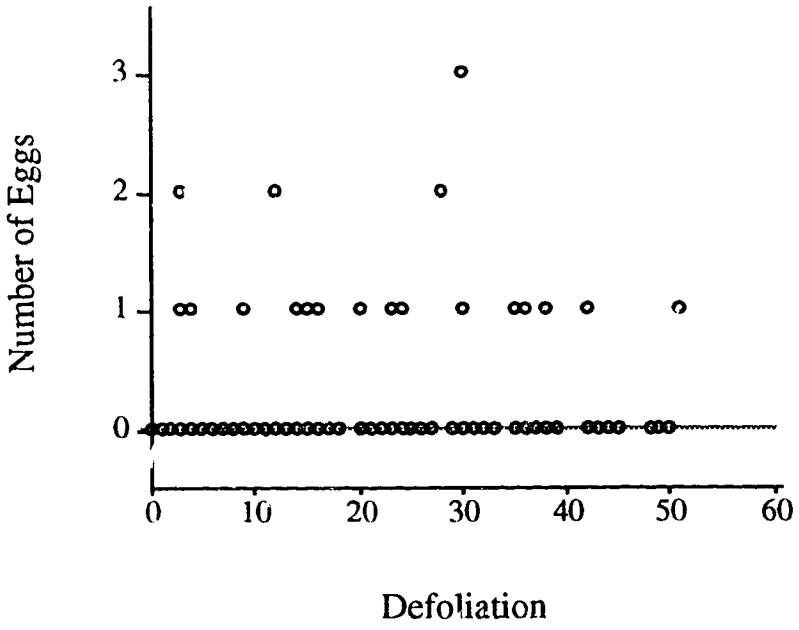

Figure 4.9. Number of *P. pachypyga* eggs laid as a function of defoliation of balsam poplar leaf clusters.

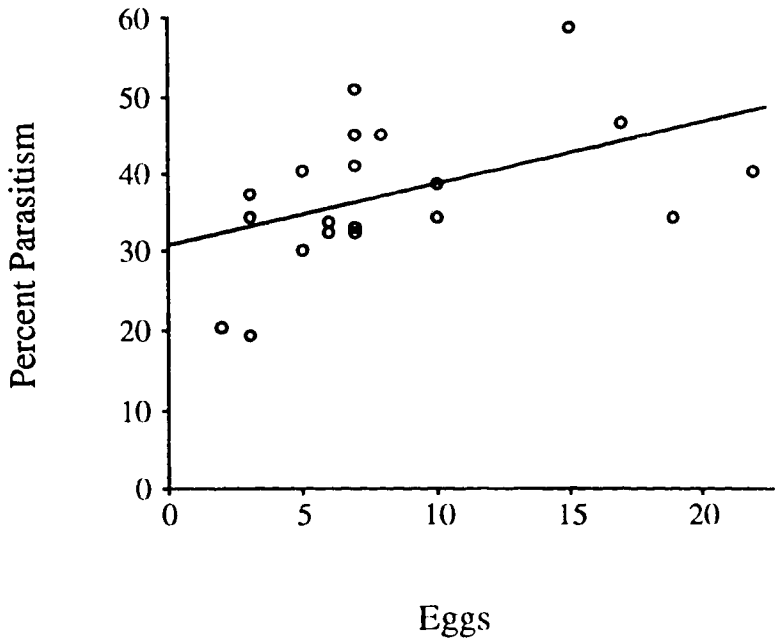

Figure 4.10. Parasitism as a function of the number of eggs laid per tree on aspen poplar trees y = 0.776(eggs) + 30.904, r2 = 0.217.

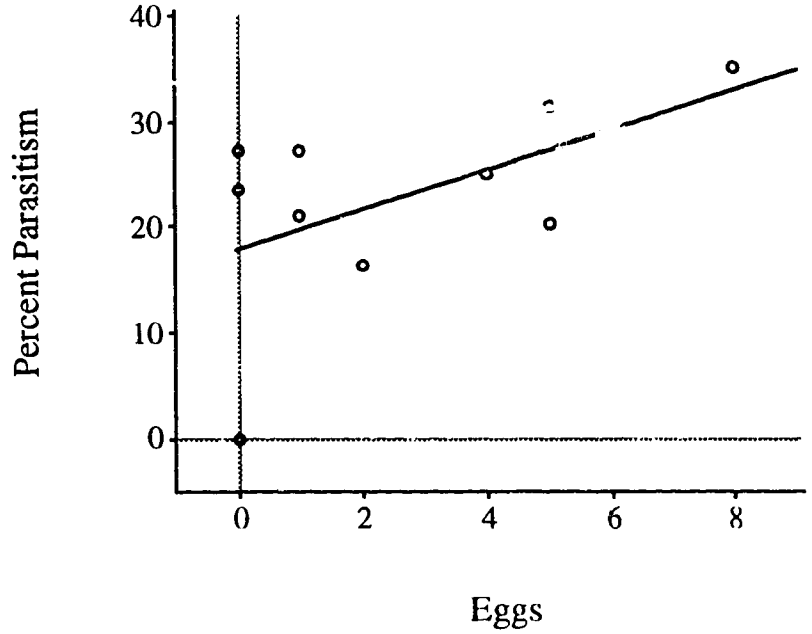

Figure 4.11. Parasitism as a function of the number of eggs laid per tree on balsam poplar trees y = 1 882(eggs) + 17.85, r2 = 0.289.

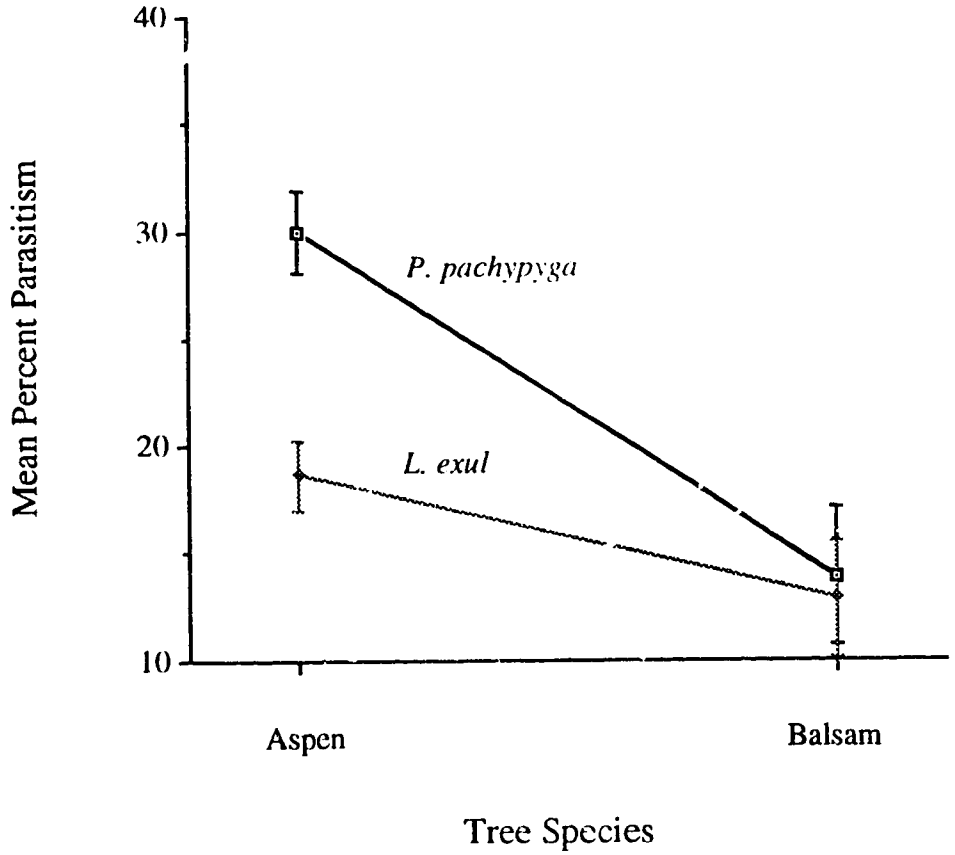

Figure 4.12. Mean parasitism (+/- SE) of FTCs by *L. exul* and *P. pachypyga* flies on aspen poplar and balsam poplar trees. There is a significant interaction between fly species and tree species (F=5.0226, df=1, P=0.029).

Chapter 5 General Conclusions

5.1 Summary

The parasitoids *L. exul* and *P. pachypyga* cause significant parasitism of forest tent caterpillar (FTC) *Malacosoma disstria* populations. Each species, however, responds differently to the host caterpillars feeding on different tree species. The flies differ: (a) in the way they find their hosts, (b) in the number of eggs laid on different species of host plants, and (c) in the resulting parasitism levels they cause. These differences in parasitoid behaviour may have implications for host-parasitoid interactions at both the level of the individual as well as that of the population.

In Chapter 2, I demonstrated that *L. exul* and *P. pachypyga* locate FTCs in different ways. *L. exul* locates its host by being attracted to leaf-damaged plants, regardless of tree species. In contrast, *P. pachypyga* locates host caterpillars by being attracted to the principal host plant of the FTC, aspen poplar trees, but not to balsam poplar trees. These host-finding mechanisms represent two different ways of accomplishing the same task, and both mechanisms have advantages and drawbacks. For example, responding to damage on more than one tree species permits *L. exul* to find its host even if the host is feeding on an alternate host plant species. However, by being attracted to damage on any plant species *L. exul* might waste eggs by responding to leaf damage on some plant species that was not caused by FTCs. Similarly, by responding to leaf damage on aspen trees *P. pachypyga* would be attracted to only the principal host plant of the FTC resulting in an efficient host-finding strategy. However, this fly species might respond to damage on aspen not caused by FTCs, but by other defoliators. Furthermore, if FTCs

'wandered' from the principal host plant to feed on other plant species they would be relatively safe from parasitism by *P. pachypyga*.

In Chapter 3, I examined the oviposition preference of *L. exul* between FTC damage on aspen poplar and damage on balsam poplar leaf clusters. *L. exul* were attracted to both damaged aspen and damaged balsam poplar trees (Chapter 2), and this was reflected in there being no difference in the number of eggs laid on aspen poplar and balsam poplar leaves. The flies laid their eggs on defoliated leaves, regardless of tree species. Gravid females also had similar search strategies on the different plant species as evidenced by behavioural kinematograms. Tarsal contact with recent leaf damage was found to be highly correlated with oviposition on both plant species.

In Chapter 4, I described the spatial distribution of eggs and resulting parasitism rates on field trees. While the number of *L. exul* eggs found on leaf clusters was low, there was no difference in the number of eggs found on aspen and balsam poplar trees, the same pattern as seen in laboratory studies. Parasitism rates by *L. exul* were also not different on aspen and balsam poplar trees. Similar to Parry (1994, 1995) who found the tachinid *P. pachypyga* to be the most important parasitoid attacking FTCs in central Alberta, *P. pachypyga* was the dominant parasitoid attacking caterpillars at my Ministik Hills study site. *P. pachypyga* laid more eggs on aspen poplar trees and caused significantly higher rates of parasitism on aspen poplar compared to balsam poplar trees. This suggests that *P. pachypyga* is more tightly linked to the FTC-aspen poplar system than is *L. exul*.

FTCs feeding on different plant species then experience different parasitism risks from these two tachinid parasitoids. Because *L. exul* parasitizes similar numbers of caterpillars on both aspen and balsam poplar trees, FTCs dispersing from aspen poplar trees to

alternate food sources would not be in enemy-free space from *L. exul*. *P. pachypyga*, however, parasitizes greater numbers of caterpillars on aspen poplar; thus FTCs 'wandering' to feed on balsam poplar trees would be 'wandering' into relatively enemy-free space from this parasitoid. As *P. pachypyga* is the dominant parasitoid in central Alberta, the greater selective pressure imposed on FTCs by *P. pachypyga* may result in a maintenance of 'wandering' behaviour from one plant species to another upon reaching an instar susceptible to parasitism.

5.2 Future research

Numerous questions remain to be answered concerning the FTC, tachinid parasitoid, poplar tree tritrophic interaction. First, FTCs feed preferentially on aspen poplar tree leaves when compared to balsam poplar leaves. The caterpillars risk of parasitism, however, is substantially higher on aspen poplar compared to balsam poplar trees. Therefore, the ecological and evolutionary constraints on caterpillar foraging is, in part, derived from a trade-off between foraging efficiency and parasitism risk. It would be advantageous to determine exactly when the benefits of feeding on a higher quality food source are outweighed by the risk of being parasitized. Questions related to this topic are: Do caterpillars avoid tree leaves that have been previously fed upon, thereby avoiding parasitoid eggs? Do parasitoids emerging from FTCs that have been feeding on balsam poplar trees have smaller sizes, lower fecundity, and lower fitness than parasitoids using aspen poplar feeding larvae as hosts?

Second, the behavioural determinants of FTC larval 'wandering' have not been examined (Fitzgerald 1995). Parry and Spence (unpublished data) have suggested that larval 'wandering' is not related to food availability or conspecific density but due to selective

pressures imposed by avian predators at low larval densities (Parry 1994). While I have presented evidence for habitat-specific parasitism on aspen poplar trees which possibly enhances a behavioural adaptation to move into relatively enemy-free space, other factors such as defensive secondary compound production in plants may also encourage increased larval movement. Secondary compound production in aspen poplar trees in response to FTC feeding damage is poorly known. Studies have focused on plant secondary chemistry in response to simulated herbivory, that is mechanical damage. Real herbivory may have substantially different effects on host plants than does mechanical damage. The interaction between all selective pressures (predators, parasitoids, plant compounds, etc.) on caterpillar foraging and larval movement remain to be identified and ranked in importance.

This is not a comprehensive list of all possible questions yet to be addressed on the FTC, tachinid parasitoid, poplar tree tritrophic interaction. In fact, the research presented in this thesis should be viewed as an introductory foray into this system. Further behavioural studies are required on the parasitoids that help to suppress FTC populations.

5.3 Literature cited

Fitzgerald, T. D. 1995. The Tent Caterpillars. Ithaca, New York: Cornell University Press.

Parry, D. 1994. The impact of predators and parasitoids on natural and experimentally created populations of forest tent caterpillar, *Malacosoma disstria* Hübner (Lepidoptera:Lasiocampidae). M.Sc. Thesis. University of Alberta, Edmonton, Alberta, Canada. 91 pp.

Parry, D. 1995. Larval and pupal parasitism of the forest tent caterpillar, *Malacosoma disstria* Hübner (Lepidoptera:Lasiocampidae), in Alberta, Canada. The Canadian Entomologist 127:877-893.

Appendix 1.

The existence of zeroth and first order dependencies were first analyzed using the complete 3x3 transition matrix, analyzed as a contingency table (e.g. aspen poplar):

Succeeding Behr.	Touch	Oviposit	Change
Preceding Behr.			
Touch	110	51	10
Oviposit	48	60	23
Change	15	21	21

$\chi^2 = 51.846$, df = 4, $P = 0.0001$

Then test the single behaviours that occur repeatedly, designated by '*':

Succeeding Behr.	Touch	Oviposit	Change
Preceding Behr.			
Touch	110*	51	10
Oviposit	48	60*	23
Change	15	21	21*

Then test the behavioural transitions, designated by '*':

Succeeding Behr.	Touch	Oviposit	Change
Preceding Behr.			
Touch		51*	10*
Oviposit	48*		23*
Change	15*	21*	

The existence of second order dependencies were analyzed using a complete 3x9 transition matrix, analyzed as a contingency table (e.g. aspen poplar):

Succeeding Behr.	Touch	Oviposit	Change
Preceding Behr.			
Touch/Touch	39	49	10
Touch/Oviposit	22	21	5
Touch/Change	3	2	3
Oviposit/Touch	44	3	0
Oviposit/Oviposit	19	28	11
Oviposit/Change	4	12	5
Change/Touch	15	0	0
Change/Oviposit	5	9	6
Change/Change	6	5	8

$\chi^2 = 96.9295$, df = 16, $P = 0.0001$

Then test the single combinations of behaviours that occur repeatedly, designated by '*':

Succeeding Behr.	Touch	Oviposit	Change
Preceding Behr.			
Touch/Touch	39*	49	10
Touch/Oviposit	22	21*	5
Touch/Change	3	2	3*
Oviposit/Touch	44*	3	0
Oviposit/Oviposit	19	28*	11
Oviposit/Change	4	12	5*
Change/Touch	15*	0	0
Change/Oviposit	5	9*	6
Change/Change	6	5	8*

Then test the behavioural transitions, designated by '*':

Succeeding Behr.	Touch	Oviposit	Change
Preceding Behr.			
Touch/Touch		49*	10*
Touch/Oviposit	22*		5*
Touch/Change	3*	2*	
Oviposit/Touch		3*	0
Oviposit/Oviposit	19*		11*
Oviposit/Change	4*	12*	
Change/Touch		0	0
Change/Oviposit	5*		6*
Change/Change	6*	5*	

Zero counts were not analyzed due to violating the assumptions of the chi-square test.