1

of/de

1

MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS
STANDARD REFERENCE MATERIAL 1010a
(ANSI and ISO TEST CHART No. 2)

National Library
of Canada

Bibliothèque nationale
du Canada

Canadian Theses Service Service des thèses canadiennes

Ottawa, Canada
K1A 0N4

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

Host selection and oviposition behavior of a parasitic wasp,
Eurytoma obtusiventris (Hymenoptera, Eurytomidae)

by

Carol A. Ramey, B.Sc.

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 Biology

Carleton University

Ottawa, Ontario

March 11, 1991

© copyright

1991, Carol A. Ramey

National Library
of Canada

Bibliothèque nationale
du Canada

Canadian Theses Service Service des thèses canadiennes

Ottawa, Canada
K1A 0N4

The author has granted an irrevocable non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of his/her thesis by any means and in any form or format, making this thesis available to interested persons.

The author retains ownership of the copyright in his/her thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without his/her permission.

L'auteur a accordé une licence irrévocable et non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de sa thèse de quelque manière et sous quelque forme que ce soit pour mettre des exemplaires de cette thèse à la disposition des personnes intéressées.

L'auteur conserve la propriété du droit d'auteur qui protège sa thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

ISBN 0-315-68921-8

Canada

The undersigned recommend to the Faculty of Graduate Studies
and Research acceptance of the thesis

Host selection and oviposition behavior of a parasitic wasp,
Eurytoma obtusiventris (Hymenoptera, Eurytomidae)
submitted by Carol A. Ramey, B.Sc. (Co-op)

A thesis submitted to
the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for
the degree of Master of Science

Thesis Supervisor

Chair, Department of Biology

Carleton University
April 26, 1991

ABSTRACT

Eurytoma obtusiventris parasitizes larvae of the fly, *Eurosta solidaginis*, which live in galls formed on the goldenrod *Solidago altissima*. Female wasps have available both *Solidago* -based and *Eurosta* -based cues to find their hosts. Attack occurs before the gall forms. In the absence of host larvae, females landed more often and spent more time on *S. altissima* than *S. canadensis* or *Aster novae-angliae*. Wasps did not select *S. altissima* with host larvae over those without. Females repeatedly inserted the ovipositor while exploring the goldenrods. These insertions probably were sensory, and were usually brief (<30s) while insertions for oviposition were significantly longer (> 2 min). Most ovipositions occurred on plants containing host larvae. It is concluded that the plant provides important cues for host location by *E. obtusiventris*.

ACKNOWLEDGEMENTS

I'd like to thank my supervisor, Hans Damman, for his time, ideas, support and rather weird sense of humor. I'd also like to thank the other members of my committee, George Carmody and Jaroslav Picman for their assistance. JoAnne Whittingham, Shelley Ball, Steve Thomas, Naomi Cappuccino and the Carleton University Microcomputer Response Centre are owed megafavors for technical assistance, as are the regular B.E.C. crowd. The Parsnip Patch crowd deserves many beers for all kinds of technical and not-so-technical assistance. Henk Datema, Ed Bruggink, Art Goodenough and all the staff of the Carleton University environmental labs were a great help, especially at resurrecting lifeless goldenrod seed.

Thanks is due to John Hayward and my parents, Virginia and Len Thomas for providing me with the impetus to start graduate school. Thanks to John and Ted Hagmeier for assistance in obtaining funding; it saved me once or twice along the way. Financial support was provided by the National Sciences and Engineering Research Council.

Ruth Lifeso and Grey Merriam provided emotional support when I needed it the most, and expected it the least.

Finally, this thesis is dedicated to my husband, Darrin Ramey, whom I used to have a life with. I promise him a life together again soon, to do all the things that have been on the back burner for the past two years. I appreciate all his patience and support. I truly wouldn't have made it without him.

TABLE OF CONTENTS

	Page
Acceptance Sheet	ii
Abstract	iii
Acknowledgements	iv
Table of Contents	v
List of Tables	vi
List of Figures	vii
 Introduction	 1
 Methods and Materials	 14
 Results	 23
 Discussion	 49
 Conclusions	 67
 References	 68

LIST OF TABLES

TABLE	DESCRIPTION	PAGE
1	Contents of preliminary galls collected in Ottawa.	15
2	Contents of galls collected at five sites in 1989 and 1990.	25
3	Oviposition records of plants that formed galls in 1989.	27
4	χ^2 analysis of the first plant landed on by wasp females.	31
5	Nested analysis of variance for the number of landings on different plant species.	32
6	Nested analysis of variance for the amount of time spent on different plant species.	35
7	Goodness of fit test for the number of landings on <i>S. altissima</i> with and without host larvae in 1989.	39
8	Kruskal-Wallis statistic for the amount of time on <i>S. altissima</i> with and without host larvae in 1989.	41
9	Goodness of fit test for the number of pokes and ovipositor insertions on <i>S. altissima</i> with and without host larvae in 1989.	42
10	Analysis of variance for landings, time on plant, number of pokes and ovipositor insertions on <i>S. altissima</i> with and without host larvae in 1990.	44
11	Kruskal-Wallis statistic for the duration of ovipositor insertions on <i>S. altissima</i> with and without host larvae in 1989.	46
12	Mann-Whitney U-test for duration of ovipositor insertions on <i>S. altissima</i> with and without host larvae in 1990.	48

LIST OF FIGURES

FIGURE	DESCRIPTION	PAGE
1	Emergence dates of insects from galls collected in 1989 and 1990.	24
2	Gall diameter and height by contents for Albion and Ithaca site 1.	29
3	Number of landings on <i>S. altissima</i> , <i>S. canadensis</i> and <i>A. novae-angliae</i> .	33
4	Amount of time spent on <i>S. altissima</i> , <i>S. canadensis</i> and <i>A. novae-angliae</i> .	34
5	Number of pokes and ovipositor insertions on <i>S. altissima</i> with and without hosts in 1989.	43
6	Number of ovipositor insertions on <i>S. altissima</i> with and without hosts in 1989.	45
7	Hypothesized sequence of host location for <i>E. obtusiventris</i> .	51

INTRODUCTION

The ability of insects to locate hosts is crucial to the survival of all species. This includes the selection of plants by herbivorous insects and of host insects by their parasites and predators. Recent work has made it clear that these two situations cannot be dealt with in isolation (Price, 1980). The selection of plants by herbivores can be influenced by natural enemies (Damman, 1987). Similarly, locating a host's food plant is a critical step for many parasites and predators (Vinson, 1976). While this paper will concentrate on the location of host insects by parasitoids (i.e. those parasites which kill their host insects), many of the processes apply equally well to the location of plants by insect herbivores. In both cases the insect not only needs to locate a host, but needs to optimize its search behavior to find a sufficient quality and quantity of hosts for survival. In the case of parasitoids, searching involves ovipositing females finding host insects (usually herbivores) for their offspring to live on. For many parasitoids, a female's choice of oviposition site determines the individual host insect on which her offspring will live. Thus, the success of her offspring depends on a female's ability to select suitable hosts. A female should tailor her behavior to maximize the number of hosts successfully parasitized (Jaenike, 1978). A female may use random or non-random search patterns. Non-random searching can increase the search efficiency over random searching. Non-random searching may occur in response to stimuli correlated to the host insect.

Host-insect selection by parasites (including parasitoids) encompasses three steps: a) host habitat location, b) host location, and c) host acceptance (Vinson, 1976). Most parasitoids will locate a host insect's habitat as the first step in searching. Parasitoids may not choose the most acceptable host, but

one in a preferred habitat. The ability of a parasitoid to disperse, especially through flight, will influence its range of habitat choices. Choice of habitat is limited for those with little or no flight ability. A variety of environmental parameters such as temperature, humidity, light, or wind can also affect habitat location: e.g., *Pieris napi japonica* larvae suffer less braconid parasitism on shaded plants than well-lit ones, and *Pieris rapae* larvae are immune to attack when on foodplants hidden by overshadowing vegetation (Sato and Ohsaki, 1987).

While habitat choice is generally made in flight, a parasitoid may locate an individual host insect while in flight or after landing on a plant. *Sympeisis sericeicornis*, a parasitoid of leafminers, detects mines during flight (Casas, 1989). Alternatively, *Cardiochiles nigriceps* does not react to chemicals from its host insect before physical contact is made (Vinson, 1976). Parasitoids may detect the host insect itself or may detect other cues related to the host. For example, *Apanteles kariyai* responds to the fecal pellet of its host (Takabayashi and Takahashi, 1989).

One of the important cues used by many parasitoids in habitat and host location is the food plant of the host insect. Locating a host's food plant, rather than searching all available plants at random, may increase the searching efficiency of a parasitoid. Many parasitoids are attracted to the food plant of the host in the absence of host larvae. *Itopectis conquisitor* lands more frequently on Scots pine than on red pine, even in the absence of host larvae, explaining the higher parasitism rate on Scots pine (Arthur, 1964). The braconid parasitoid, *Peristenus pseudopallipes*, shows a clear attraction for its host's

food plant in an olfactometer (Shahjahan, 1974), as does the ichneumonid, *Campoletis sonorensis* (Elzen, *et al.*, 1983). *Cardiochiles nigriceps*, a parasitoid of the tobacco budworm, is attracted to the tobacco plant regardless of the presence or absence of the budworm (Vinson, 1975). Some parasitoids respond to the host insect's effect on the plant. *Drino bohemica* preferentially attacks host larvae on damaged plants, indicating an ability to distinguish between plants of a single species, based on a criterion that reflects the likelihood of host presence (Monteith, 1964).

Parasitoids of endophytes presumably use host-related cues, rather than the host insect itself, because the host insect (and its odors) are enclosed inside plant tissue. Plant odors are capable of masking the odors of even external phytophagous insects (Monteith, 1960). Some endophytes, such as leaf-miners and gall-makers create visible signs of their presence, which may be used by enemies. Many endophytes, such as stem-borers, do not leave visual cues for parasitoids to use. For these parasitoids particularly, the host's food plant may be an important cue in host location.

Insects rely on a variety of sensory inputs to locate their hosts. Habitat selection may involve long range cues, which are detected before the insect lands on a plant. Long range cues may be chemical, vibrational or electromagnetic (such as color or infrared detection), including visual determination of plant or host-insect morphology. Host location before landing may also involve long-range cues. After landing, short-range cues are used. Short range cues may also be chemical or vibrational, or include tactile cues, such as shape, pattern and texture. Vinson (1984) proposes three strategies of

host location by parasitoids. The parasitoid may detect its host insect directly from long range by sound or chemical cues. Alternatively, a series of stimuli might each progressively add to each other as a parasitoid approaches its host. Finally, a series of discrete stimuli, each leading to the next may lead a parasitoid to the host.

Sensory input may be detected by a parasitoid in a variety of ways. Antennae are important in detection of long- and short-range chemical cues, as well as in detecting tactile stimuli (Vinson, 1975). Tarsi may be used to detect tactile stimuli, such as host-generated vibrations. There is evidence that sight is used by some species. The ovipositor is used as a sensory organ by many species. What it detects is not well understood, but it is known to respond to both chemical and physical cues (Vinson, 1985). Wasp parasitoids may use any combination of these stimuli (Vet and van Alphen, 1985).

Price (1980) states that at least three trophic levels, plants, herbivores and the natural enemies of the herbivores, must be considered in understanding plant-herbivore interactions. While the above discussion of host location by parasitoids emphasizes the importance of the host's food plant to searching parasitoids, the relationship must also be considered from the other perspective, that is, that natural enemies, including parasitoids, may be exploited and encouraged by plants as a defence against herbivores. Abrahamson *et al.* (1983) postulate that parasitoids may be part of a goldenrod's battery of defenses against the gall-fly, *Eurosta solidaginis*. It is clear that the gall-fly is detrimental to the plant, resulting in fewer resources being allocated to seed production (Abrahamson *et al.*, 1988). While overall

parasitism reduces this cost to the plant, the effects of individual enemies are unknown (Stinner and Abrahamson, 1979). If a natural enemy, such as a parasitoid) lessens the impact of herbivory on a plant, and if a plant character encourages that enemy, there should be a selective pressure for that character. Plants may act on the herbivore or the enemy to influence their interaction in a number of ways. Plants may decrease their nutritional quality or produce specific compounds that slow herbivore development. In some cases this may benefit the plant by prolonging larval developmental time, thus increasing mortality from natural enemies (Moran and Hamilton, 1980). Plants may attract enemies with chemical cues, such as food or non-food compounds, or with physical cues. Alternatively, plants may impede natural enemies with some of the traits which are used to impede herbivores (Price, 1980). Such morphological features as trichomes, hairs or secreted resins may inhibit enemy movement, while plant toxins ingested by herbivores may render them toxic to natural enemies (Vinson, 1976).

Plants should most effectively exploit the parasitoids of their herbivores whenever the plant is an important search cue for the parasitoid. It would be expected *a priori* that this would be true for the parasitoids of non-galling or non-mining endophytic herbivores, because the herbivore is enclosed in the plant and leaves no obvious external signs of its presence. Hawkins (1988) reports that leaf miners and gall formers support more parasitoids than external feeders or other endophytes do. This suggests that other endophytic herbivores are well concealed from searching parasitoids. Therefore, indirect cues, such as the food plant of the herbivore, may be important to these parasitoids.

An important criterion in choosing a parasitoid of an endophytic herbivore for study would be its ease of collection. Parasitoids may be more easily obtained than predators, because they are contained within the herbivore. One easily collected parasitoid of an endophyte is the wasp, *Eurytoma obtusiventris* (Eurytomidae), which attacks larvae of the ball gall-fly *Eurosta solidaginis* (Tephritidae). *E. obtusiventris* attacks young gall-fly larvae, before the gall is formed. Therefore, when the wasp searches for the herbivore, the gall-fly larva is equivalent to a non-galling endophyte. However, the formation of the gall later in the season makes insect collection easy. This is an ideal system to use to examine questions about herbivore location by parasitoids, particularly questions about the role of the herbivore's food plant in habitat and host location. In the case of *E. obtusiventris*, finding the gall-fly's food plant probably encompasses habitat location, as adult wasps emerge from the same galls the larvae develop on, and thus are already in a goldenrod clone. The food plant of the herbivore may also be important in host location because at the time of attack by the wasp, because there is no gall for wasps to use as a cue.

The ball-gall fly and its natural enemies

Larvae of *E. solidaginis* form ball galls on stems of several species of goldenrod, including *Solidago altissima* (Abrahamson *et al.*, 1983) *S. canadensis* (Hartnett and Abrahamson, 1979), *S. gigantea* (Miller, 1959) and *S. ulmifolia* (Miller, 1959). The gall-fly has a wide distribution, ranging

from British Columbia to Nova Scotia. In the United States it is found south to Virginia, and west to Texas and Washington (Uhler, 1951; Miller, 1959). Generally, different species of goldenrod are used as food plants in different parts of its range; but the use of species names, especially *S. altissima* and *S. canadensis* is not consistent (Semple and Ringius, 1983) and there is some ambiguity as to which plant species are used in different areas. In northeastern North America, the gall-fly uses *S. altissima* and *S. canadensis*. In Ontario, these plant species are morphologically distinct. While at least eleven species of goldenrod are found in the Ottawa, Ontario area (Semple and Ringius, 1983), *S. altissima* is used almost exclusively by ball-gall flies, though a few specimens have been found on *S. canadensis* (H. Damman, personal communication).

The life history of *E. solidaginis* was detailed by Uhler (1951). Adults emerge from the galls in May and June, mate, then oviposit between the leaves of the terminal buds on young goldenrod. Females may be distinguished from males by the presence of an ovipositor at the caudal tip of the abdomen. Eggs hatch in the terminal bud, then the new larvae tunnel into the stem, where they develop over one year. Gall-flies pass through three larval instars during the summer, then overwinter as third instar larvae.

In response to the second instar larva, the goldenrod stem swells to form the ball-gall (Walton, 1988). The gall is induced in the plant by the gall-maker and gall size is a heritable character of the fly (Weis and Abrahamson, 1986). However, plant genotype affects the duration of the period between oviposition and gall formation (Weis and Abrahamson, 1985; Weis and Gorman, 1990) with

late cohorts (plants which delay gall formation) having smaller galls. Thus, the gall phenotype is influenced by both plant and gall-maker genomes.

In late summer the third instar larva chews an exit tunnel from its cavity to the surface of the ball-gall, leaving a thin membrane over the entrance (Uhler, 1951). The larva pupates in the early spring and the adult emerges through the prepared exit tunnel.

Plant selection by gall-flies is not random (Anderson *et al.*, 1989). Resistance to gall-flies is a heritable genetic trait of the plant (Maddox and Root, 1987). Resistant genotypes produce necrotic tissue around the larva, while susceptible ones are taller and grow faster at the time of oviposition (Anderson *et al.*, 1989). Walton *et al.* (1990) confirm that gall-flies preferentially puncture tall plants. The rate of gall formation is higher on tall plants. When gall-flies are forced to oviposit on an unpreferred clone in the greenhouse, one third of the plants form galls (Abrahamson *et al.*, 1988). Variance in puncturing by gall-flies is primary in determining levels of gall formation (Anderson *et al.*, 1989). Gall-fly survival, and the overall pattern of dispersion, does not change with density (McCrea and Abrahamson, 1987). While 77% of the variance in gall-fly density is explained by plant density, there is no density dependant response by the gall-fly to the plant (Abrahamson *et al.*, 1983; Walton, 1988). Levels of infestation, oviposition and the level of gall formation differ between clones (McCrea and Abrahamson, 1987; Anderson *et al.*, 1989).

Part of plant discrimination by gall-flies occurs after ovipositor insertion (Anderson *et al.*, 1989). Twenty-three percent of all plants punctured receive no eggs. This suggests that female gall-flies use the ovipositor to discriminate

plants. Ritter and Vasey (1989) found that the gall-fly ovipositor has trichoid sensillae, which act as mechanoreceptors, campaniform sensillae, which have a chemosensory function, and small ampulla-like structures of unknown function.

The gall has no effect on the total production by the plant, but does decrease the allocation to leaves and inflorescences while increasing the allocation to the shoot (Hartnett and Abrahamson, 1979). The resources allocated to vegetative reproduction are increased. Nitrogen and phosphorus but not potassium are concentrated in the gall (Abrahamson and McCrea, 1986), but nutrient supplements do not increase gall growth. In addition, supplemental nutrients do not increase plant resistance to the gall maker (Abrahamson *et al.*, 1988), or affect gall or larva size and growth. Stinner and Abrahamson, (1979) report that galls attacked by any of three insect enemies (which they collectively call parasites) accumulate less energy than parasitized ones. Increasing parasitism results in decreased energy and nutrient cost to plant (Stinner and Abrahamson, 1979). Parasitized galls concentrate nitrogen but not phosphorus over the levels of galls containing unparasitized gall-flies (Abrahamson and McCrea, 1986). Weis and Abrahamson (1985) report that parasite attack does not stop gall growth and thus does not benefit the plant. They conclude that the observed energy savings in parasitized galls is due to the increased vulnerability of smaller galls to parasitoids. They do not report on the effect of parasitism on plant reproduction, or the effect of individual enemy species.

Two parasitoids, *Eurytoma gigantea* and *E. obtusiventris* attack *E. solidaginis*. Larvae of the beetle, *Mordellistena unicolor*, live in the goldenrod stem and are predators of all gall inhabitants (Uhler, 1951). Other predators include the downy woodpecker (*Picoides pubescens*) and the black-capped chickadee (*Parus atricapillus*) (Uhler, 1951).

E. obtusiventris and *E. gigantea* females are morphologically distinct; *E. obtusiventris* is 3.5 mm long (Gahan, 1934), while *E. gigantea* is about 6 mm (Walsh, 1870), with a large, sharply pointed abdomen. The male of *E. gigantea* is undescribed.

Bugbee (1967) surmised that *E. obtusiventris* is parthenogenetic. There is a single report of a dead *E. obtusiventris* male, from a gall dissected in August, in London, Ontario (Judd, 1953). However, he does not describe it or say how it was distinguished from *E. gigantea* males, which also would have been present in the gall at that time of year. The life history of *E. obtusiventris* has not been detailed, but it reportedly pupates in May (Hughes, 1934). Females emerge in the late spring shortly after *Eurosia*. Uhler (1951) describes it as an endoparasitoid, which induces the gall-fly larva to pupate in the late summer. The wasps overwinter as larvae in the gall-fly puparium. Galls parasitized by *E. obtusiventris* have a characteristic resinous interior (Milne, 1940). The females have short ovipositors (length = 2.69 ± 0.19 mm) (Weis and Abrahamson, 1985). Despite this, all gall-fly larvae are vulnerable to *E. obtusiventris*, because oviposition occurs in the first instar larva, before gall formation begins (Abrahamson *et al.*, 1989). This is reflected in a random distribution of *E. obtusiventris* among early and late cohorts of galls (Weis and

Abrahamson, 1985). *E. obtusiventris* does not affect gall growth and is randomly distributed among galls of various sizes (Weis and Abrahamson, 1985). While all larvae are vulnerable to attack by *E. obtusiventris*, the wasp is not randomly distributed among galls in the spring (Abrahamson, *et al.*, 1989). *E. obtusiventris* tends to emerge from larger galls, as do gall-flies, probably due to attack of *E. gigantea* (21.9% of *E. obtusiventris* mortality) and *M. unicolor* (28.6% of *E. obtusiventris* mortality) on smaller galls. Abrahamson *et al.* (1989) conclude that *E. obtusiventris* places no selective pressures on the gall-flies for gall size. Cane and Kurczewski (1976) report that *E. obtusiventris* occurs on lower galls.

Other enemies of the ball gall-fly include *E. gigantea* which emerges in July to attack gall fly larvae after galls have reached their maximum size (Weis and Abrahamson, 1985). *E. gigantea* can only parasitize small galls because of their short ovipositors (Weis *et al.*, 1985; Weis and Abrahamson, 1985). Thus, *E. gigantea* tends to be concentrated on late cohorts. This creates a selection pressure favoring gall-flies that form larger galls early in the season (Abrahamson *et al.*, 1989). Cane and Kurczewski (1976) report that 0.4% of galls contained both wasp larvae. This is less common than expected by chance, probably because the *E. gigantea* larva destroys the *E. obtusiventris* larva. Parasitized and unparasitized gall-fly larvae may be consumed by the beetle, *M. unicolor* at any time during the season. Parasitoid density (*E. obtusiventris*, *E. gigantea* and *M. unicolor*) increases with gall-fly density. As in the case of gall-flies, there is no density dependence (Abrahamson *et al.*, 1983).

Chickadees and downy woodpeckers are common predators of ball-galls in Ontario (Schlichter, 1978). Downy woodpeckers make small regular holes and tend to attack at the forest edge, while chickadees make large irregular holes and attack throughout a field (Schlichter, 1978). It was initially proposed that birds gain access to the gall by enlarging the exit tunnel (Moeller and Thogerson, 1978). Subsequently, it has been shown that downy woodpeckers drill their own hole 47% of the time (Confer and Paicos, 1985). Downy woodpeckers show a preference for larger and taller galls at some sites (Moeller and Thogerson, 1978; Confer and Paicos, 1985; Walton, 1988) and prefer stems with multiple galls (Cane and Kurczewski, 1976). Birds attack a greater proportion of galls in fields that have larger galls, though bird attack is not correlated with gall density (Abrahamson *et al.*, 1989). Bird selection for larger galls (Abrahamson *et al.*, 1989) counteracts the selection by *E. gigantea* for smaller galls (Weis and Abrahamson, 1985).

Parasitoids seem to have some immunity to birds. Downy woodpeckers abandon galls with living larvae 34% of the time if the contents are parasitized, but only 12% of the time if they are not (Confer and Paicos, 1985). In addition, because downy woodpeckers use the exit tunnel for 53% of their excavations, *E. obtusiventris* should suffer considerably less predation, as it does not make exit tunnels. Cane and Kurczewski (1976) report that downy woodpeckers rarely consume *E. obtusiventris*, possibly because the premature pupa or the varnish like coating inside parasitized galls is distasteful.

E. obtusiventris could use either *Solidago* - based or *Eurosta* -based cues to locate gall-fly larvae, or a combination of both. One would suspect a

priori that the plant would be an important cue to *E. obtusiventris*. *E. obtusiventris* adults, emerging from dead *S. altissima* stems, are already in the correct habitat for their larvae to develop, so they need only locate *S. altissima* within that habitat. The ball-gall is not visible at the time of *E. obtusiventris* attack, so it is not a cue for host location. Additionally, as the host is enclosed inside the stem it is unlikely that *E. obtusiventris* homes in directly on a chemical, and certainly not a visual cue from the gall-fly. This leaves plant-based cues as the most likely ones to lead *E. obtusiventris* to its host.

My objective was to determine which cues were important to *E. obtusiventris* in locating host larvae. A comparison of the response of wasps to three plant species, *S. altissima*, *S. canadensis* and *Aster novae-angliae*, determined if selection of plants by wasps was random, or if there was a preference for *S. altissima* over other old field species. Secondly, host presence/absence experiments determined if selection of *S. altissima* was random, or if there was a preference for *S. altissima* containing gall-fly larvae over those without host larvae.

METHODS AND MATERIALS

Insect collection and rearing

The data were collected over the summers of 1989 and 1990. Preliminary samples of galls were collected in April, 1989 at four sites in the Ottawa area: Conroy Road pit, Baseline Road, Dolman Ridge Road, and Albion Road. I dissected these galls to determine the proportion with living contents. I used this to estimate the number of galls needed to obtain adequate numbers of insects for the experiments. Preliminary samples of galls in Ottawa contained from 25% to 90% living contents (Table 1), with 0% to 65% being pupae. At the end of April all gall-flies were in the pupal stage (Uhler, 1951), so the total number of pupae should include the pupae of gall-flies and both *Eurytoma* parasitoids. Previous censuses in Pennsylvania (Abrahamson *et al.*, 1989) and New York (Cane and Kurczewski, 1976) have shown that galls may contain up to 25% *E. obtusiventris*.

Based on the preliminary samples, 400 galls were collected from May 5 to 8, 1989, at Albion road, Ottawa. I expected up to 100 *E. obtusiventris* to emerge from these galls. Galls were taken at random along transects through the field. Because *E. obtusiventris* has not been reported previously in the Ottawa area, additional galls were collected at the nearest reported site. In early May, galls were collected at two sites near Ithaca, N.Y. (Ithaca sites 1 and 2). In February, 1990, galls were collected at two additional sites (numbers 3 and 4) near Ithaca, N.Y. Gall length and width were measured to ± 0.1 mm using Manostat calipers. At Albion road and Ithaca site 1 gall and plant height

Table 1: Contents of preliminary galls collected in Ottawa on April 26 and 27, 1989. Empty galls contained nothing or dead insects. Sample sizes are given under the site name. Larvae were probably all *Mordellistena*, and pupae should represent a combination of *Eurosta* and *E. gigantea* and *E. obtusiventris*.

	Site			
	Conroy	Albion	Dolman	Baseline
	(37)	(8)	(52)	(57)
% Empty	10.8	75.0	53.9	43.9
% Larvae	24.3	25.0	23.1	14.0
% Pupae	64.9	0.0	23.1	42.1

were measured. Each gall was placed in a 40 or 100 dram plastic vial and sealed with a plastic cap. Holes were punched in each cap, to allow ventilation. In 1989, a 5 x 5 cm square of organdy fabric was placed under the cap and the vials were housed in wooden racks outside the glasshouses at Carleton University, Ottawa. This arrangement resulted in the vials filling with rainwater, so in 1990, the vials were capped in rows on large sheets of cotton, ranging from about 0.5 x 0.5 m to 0.5 x 1.3 m in size. The edges of these sheets were stapled to wooden frames, and stood upright outside the glasshouses. This arrangement was lighter and stronger than the 1989 method, and allowed the vials to sit horizontally rather than vertically, so that they did not tend to fill with rainwater. At the end of the 1989 season, galls that formed over the summer were labelled and placed outside the glasshouses for the winter. Galls were covered with cloth sleeves to discourage predatory birds. In the spring of 1990 the galls were retrieved and housed in vials like the field collected galls. At the end of the season the field-collected galls which had bird damage from the previous winter were dissected.

Seed collection and plant rearing

Plants used in my research were reared in glasshouses from field-collected seed. Seed was collected in the winters of 1989 and 1990 from *Solidago altissima* stems with ball galls, and from *Aster novae-angliae* and *Solidago canadensis*. The seeds were germinated at four dates in early spring in one part sandy loam, two parts peat moss, and one part Perlite. After about two weeks of growth, seedlings were transferred to four or six inch plastic pots.

Plants were watered twice daily and in 1989 fertilized once a week with Plant Prod 20/20/20. In 1990 plants were fertilized only once, immediately after transfer to the larger pots. The goldenrod and aster seed germinated quickly in 1989 and plants grew vigorously, resulting in development well ahead of that in the field. Most plants from the first three germination dates were in flower or bud at the time of gall-fly emergence. In 1990, germination was not obtained until the later plantings, resulting in plants which were developmentally behind the field goldenrods.

Insect emergence, mating and oviposition

Galls were checked every two to three days in the spring until the first insects emerged, then they were checked every morning. On emergence the vial containing the insect was moved into the glasshouse. They were provided with 2 pieces tissue paper, one saturated with water and the other with approximately 25% honey solution. As gall-flies emerged one male and one female were placed together in a vial until they copulated. As time permitted, females were mated to a second male.

Mated gall-fly females were placed in plastic cylinders, 10 cm in diameter, containing a *S. altissima* plant. The cylinders were either 30 cm or 45 cm high. The tops were covered with plastic mesh and there were two 5 cm ventilation holes in the sides per 10 cm of height, also covered with plastic mesh. Females were left in the cylinders until they had oviposited at least twice. Ovipositor insertion was easily determined as each insertion of the ovipositor left a noticeable scar on the growing tip. Gall-flies oviposited on 171 *S.*

altissima in 1989, and 75 in 1990. Gall-flies were also placed in cylinders over *S. canadensis* to determine if they would oviposit on that species. I kept insects and plants in separate glasshouses to prevent indiscriminate oviposition by escaped gall-flies. In 1989, gall-flies would not oviposit on *S. altissima* once the flower buds had begun to form. Gall-flies oviposited readily on the plants in 1990. No oviposition occurred on *S. canadensis*.

Because there was no published data showing that *E. obtusiventris* is parthenogenetic, and because the male of *E. gigantea* is undescribed, I made no assumptions about the gender of the *E. obtusiventris* which emerged or the species of any male wasps which emerged. To determine if there are two superficially similar sexes of *E. obtusiventris*, I placed newly emerged *E. obtusiventris* in vials with each other. When wasps matching the description of the males in this genus began to emerge, their species was determined by placing them in vials with *E. obtusiventris* alone and with both *E. obtusiventris* and *E. gigantea* females. The wasps were observed for several hours and matings were noted.

Response to different plant species

To determine if wasps selected plants randomly, equal numbers (either two or three) of *S. altissima*, *S. canadensis* and *Aster novae-angliae* were positioned randomly in a 0.8 m³ cage. The cage consisted of a wooden frame covered on four sides with cotton gauze. The front and one side were covered with plexiglass to allow observation of the interior. The availability of *S. canadensis* and *A. novae-angliae* in addition to *S. altissima* provided

E. obtusiventris females with a choice of plants that reflected the choice in the field. *S. canadensis* has been reported as a food-plant for ball-gall flies in various portions of their range. *A. novae-angliae* is not a food-plant, but is common in Ottawa fields, and has a growth form similar to *Solidago*. Preliminary work suggested that plant height affected plant choice. Wasps never landed on shorter plants so plants were placed on boxes, such that the tops all were approximately the same height. A vial containing a wasp was placed near the center front of the floor of the cage and the top was removed. The wasp was observed and her behavior recorded for approximately 1 hour. To determine if pre-landing choice of plants was random, the first plant that each female chose was recorded. I considered a plant chosen if a female walked or flew onto the plant and did not immediately leave it again. I recorded the total number of landings on each plant species. If a female did not land on at least one plant during the hour in the cage, the run was not used in the analysis. I noted the time that a female spent on each plant, allowing me to estimate the total time a female spent on each species. Behaviors were either timed with a stopwatch and recorded on paper or into a tape recorder, or were entered directly on to an Atari Portfolio computer which automatically recorded the time.

Host presence/absence experiments

Equal numbers of *S. altissima* on which gall-flies had oviposited, and *S. altissima* that had never been exposed to gall-flies were placed in the cage to determine if wasp behavior on *S. altissima* was independent of the presence

of gall-fly larvae. In 1989 two plants of each treatment were used. In 1990 three plants were used. Experiments were conducted as soon as *E. obtusiventris* began to emerge and continued until all the wasps had died. At this time gall-fly larvae in the experimental plants should have been at about the same stage of development as in the field, i.e. in the first instar, prior to gall formation. Manipulations and observations were conducted as in the plant-species experiment. In addition to the number of landings and time on plant, the number of ovipositions and the duration of ovipositions were compared. I scored oviposition when a female poked the plant with the tip of her abdomen and then inserted her ovipositor. The numbers of pokes and ovipositor insertions were counted and analyzed separately. Ovipositor insertions were timed from the moment that a female pressed her abdomen against the plant until she walked away. During this time she usually remained motionless and I assumed that her ovipositor was inserted in the plant for the entire time.

E. obtusiventris oviposits before the gall becomes visible, so I was unable to tell whether gall-fly oviposition had been successful at the time my host presence/absence experiments were conducted. In 1989, 19 out of the 171 *S. altissima* exposed to gall-flies developed galls (11.1%). Many runs of the experiment were done with goldenrods that never developed galls. These runs were eliminated, leaving five runs on one combination of plants. Females landed on at least one plant in four out of five runs of the experiment. The run in which the female did not land on any plant was not used in analysis. To accommodate variable gall-fly oviposition success, the plants were divided into three treatments: a) No gall-fly oviposition indicates *S. altissima* which were never exposed to gall-flies, b) Unsuccessful gall-fly oviposition or Not

Successful oviposition indicates plants exposed to gall-flies, which never developed a gall, and c) Successful oviposition, consisting of plants which developed galls after exposure to gall-flies. In 1990, two out of 75 *S. altissima* developed galls (2.67%). By chance, neither of the two galled plants were chosen for the experiments in 1990. Therefore, galls were classified as Unsuccessful oviposition and No oviposition. Thirteen runs of the experiment were used in the analysis.

Statistical Analyses

Gall diameter and height were compared between the three sites used in 1989, and between the inhabitants of the galls by analysis of variance (ANOVA). Data from the two years of the experiment were compared by nested ANOVA, with the years nested in the treatments. This tested for differences between the two years in the wasps' response to a given treatment.

Wasp behavioral data were tested to see if they conformed to the assumptions for ANOVA, particularly the assumption of homogeneity of variance. Data for the plant species experiments met the assumptions, so the number of landings on each species of plant and the time spent on each species were compared by ANOVA using the SuperANOVA 1.1 program for the Macintosh computer. A χ^2 analysis was done, using StatView 1.2, to test if the choice of first plant was random.

The post-hoc categorization of the plants in the 1989 host presence/absence experiment resulted in an unbalanced design; there was one plant with successful gall-fly oviposition, one with unsuccessful gall-fly

oviposition and two with no gall-fly oviposition in the cage during each run. The assumptions for ANOVA are robust as long as the group sample sizes are equal (Zar, 1984). However, the assumption of homogeneity of variance must be strictly adhered to in unbalanced designs. The 1989 host presence/absence data did not meet the assumptions for ANOVA, so the discrete data, such as the number of landings and pokes and ovipositor insertions, were analyzed by replicated goodness-of-fit tests (G-statistic). The G-statistic determines if the overall significance of the data (G_T) is consistent across the replicates by separating out the heterogeneity (G_H) of different replicates (females) from the total statistic (G_T), leaving the statistic for the pooled data (G_P) (Sokal and Rohlf, 1981). Continuous data, such as the time on plant and duration of ovipositor insertion were analyzed by Kruskal-Wallis test, which compares the ranks of the data, using the StatView program. The 1990 host presence/absence data were analyzed by ANOVA, as the design was balanced and the assumptions were met, except for the duration of the ovipositor insertions, which were analyzed by Mann-Whitney U-test. Differences were considered significant if $\alpha < 0.05$.

RESULTS

Insect emergence and mating

In both 1989 and 1990 gall-flies emerged first, in late May and early June (Figure 1). Males began emerging at the same time or a few days before the females. In 1989, the proportion of flies emerging from the galls ranged from 2.0% at Ithaca site 1 to 12.1% at Ithaca site 2 (Table 2). In 1990 the gall-fly emergence was similar to 1989, except that about 23% of all galls at each site contained gall-flies, giving a total of 201 flies. Males and females mated readily regardless of which site they were collected from.

Three morphologically distinct wasps emerged from the galls. One to three weeks after the emergence of the gall-flies, wasps matching Gahan's (1934) description of *E. obtusiventris* began to emerge (Figure 1). About two weeks later *E. gigantea* females emerged. The third type of wasp was similar to *E. obtusiventris* in size and body shape, except that the antennae were densely covered with long hairs and that each antennal segment tapered towards the distal end. These were determined to be males, as this antennal structure is characteristic of other males of this genus (Walsh, 1870). These males mated readily with the *E. gigantea* females. No matings were observed for *E. obtusiventris*, either with these males or between *E. obtusiventris* individuals. This confirms that all the males were *E. gigantea* and that there are not two morphologically similar sexes in *E. obtusiventris*.

No *E. obtusiventris* emerged from the galls collected in Ottawa (Table 2). For this reason, no galls were collected in Ottawa in 1990. The level of

Figure 1: Emergence dates of insects from galls collected in 1989 and 1990. Bars enclose the first to last dates of emergence. Each site is shown as a separate bar, in top to bottom order, as listed in Table 2. Sites with no emergence (Table 2) are omitted. Dates when galls first appeared are indicated with arrows for each year ('89 and '90).

Table 2: Contents of galls collected at 5 sites for 1989 and 1990. The percentage of galls containing each species is given, as is the total number of galls collected at each site.

	Site				
	1989 Albion	Ithaca1	Ithaca2	1990 Ithaca3	Ithaca4
<i>Eurosta</i>	7.5	2.0	12.1	23.8	22.9
<i>E. gigantea</i>	7.1	0.9	3.1	6.6	0.0
<i>E. obtusiventris</i>	0.0	3.0	3.8	0.6	0.0
<i>M. unicolor</i>	4.8	24.8	14.3	5.0	0.6
<i>Schizoprymnus</i>	5.3	0.0	3.0	6.1	0.6
Bird	9.8	0.0	0.0	11.2	0.0
Other	1.8	0.0	1.5	0.8	1.2
Total	400	101	133	623	164

E. obtusiventris emergence from the Ithaca galls ranged from 0.6% at sites 3 and 4 in 1990 to 3.8% at site 2 in 1989.

In addition to the gall-flies and wasps, significant numbers of *M. unicolor* and *Schizoprymnus* sp. emerged. *Schizoprymnus* is mentioned as being present in London, Ontario galls (Judd, 1953) but is not mentioned by any other author. Judd (1953) reported that it was probably a parasitoid of *M. unicolor*. Both *E. gigantea*, and *Schizoprymnus* readily mated in vials. Miscellaneous contents included several unidentified chalcids and the hymenopterans *Heteropilus languriae* (identified by Marsh), *Lasioptera* sp. (identified by J. Huber) *Ancistrocerus* sp., *Glypta* sp. (identified by D. Wahl), and *Dibrachys* sp. and *Ophiomyia* sp.

Levels of bird predation on galls ranged from 0% to 11% (Table 2). In Ithaca galls, all holes were small and regular, as is characteristic of woodpecker attack. The Ottawa galls showed evidence of both downy woodpecker and chickadee predation. Thirty-nine galls were opened by birds. Twenty-seven showed large, irregular holes characteristic of chickadees, 9 showed woodpecker holes and 3 were indeterminate. Dissection of the galls indicated that both birds found the central chamber without using the gall-fly exit tunnel. Chickadees used the exit tunnel exclusively 8 times, while woodpeckers used it 7 times. Three galls attacked by chickadees had a second hole in addition to the one through the exit tunnel.

Nineteen galls were created in the 1989 experiments (Table 3). One was accidentally destroyed during the summer of 1989. Of the 18 left outside for the winter, 13 were recovered in the spring of 1990 and 5 could not be found. Six gall-flies and one *E. obtusiventris* emerged from these galls and were used

Table 3: Oviposition records for plants which formed galls in 1989 and emergence record for 1990. Numbers separated with a "/" (ie 55 / 443) indicate that two individuals oviposited on a plant. Es: *Eurosta solidaginis*, Eg : *Eurytoma gigantea*, Eo : *Eurytoma obtusiventris*. Plants B5 and B26 could not be distinguished from each other in the spring. A gall-fly male emerged on May 25 and a female on May 28 from that pair of galls.

Plant	Gall-fly oviposition		Other oviposition		Winter	1990 Spring emergence
	Number	Date	Number	Date		
A3	330	May 31			Broken off	
A4	443	May 31			Outside	
A17	55 / 443	June 5	Eg 195	July 7	Outside	
A20	355 / 332	June 7	Eo 26 / 93	July 7	Outside	Es female May 25
A22	129	June 1			Outside	
B2	330	June 3			Outside	
B4	55	June 7	Eo 26	June 30	Outside	Es male May 24
B5	55	June 6	Eo	June 27	Outside	Es m/f May25/28
B6	H1	June 12	Eo 26 / 93	July 7	Outside	Lost
B13	55	June 8	Eo 26 / 93	July 7	Outside	Eo June 14
B26	330	June 2	Eg RT16	July 10	Outside	Es m/f May25/28
B28	355	June 7	Eo 81	June 30	Outside	Es male May 27
B30					Outside	
D2	443	May 31			Outside	
D4	443	May 31			Outside	
D7	443	June 3			Outside	Es female May 27
D16	1/ 55	June 13 / 7	Eo 26 / 93	June3/July1	Outside	
D18	355 / 332	June 6	Eg RT11	July 6	Outside	
E6	443	May 31	Eo 446	June 16	Outside	

with insects from the Ithaca galls in the 1990 experiments. Thus, unmated *E. obtusiventris* produced viable eggs.

Effect of gall size and position

Gall diameter and height differed significantly at the three sites used in 1989 ($p = 0.0001$), so data were not pooled. At Ithaca site 2, where only gall diameter was measured, there was no difference in the diameters of galls with different inhabitants. At the other sites, 84% of the variance on plant height was explained by gall height ($r^2 = 0.84$), so only the gall-height data will be presented. At Ithaca site 1 (Figure 2), galls containing *E. obtusiventris* were lower on the the stem than galls with other contents. At this site the mean diameter of galls containing *E. obtusiventris* was intermediate (15.5 mm) between the other classes of galls. At both Ithaca site 1 and Albion road, empty galls were smaller than galls with living inhabitants. At Albion road, galls attacked by birds were larger and higher than those birds did not attack.

Response to different plant species

There was no difference between 1989 and 1990 in the number of landings on each plant species ($p = 0.91$) or the amount of time spent on each species ($p = 0.41$), so data for the two years were pooled for further analysis. In the plant species experiments, *E. obtusiventris* females spent $79 \pm 21\%$ (mean $\pm$ s.d.) of their time on the cage walls or roof. There was no difference between 1989 and 1990 ($t_{16} = -0.821$, $p = 0.42$).

Figure 2: Gall diameter (open bars) and gall height (shaded bars) (mean $\pm$ s.e.) by gall contents (or fate) for galls collected at Albion and Ithaca site 1 in the spring. Bars with an * are significantly different from other galls. At Albion, the diameter ($p = 0.0001$) and height ($p = 0.039$) of galls attacked by birds, and the diameter of galls with nothing in them ($p = 0.003$) were significantly different from all other galls. At Ithaca, the height of galls attacked by *E. obtusiventris* ($p = 0.024$) and the diameter of galls with nothing in them ($p = 0.017$) were significantly different from all other galls.

A χ^2 -test indicated that wasp females prefer *S. altissima* over both other species. Females chose *S. altissima* as the initial landing site more often than *S. canadensis* or *A. novae-angliae* (Table 4). Females landed more frequently on *S. altissima* than on *S. canadensis* or *A. novae angliae* (Table 5). Females landed equally frequently on *S. canadensis* and *A. novae angliae* (Figure 3). In spite of significant day-to-day and female-to-female variation in the total number of landings, females consistently preferred *S. altissima* over the other two species. There was no plant species by female interaction (Plant sp. * Eo), indicating that the response was consistent among all females (Table 5). A similar trend was reflected in the amount of time that *E. obtusiventris* spent on the three species (Figure 4), but differences between females and differences between dates were not significant (Table 6). Again, there was no female by plant species interaction, indicating that the response was consistent in all females. Females spent more time on *S. altissima* than *A. novae-angliae*, but the time spent on *S. canadensis* was not significantly different from either of the other species.

Oviposition behavior

Ovipositor insertions occurred on *S. altissima* with successful gall-fly oviposition, unsuccessful gall-fly oviposition and no oviposition by gall-flies. As females moved over the plants they continuously tapped the surface of the plant with their antennae. Females moved up and down the stem and growing tip several times exploring all sides of the plant. Searching was concentrated on

Table 4: χ^2 analysis of the first plant species landed on by each wasp female.
Observed number of landings is the sum of all first landings on each species.

First landing	Plant species			Σ
	<i>S. altissima</i>	<i>S. canadensis</i>	<i>A. novae-angliae</i>	
Observed	12	3	4	19
Expected (E_i)	6.33	6.33	6.33	19
Chi-Square = 7.68 d.f. = 2 p = 0.02				

H_0 : The first plant landed on is a random choice, thus the probability of a female landing first on any species is 1/3.

Table 5: Nested ANOVA testing for differences between the number of landings on *S. altissima*, *S. canadensis* and *A. novae-angliae*, for different species of plants, different wasp females, different dates nested in female (Date (Eo)) and the plant by wasp interaction (Plant sp.*Eo). d.f. = degrees of freedom.

Source	d.f.	Sum of Squares	Mean Square	F-Value	P-Value
Plant species	2	29.674	14.837	11.561	0.0005
<i>E. obtusiventris</i>	8	43.527	5.441	4.240	0.0041
Date (Eo)	7	130.167	18.595	14.490	0.0001
Plant sp. * Eo	16	23.130	1.446	1.126	0.3952
Residual	20	25.667	1.283		

Figure 3: Number of landings (mean $\pm$ s.e.) on *Solidago altissima*, *S. canadensis* and *Aster novae-angliae* by wasp females (n = 19). Linear comparisons of means (Table 5) indicate *S. altissima* was different from *S. canadensis* (p = 0.0011) and *A. novae-angliae* (p = 0.0002), but the latter were not different (p = 0.5305).

Figure 4: Amount of time (mean $\pm$ s.e.) spent on *S. altissima*, *S. canadensis* and *A. novae-angliae* by wasp females (n = 19). Linear means comparisons (Table 6) indicate wasps spent more time on *S. altissima* than *Aster* (p = 0.01), but *S. canadensis* was not different from *S. altissima* (p = 0.12) or *Aster* (p = 0.23).

Table 6 : Nested ANOVA testing for differences in the amount of time (min) spent on *S. altissima*, *S. canadensis* and *A. novae-angliae*, for different species of plants, different wasp females, different dates nested in female (Date (Eo)) and the plant by wasp interaction (Plant sp.*Eo). d.f. = degrees of freedom.

Source	d.f.	Sum of Squares	Mean Square	F-Value	P-Value
Plant species	2	470.473	235.236	4.070	0.0329
<i>E. obtusiventris</i>	8	778.979	97.372	1.685	0.1639
Date (Eo)	7	213.744	30.535	0.528	0.8028
Plant sp. * Eo	16	508.876	31.798	0.550	0.8855
Residual	20	1155.991	57.8		

the top 1/3 of the plant and on the stem and growing tip. Oviposition involved two distinct steps. First, the wasp flexed her abdomen ventrally, 90° to her thorax, and poked the plant with the caudal tip of her abdomen. Secondly, she pressed her abdomen against the surface of the plant, inserting the ovipositor. This behavioral sequence was repeated as a female moved up the stem. Typically she poked the stem and inserted her ovipositor briefly, then moved a few millimeters up the stem and repeated the sequence. In the two seasons of data collection, poking and ovipositor insertion were observed on *S. canadensis* on only one occasion when a female poked and inserted her ovipositor briefly two times in succession. Females never inserted their ovipositors into *A. novae-angliae*.

In 1989, insertions were divided into two distinct types. Short insertions (75.8% of all insertions) lasted less than 1 minute. These insertions occurred on all areas of the stem and growing tip of *S. altissima*. Most of the short duration insertions occurred on the plants with unsuccessful gall-fly oviposition or no oviposition by gall-flies. Longer insertions, lasting between 2 and 6 min in length, represented 15.2% of all insertions. These long insertions occurred on all three treatments of *S. altissima*, but most were on the plant with successful gall-fly oviposition. The remaining 9.1% were between 1 and 2 minutes and occurred only on the plant with unsuccessful gall-fly oviposition. When the gall developed on the plant with successful gall-fly oviposition, it formed on the area of the stem where females were observed making long ovipositor insertions. Long insertions probably represent actual oviposition. After long insertions females often climbed to the top of the plant or tip of a leaf and groomed themselves. This was usually followed by flight away from the plant. Females

were occasionally observed hovering close to a plant. Females frequently circled plants during flights. If another plant was in physical contact, wasps occasionally walked from one plant to another.

In two cases, long ovipositions represented the first insertion on the stem; one was during the female's first landing on the plant. Female 81 landed on the plant with successful gall-fly oviposition four times. She poked the plant three times, the first two of which were followed by long insertions in the area where the gall eventually formed. Similarly, female 93's first insertion on the plant with successful gall-fly oviposition was a long one in the same area. Both females made short insertions on the plant after the long insertions. In contrast, the other two females made no ovipositor insertions in the plant with successful gall-fly oviposition, despite landing on it twice each.

Host presence/absence experiments

The *post-hoc* categorization of the treatments makes the interpretation of the plant with unsuccessful gall-fly oviposition somewhat involved, as the exact status of each plant at the time of the experiments could not be determined. The plant with unsuccessful gall-fly oviposition represents a situation where either the gall-fly did not lay an egg, or the offspring died sometime before gall formation was stimulated. If the gall-fly did not lay an egg, and there was no plant response to gall-fly ovipositor insertion, then the plant with unsuccessful gall-fly oviposition represented the same treatment as the plants with no gall-fly oviposition. If an egg was laid, and was still alive when the experiment was conducted, the plant represented the same treatment as the plant with

successful gall-fly oviposition. If an egg or larva died before the experiment was conducted, the plant with unsuccessful oviposition could not be classed with either of the other treatments. In 1989 this group consisted of one plant, and could not be classified easily as successful oviposition or no oviposition, as the wasps' responses to it were not consistent with either of these two cases. It may be that a gall-fly larva developed on this plant, but died shortly prior to the experiments. The presence of a dead larva might produce a wasp response intermediate between the response to a living larva (successful oviposition) and no larva at all (no oviposition). In 1990, there were nine plants with unsuccessful gall-fly oviposition, and may have been a combination of the above possibilities. The general low rate of gall formation in 1990 suggested that most of these plants contained no larvae. The lack of a significant difference in the amount of time and number of landings on the plants, group was consistent with this hypothesis (see below).

In 1989, females spent a significant portion of their time ($40.10 \pm 13.74\%$) on the cage. This was only about half the amount of time spent on the cage in plant species experiments. There was no significant difference in the number of times *E. obtusiventris* landed on *S. altissima* with successful gall-fly oviposition, unsuccessful oviposition or no gall-fly oviposition (Table 7). The total G-statistic (G_T), testing the hypothesis that females land in a 1:1:2 ratio on the one plant with successful oviposition, one plant with unsuccessful oviposition and the two plants no gall-fly oviposition, was not significant. Similarly, while three of the four females spent more time on *S. altissima* with successful gall-fly oviposition than the other two treatments,

Table 7: Replicated goodness of fit test (G-statistic) for the number of landings on *S. altissima* with Successful oviposition, Unsuccessful oviposition and No oviposition by gall-flies. Data were transformed to (number of landings +1) for analysis, as zeros result in error in log calculations. d.f. = degrees of freedom.

Data Summary

Female	Number of Landings Gall-fly oviposition			Σ
	Successful	Not Successful	None	
26	2	4	2	8
81	4	1	9	14
93	2	0	2	4
RT1	2	0	2	4
Σ	10	5	15	30

G-statistic

Test	d.f.	G-value	P-value
Pooled (G_P)	2	1.477	0.48
Heterogeneity (G_H)	6	5.627	0.47
Total (G_T)	8	7.104	0.53

H_0 : Females land at random on the three treatments of *S. altissima*, giving a ratio of 1:1:2 for Not Successful:Successful:None. (There were twice as many None plants as Successful and Not successful).

the overall Kruskal-Wallis did not show a significant difference in the amount of time females spent on the three treatments (Table 8). The mean amount of time spent on the plant with unsuccessful oviposition was intermediate between the plant with successful oviposition and those with no gall-fly oviposition. Pairwise Mann-Whitney U-comparisons did not show it to be significantly different from either of the other two treatments.

The frequency of each oviposition behavior, pokes and ovipositor insertions, were almost identical, as poking was almost always followed by ovipositor insertion. Therefore, the two sets of data were interpreted together. In 1989 the overall G-test showed a significant difference (Table 9) in the number of pokes and ovipositor insertions in the three treatments of *S. altissima* (Figure 5). However, there were also significant differences between females (G_H). The overall significance of G_T was due to the response of female 26 alone (Table 9). G-tests of the individual females indicated that only female 26 deviated significantly from the 1:1:2 ratio. The other three females did not deviate significantly from this ratio. In 1990, there was no significant difference in the number of pokes or insertions between the plants with unsuccessful gall-fly oviposition and no gall-fly oviposition (Table 10).

Ovipositor insertions were longer on plants with successful gall-fly oviposition (Figure 6). The overall Kruskal-Wallis test was significant, as were all pairwise Mann-Whitney U comparisons (Table 11), confirming that most long insertions occurred on the plant with successful oviposition, while most short insertions occurred on the plants with unsuccessful oviposition or no gall-fly oviposition. Short insertions (<1 min) are probably sensory, as they were most frequent and occurred on all treatments of *S. altissima*. Long insertions

Table 8: Amount of time (min) wasp females spent on *S. altissima*, with Successful oviposition, Unsuccessful oviposition and No oviposition by gall-flies. a) Summary of data, giving N (sample size), values for individual females (value = total time on treatment/number of plants in treatment), mean, standard deviation, sum and mean of the ranks for each treatment. b) Kruskal-Wallis test for differences in the mean ranks. d.f. = degrees of freedom.

a) Data Summary

Treatment	Time on plant (min)							Σ Rank	Mean Rank
	N	26	81	93	RT1	Mean	Std. Dev.		
Successful	4	1.4	20.5	24.1	20.1	16.53	10.26	46	11.5
Unsuccessful	4	35.8	7.1	0.0	0.0	10.74	17.06	29	7.25
None	8	1.7	7.7	14.1	4.5	7.00	5.91	61	7.625

b) Kruskal-Wallis Statistic

d.f.	2
H corrected for ties	2.147
P-value	0.36

Table 9 : a) Replicated goodness of fit test (G-statistic) for the number of pokes and ovipositor insertions on *S. altissima* with Successful oviposition, Unsuccessful oviposition and No oviposition by gall-flies, with b) G-statistics for each female. Data were transformed to number of pokes (or insertions) + 1 to eliminate calculation errors. P-values given are accurate for the number of pokes and insertions. d.f. = degrees of freedom.

a) Overall G-statistic

Test	d.f.	G-value		P-value (both)
		Pokes	Insertions	
Pooled (G_P)	2	22.256	26.392	< 0.001
Heterogeneity (G_H)	6	36.356	26.717	< 0.001
Total (G_T)	8	58.612	53.109	< 0.001

H_0 : Females poke and insert at random on the three treatments of *S. altissima*, giving a ratio of 1:1:2 for Unsuccessful:Successful:None. (There were twice as many plants with No oviposition as Successful oviposition and Unsuccessful oviposition).

b) Data summary by female

Female	Number of pokes (and insertions)				By Female	
	Successful	Unsuccessful	None	Σ	G	P (both)
26	0 (0)	23 (22)	0 (0)	23 (22)	53.83 (51.22)	< 0.001
81	3 (2)	3 (1)	3 (2)	9 (5)	1.36 (0.71)	> 0.50
93	3 (1)	0 (0)	8 (4)	11 (5)	3.09 (0.85)	> 0.50
RT1	0 (0)	0 (0)	0 (0)	0 (0)	0.34 (0.34)	> 0.75
Σ	6 (3)	26 (23)	11 (6)	43 (32)		

Figure 5: Number of pokes and ovipositor insertions by wasp females on *S. altissima* with Successful oviposition, Unsuccessful oviposition and No oviposition by gall-flies (n = 4). Horizontal bars represent medians, and each data point is shown. Multiple points of the same value are placed side-by-side. While the overall G-statistic was significant ($p < 0.001$), this difference was due to one female only (Table 9).

Table 10: Response of wasps in 1990 to *S. altissima* with Unsuccessful oviposition and No oviposition by gall-flies. a) Data summary and b) nested ANOVA testing for differences in number of landings, time on plant, number of pokes and number of ovipositor insertions by oviposition group (Unsuccessful oviposition and None), female (Eo), Date (nested in female) and the oviposition group by female interaction (Ovip * Eo). d.f. = degrees of freedom.

a) Data summary

	Unsuccessful		None	
	Mean	Std. Error	Mean	Std. Error
Landing	0.949	0.246	1.513	0.254
Time	2.509	0.720	5.685	1.330
Pokes	0.487	0.272	0.872	0.314
Insertions	0.282	0.164	0.962	0.270

b) Analysis of variance

	Source	d.f.	Mean Square	F-Value	P-Value
Landings	Oviposition	1	3.651	1.600	0.2108
	Eo	5	5.046	2.211	0.0648
	Date (Eo)	6	3.538	1.550	0.1770
	Ovip * Eo	5	0.786	0.344	0.8840
	Residual	60	2.282		
Time	Oviposition	1	104.903	2.231	0.1405
	Eo	5	29.889	0.636	0.6733
	Date (Eo)	6	24.318	0.517	0.7931
	Ovip * Eo	5	22.145	1.173	0.3332
	Residual	60	47.026		
Pokes	Oviposition	1	0.478	0.153	0.6972
	Eo	5	3.939	1.260	0.2929
	Date (Eo)	6	7.367	2.357	0.0413
	Ovip * Eo	5	1.883	0.602	0.6982
	Residual	60	3.126		
Insertions	Oviposition	1	0.238	0.135	0.7143
	Eo	5	2.945	1.674	0.1547
	Date (Eo)	6	4.107	2.335	0.0430
	Ovip * Eo	5	1.304	0.741	0.5957
	Residual	60	1.759		

Figure 6: Duration of ovipositor insertions (seconds) by individual wasp females on plants with Successful oviposition, Unsuccessful oviposition and No oviposition by gall-flies. Horizontal bars represent medians, and each data point is shown. Multiple points of the same value are placed side-by-side. Kruskal-Wallis statistic and Mann-Whitney U-tests (Table 10) indicated all groups are significantly different.

Table 11: Comparison of the duration (seconds) of ovipositor insertions by wasp females in 1989 on *S. altissima* with Successful oviposition, Unsuccessful oviposition and No oviposition by gall-flies. a) Kruskal-Wallis test for differences in the mean ranks. b) Paired Mann-Whitney U tests for differences in the mean ranks. Note for a two-tailed test, the larger of U and U' is used to determine the P-value. d.f. = degrees of freedom.

a) Kruskal-Wallis Statistic

d.f.	2
H corrected for ties	10.014
P-value	< 0.05

b) Paired Mann-Whitney U Tests

Comparison	Successful vs Unsuccessful	Successful vs None	Unsuccessful vs None
d.f.	4,21	4,8	8,21
U-statistic	69	29	134.5
P-value	0.05	0.035	0.015

(>2 min) were concluded to be actual ovipositions, as they occurred on the successful oviposition plant (Figure 6), in the area of the stem where the gall eventually developed. Not all insertions on the other two treatments were of the short type. There were three ovipositor insertions on the unsuccessful oviposition plant from 1.05 to 2.42 min in duration. This is longer than most of the sensory insertions but shorter than the insertions classed as actual oviposition on the plant with successful gall-fly oviposition. These insertions were located at the base of the growing tip where any larvae would be located. One long probe (2.85 min) was made on the plants with no gall-fly oviposition, indicating probes of this length can be sensory or that females may erroneously oviposit into stem tissue. In 1990, females also probed both treatments of plants. There was no difference in the length of the insertions on the two treatments of plants (Table 12).

Differences were not significant in the number of landings, or the amount of time wasps spent on plants with unsuccessful oviposition or no gall-fly oviposition in 1990 (Table 10). Similarly, differences in the number of pokes and ovipositor insertions were not significant. There was a significant effect of date on the number of pokes and insertions, but the lack of a female effect or oviposition group by female interaction suggests that the overall response is consistent. In six out of 13 trials, no pokes or ovipositor insertions occurred. A total of 38 insertions were timed. 74% were less than one minute in length. Two insertions over two minutes in length occurred; both were on the plant with unsuccessful oviposition. Differences in the duration of the insertions on the two treatments were not significant (Table 12).

Table 12: Mann-Whitney U test for the duration of ovipositor insertions by wasp females on *S. altissima* with Unsuccessful oviposition and No oviposition by gall-flies in 1990. d.f. = degrees of freedom.

	Sample size	Σ of the Ranks	Mean Rank
Unsuccessful	12	286.5	23.875
None	26	454.5	17.481

d.f.	12,26
U-statistic	208.5
P-value	0.10

DISCUSSION

Host selection by parasitoids involves a series of trade-offs between the quality and the quantity of host insects attacked. A female must choose between ovipositing on every host located, at the risk of her offspring not surviving on hosts of inferior quality, or she may reject inferior hosts, at the risk of not locating a better host later in her search. A female's decision to reject or accept a host should depend on several factors, including the quality of that host, her life span, rate of egg production, and the total number of eggs a female has to oviposit; the probability of locating another potentially more suitable host will depend on the distribution of the host. A female should develop a search behavior which optimizes these trade-offs to maximize the survivorship and fecundity of her offspring (Jaenike, 1978). The search behavior a parasitoid uses is restricted by the life style and behavior of its host. Parasitoids of endophytes probably cannot detect direct visual or chemical cues from the host and must, therefore, develop a search behavior based on other methods of host detection.

Parasitoids may search randomly or non-randomly for their hosts. Different steps in host selection may involve different strategies, and most parasitoids typically use a combination of search strategies. Non-random phases of searching may involve a variety of cues, including chemicals (Vinson, 1984) vibrotaxis, ovipositor searching, and antennal searching (Vet and van Alphen, 1985). Females may use any combination of these cues in locating their hosts. There should be strong selection for females to develop the most efficient search behavior, optimizing each step of the search to find the most and the best hosts available. The data suggest *E. obtusiventris* uses both

random and non-random searching, using a variety of sensory input in locating its host. A summary of the search process of *E. obtusiventris*, as suggested by the data, is presented in Figure 7.

Effect of gall size and position

E. obtusiventris was found in lower galls confirming Cane and Kurczewski's (1976) observation of the same. In the cage, however, females so obviously avoided shorter plants that I equalized the plant heights. This response could have been an artifact of the cage. It is unclear if wasps selected lower plants or inhibited growth resulting in lower galls. The most obvious advantage to lower galls would be the lack of bird predation, which is concentrated on higher galls. *E. obtusiventris* has been reported to avoid bird predation (Cane and Kurczewski, 1976). Choosing lower galls and the lack of an exit tunnel during the winter would facilitate this. Wasps could also avoid birds by using smaller galls, but there is no evidence for this. *E. gigantea* probably consumes some *E. obtusiventris* in smaller galls, and overall survival seems to be less in small galls. Thus, small galls are probably not favoured.

Response to different plant species

E. obtusiventris adults, emerging from ball-galls, are already in the old field where they will encounter the gall-fly larvae. Therefore, habitat selection for this species should be oriented towards locating the correct plant species in a field of many goldenrod and other similar species. *E. obtusiventris* could

Figure 7: Hypothesized sequence of host location for *E. obtusiventris*.

distinguish between old field species in the cage, showing a distinct preference for *S. altissima* over *S. canadensis* or *Aster novae-angliae* in the absence of host larvae (Table 5). Thus, *E. obtusiventris* used plant-based cues as part of its host location behavior.

Wasp females discriminated among plant species before landing, as reflected in the larger number of landings on *S. altissima*. In the artificial environment of the cage, with a limited number of plants, the increased numbers of landings on *S. altissima* could reflect females learning the locations of preferred plants and avoiding other plants during the hour period in the cage. However, females usually landed first on *S. altissima*, indicating wasps preferred *S. altissima* prior to any learning effects. In a dense field of goldenrod this orientation need not be effective over a long distance. The observation of females hovering and circling around plants suggests that they may discriminate between species at relatively close range. This method of discrimination has been established for other parasitoids (Vinson, 1976). The lack of a preference for *S. canadensis* over *Aster novae-angliae* was unexpected because *S. canadensis* is an alternate food plant to gall-flies in some areas, although not in the Ithaca area where *E. obtusiventris* was collected. Gall-flies which use other species of goldenrod appear to be a distinct subspecies (Tim Craig, personal communication). It is possible *E. obtusiventris* is specific to a race of gall-fly on a specific goldenrod species. Alternatively, the confines of the cage may have affected the wasps' ability to discriminate the source of chemical cues.

The specific chemical or physical cues used by *E. obtusiventris* to identify *S. altissima* before landing were not determined. Wasps were tested in an

olfactometer to determine if *S. altissima* leaf or stem tissue emitted attractive chemicals, but wasps would not enter either path (with or without plant tissue) available to them. While this was probably due to an aversion to the olfactometer itself, it does not suggest that there was an overwhelming chemical stimulus attracting wasps to *S. altissima*. The significant number of landings on other species also suggests this. Discrimination of plant species before landing, therefore, could be due to chemical or physical cues or a combination of both. Pre-alighting discrimination leading to non-random landing has been documented in many insects (Rausher *et al.*, 1981). Insects respond to physical characteristics of plants. For instance, *Battus philenor* butterflies orient to the terminal leaf buds of its food plant (Papaj, 1986), and sawflies prefer long shoots as oviposition sites (Craig, *et al.*, 1989). There is also abundant evidence of response to chemical cues, such as the attraction of the wasp, *Camponotus sonorensis*, to volatile and contact chemicals from its host's food plant (Elzen, *et al.*, 1983).

Females also preferred *S. altissima* after landing. Even in the absence of host larvae, wasps stayed significantly longer on *S. altissima* than *A. novae-angliae*, suggesting that females used plant-based cues to distinguish species after landing. *S. canadensis* was indistinguishable from either of the other two species, as reflected in the amount of time wasps spent on the plants. However, females seemed to discriminate *S. canadensis* from *S. altissima*, because only two ovipositor insertions were observed on *S. canadensis* in 22 runs over two summers. No insertions were observed on *A. novae-angliae*, suggesting females could accurately identify this species as well. This discrimination was clearly not based on ovipositor sensation. It was not based

exclusively on the lack of larval vibrotaxis. If plants were rejected based on lack of larval vibrotaxis alone, all three species should have been rejected at the same rate, because none of the plants contained larvae. Thus, antennal sensation was the most likely basis of discrimination. Antennae may detect chemical or tactile cues (Vinson, 1984). *E. obtusiventris* may have detected chemicals on the surface of the plant that are not apparent before it landed. Alternatively, females may have detected morphological differences in the plants, possibly the more general pubescence of *S. altissima* over *S. canadensis* (Semple and Ringius, 1983).

The overall plant selection process of *E. obtusiventris* involved detecting, in flight, a plant likely to be *S. altissima* and landing on it. Short range cues, probably detected through the antennae, were then used to reject non-*S. altissima* (Figure 7).

The interactions between plant, herbivore and the herbivore's natural enemies are not fully understood. Why does *E. obtusiventris* attack before the gall forms, not taking advantage of this obvious cue? Price *et al.*, (1987) suggest that the gall provides nutrition and a suitable microhabitat to the gall-maker. They do not, however, rule out protection from enemies and suggest further investigation. Hawkins (1988) shows that gall-makers have a greater diversity of parasitoids than non-galling endophytes and discounts the protection role. Clancy and Price (1987) show that parasitoids of the leaf-galling sawfly, *Pontania* sp., preferentially attack large galls which contain large host larvae. This suggests that a large gall acts as a cue for enemies, rather than deterring them. If the gall does not confer protection, why does *E. obtusiventris* attack before it forms, and not exploit this obvious cue? The

ball gall induced by the gall-fly is thicker than many other galls (such as the spindle galls produced on goldenrods by *Epiblema scudderiana*) and probably does confer protection from parasitoids. *E. gigantea*, which is many times larger than *E. obtusiventris*, is limited in the size of galls it can attack by its ovipositor length. Thus, *E. obtusiventris* probably attacks before gall formation because it cannot oviposit through the thick gall wall.

For a plant to exploit a parasitoid, the parasitoid must confer a reproductive advantage to the plant, and the plant must have the means to utilize the parasitoid. Establishing that *E. obtusiventris* uses plant cues in host location makes it possible for the plant to influence, and thus manipulate the wasp. It is not in the scope of this study to determine if *S. altissima* does encourage parasitism, but because *S. altissima* is a cue to *E. obtusiventris*, the plant has the means to influence the wasp. While Stinner and Abrahamson (1979) suggest that the overall decrease in the energetic cost of parasitized galls to the plant is because these galls are smaller, they do not discriminate among the beetle and the two wasps. Only galls attacked by *E. gigantea* and *M. unicolor* are smaller than unattacked galls, and thus little can be concluded about the effect of *E. obtusiventris* on the cost of the gall to the plant.

Host presence/absence experiments and oviposition behavior

After selecting *S. altissima*, the second level of discrimination for *E. obtusiventris* is to identify plants with gall-fly larvae. As in the discrimination of plant species, discrimination between *S. altissima* with and without host larvae can occur pre- and post-alighting.

E. obtusiventris did not discriminate between *S. altissima* with or without host larvae before landing on the plant. In 1989, there was no difference in the number of landings on the three treatments of *S. altissima*. Further, the lack of consistency in the response of the four females did not indicate any general trend. Similarly, in 1990, females did not discriminate between plants before landing. Thus, there is no evidence that *E. obtusiventris* was attracted to the plant by chemicals produced in response to gall-fly attack. Such a lack of preference is also shown by *Agathis* sp., a braconid which shows no preference for umbels with host insects over those without (Thompson, 1986).

Post-landing discrimination between *S. altissima*, and host-larva location appears to have occurred by two methods, 1) females could detect a host directly, from cues originating at the gall-fly larvae, or if the host was not detected directly, females either could reject the plant or 2) search it for a host using other cues. In the latter case, a female's decision to remain or abandon the plant would depend on a number of factors. The probability of the presence of a host, and the time required to locate an initially undetected host should be weighed against the chances of finding a host on a new plant. This trade-off would be influenced by the female's ability to detect hosts directly. If all hosts were immediately and directly detectable, then all plants except those with hosts should have been abandoned quickly. If all hosts were not immediately detectable, females should have spent time searching plants, even those without hosts. The time spent searching a plant would depend on the probability of locating a host on that plant versus the probability of finding one in a new search. The data suggest that females utilized both of these strategies.

E. obtusiventris females could directly detect a host larva, but not consistently. In 1989, females located the host directly after landing in at least two cases. Females not only located the correct place on the stem, but made long ovipositions on the first insertion, indicating that females could home in on the larva accurately. Thus, while it is frequently used, ovipositor probing was not an essential search mechanism. Wasps did not locate the host in all cases when it was available, suggesting the direct stimulus was not always present. The most likely cue that would lead a wasp, directly and accurately, to an endophytic host is vibrotaxis. It would indicate not only the host's presence but its location within the plant. Vibrotaxis is also likely to be intermittent, detectable only when the host is active. The braconid parasitoid, *Biosteres longicaudatus*, responds to the vibrations produced when its host, an endophytic fly larva, chews on fruit tissue (Lawrence, 1981). It is unlikely that a chemical cue from the larva would allow wasps to locate hosts directly. It is difficult to envision a larval chemical which could be detected through the stem, with the accuracy indicated by the oviposition behavior. It is also unlikely that such a chemical cue would be intermittent.

In most cases, a gall-fly larva was not located directly, and in this situation females usually searched the plant using their antennae and ovipositor (Figure 7). In 1989 females spent a significant amount of time searching *S. altissima*, even when there was not a host present, rejecting plants without hosts no more quickly than those with hosts. However, three out of four females did spend more time on *S. altissima* with host larvae present and the trend in the means is from plants with successful gall-fly oviposition to plants with unsuccessful oviposition to those with no oviposition. While differences were not significant,

the consistency in the females' response suggests the effect may be real, but not detectable with this sample size. The 1990 data also suggest that wasps did not discriminate between plants with unsuccessful oviposition and those which have never been visited by a gall-fly. The lack of difference could have been due to the plants with unsuccessful oviposition being a combination of treatments, but even if only some plants contained larvae, the mean time spent on these plants should still have been higher than the mean time spent on plants with no gall-fly oviposition if wasps were discriminating between plants with and without hosts. Not only was there no difference in the amount of time wasps spent on plants with unsuccessful and no oviposition, but the mean was lower for the plants with unsuccessful gall-fly oviposition, suggesting that there was no difference in wasp females' response to the treatments. Thus, in the absence of direct host detection, probably through vibrotaxis, females chose to continue to search for larva.

There is no information on how much of the time gall-fly larvae are inactive, and presumably not detectable through vibration. Two out of four wasps exposed to the plant with successful gall-fly oviposition did not locate the larva immediately. Of the two that did locate the larva with their first probe, one located it only during her second landing on the plant. These inconsistent responses suggest that females relying on direct detection alone would miss significant numbers of larvae. The lack of a significant difference in the amount of time spent on the three treatments suggest that females searched all plants equally carefully, whether a larva was detected or not. This behavior would not only increase the chances of finding a host through other cues, but simply by spending more time on the plant females would increase the probability of

detecting a period of larval activity. Clearly, an important method of searching was ovipositor insertions, as evidenced by the large number of short insertions (Tables 10 and 12), and the systematic pattern of stem searching exhibited by some females. The lack of a difference in the number of pokes and ovipositor insertions in all females except one also indicates that females thoroughly searched all plants.

Ovipositor insertion is probably not the only mechanism used in searching. Ovipositor searching was a slow process. The decision to continue probing or to abandon the plant could have been made on a variety of criteria. The data indicate a high degree of variability in the probing response of different females. In 1989, one female probed the plant with unsuccessful gall-fly oviposition to the exclusion of all others, while another did not probe any plant. Similarly, in 1990, while the overall differences were not significant, females were highly variable in their response, probing only half of the available plants with unsuccessful oviposition. This inconsistency suggests that females were using other criteria besides ovipositor insertion to evaluate plants, particularly in deciding how long to continue probing a plant. Possible criteria could include the time spent searching or chemical or physical cues from the plant detected with the ovipositor or antennae. The constant use of the antennae emphasizes their importance to the searching female.

While the search behavior utilized by *E. obtusiventris* in these experiments helps insure that females did not miss inactive larvae, it was highly inefficient. In the plant species experiments, females spent 41.6% of their time on the wrong species of plant. This was better than random ($\chi^2 < 0.001$), which would have resulted in 67% of the total time spent on plant species other

than *S. altissima*. In the host presence/absence experiments females spent 52.1% of their time on *S. altissima* without host larvae. Combining the two experiments, only 28.0% of the wasps' time on plants was spent on *S. altissima* with a host. The cage probably did not accurately reflect the field situation. It is unlikely that a field would contain equal numbers of randomly distributed species. The species composition and relative numbers of each species varies greatly from field to field. Most of the collection sites were dominated by *S. altissima*. Further, goldenrods are clonal plants (Semple and Ringius, 1983). Locating one stem with a larva may indicate a high probability of an adjacent, genetically identical stem also being likely to contain a larva. The tendency to search all *S. altissima* located may reflect a stem selection process based on searching all the stems in a clone. In such a case the flight pattern of the wasps would be crucial. The cage used in these experiments was not appropriate for evaluating flight patterns of wasps. Most flights were to or from the wall of the cage. A large number of the inter-plant flights were very short, lasting less than one second. Females often landed in the same place from which they had taken off or on a stem or leaf only a few inches away. This flight pattern suggests that their search behavior may be adapted to the clonal growth pattern of goldenrods. A second reason for thorough searches on plants where a larva has been located is the chance of finding a second host on the same plant. Uhler (1951) found that gall-fly females oviposit a mean of 1.2 eggs per stem. Thus, finding one larva indicates a significant chance of finding another on the same stem.

Why does *E. obtusiventris* not discriminate among *S. altissima* with and without hosts? They may lack any means of doing so, other than vibrotaxis,

which is sufficiently unreliable to warrant females searching all *S. altissima* encountered. Alternatively, wasps may discriminate among *S. altissima* on criteria other than the presence of the host larva itself. Gall-flies do not select plants at random, preferring certain plant traits, such as taller stems (Walton, et al., 1990), and preferring susceptible plant genotypes (Anderson et al., 1989). However, there may be no advantage to non-random selection of *S. altissima* in the absence of host-based cues. The lepidopteran, *Greya subalba* distributes its eggs so as to provide its parasitoid with as little information as possible about larval distribution (Thompson, 1986). If selecting plants based on cues which are not directly from the host does not increase the chances of finding a host, then there would be no selective pressure to develop such discrimination.

All *S. altissima* seed used in this experiment was from susceptible genotypes (i.e., seed was from galled plants), so this experiment could fail to detect pre-alighting discrimination of *S. altissima* on criteria other than host presence. Further, I chose plants at random for use in the cage, so, other than equalizing the heights, their characteristics were not controlled. *E. obtusiventris* may not select plants with host larvae, but select plants on the same criteria used by gall-flies. In this case wasps would search plants that are most favorable to the gall-fly. Wasp females would be just as likely to select a *S. altissima* without a larva as one with a larva as long as they were equally attractive to gall-flies. Therefore, while the data show no evidence for pre- or post-alighting discrimination among *S. altissima*, they do not rule out such discrimination. Identifying plants favoured by gall-flies could be a great advantage to searching wasps in the absence of other cues.

Follow up studies

E. obtusiventris is almost ideal as a study of a parasitoid of an endophyte. They are easy to locate and collect while inside the gall, and cannot be confused with any other species. The other eurytomid, *E. gigantea*, is easily distinguished because it emerges much later, females are much larger, and males have antennae distinct from those of the females (Walsh, 1870). In addition, *E. obtusiventris* is easy to work with, responding to plants and ovipositing in the cage. It is parthenogenetic, as confirmed by the 1990 emergence of a wasp from an egg laid by an unmated female. This eliminates the time-consuming and uncertain process of mating females. Despite these advantages, several factors complicated my experiments. Wasps were difficult to obtain because Ottawa is outside the range of *E. obtusiventris*, and because the overall emergence was lower than expected from the literature. One to 25% of all galls have been reported to contain *E. obtusiventris* (Abrahamson *et al.* 1989; Cane and Kurczewski 1976). Uhler (1961) reports 30% in 13 years of cumulative data from Ithaca. D. Sumerford (personal communication) reports a large decrease in *E. obtusiventris* populations and a corresponding increase in *E. gigantea* in 1988 and 1989, due to the severe drought in the summer of 1988. My data confirm these changes in population. The emergence of other species was also lower than or equal to the levels reported in the literature. This could stem from natural population fluctuations that could only be corrected for by collecting large numbers of galls. Alternatively, the storage arrangement in the vial racks could have caused mortality. In 1989, vertically stored vials often filled with rainwater and a few

galls became moldy. This problem was eliminated by horizontal storage in 1990, but only the levels of gall-fly emergence improved.

The low rate of gall formation on *S. altissima* also complicated the experiments. Several factors which could lead to gall failure were controlled for in advance. Seed was taken from *S. altissima* with ball galls to insure that plants were susceptible to gall-flies. I observed the gall-flies mating so that mated and unmated females were known. I attributed the disappointingly low gall formation in 1989 to the plants being almost in flower. Gall-flies would not oviposit on plants in bud, so plants were started at later dates in 1990. Also, in 1990, plants were not given fertilizer, so they would be less vigorous, even though Abrahamson *et al.* (1988) report that supplemental nutrients do not increase plant resistance to the gallmaker.

Either females did not lay eggs in the plants despite inserting the ovipositor, or the offspring died at some point before gall formation began. The two females which produced the two galls in 1990 were also exposed to 12 other plants, none of which formed galls. Therefore, gravid females produced high numbers of unsuccessful punctures. Gall-fly females may probe and then reject plants (Ritter and Vasey, 1989), and Anderson *et al.* (1989) report that females rejected 23% of preferred plants. Walton *et al.* (1990) report that gall formation is a function of plant height. At 10 cm about 20% of probed plants form galls, while at 18 cm about 60% form galls. While I did not measure the heights of plants at oviposition, most were over 10 cm in height when exposed to gall-flies. Some *S. altissima* genotypes are resistant to gall-flies. Anderson *et al.* (1989) found that dead gall-fly larvae in resistant plants were surrounded

with necrotic plant tissue. All *S. altissima* used in this study were grown from the seed of galled plants.

Because of low wasp emergence and low rates of gall formation, the host presence/absence experiments should be repeated to give a larger sample exposed to plants with successful oviposition, and to determine if the consistency observed in three of the four females used represents a general response. A larger sample size would also increase the power of the statistical analysis. If a non-destructive technique could be found to determine the presence of gall-fly larvae, then all plants could be classified as containing or not containing a gall-fly larva. No plants would be classified as having unsuccessful gall-fly oviposition and a balanced design could be achieved.

The olfactometer experiments should be repeated to determine if *E. obtusiventris* chooses plant species based on chemical or morphological cues. The techniques should be altered to overcome the wasp's aversion to the olfactometer tubing. Evidence for morphological discrimination might be obtained by exposing wasps to different varieties of *S. altissima* and *S. canadensis*, which overlap in characteristics such as pubescence. It would be necessary to quantify plant characters for this comparison. The mechanism wasp females use to distinguish plant species could be explored by making plant extracts, applying them to other plant species and determining if they affect the wasps' response. This was tried in 1990, using ethanolic extracts of stem and leaf tissue. However, wasps showed a significant response to an ethanol control, so the data were not presented.

The role of vibrotaxis in host location could be further elucidated by killing or anesthetizing gall-fly larva just before *E. obtusiventris* was placed on the

plant, to determine if this eliminated wasps finding the host on the first ovipositor insertion. It would be necessary to immobilize the larva without affecting the plant, physically or chemically. Alternately, if larval activity could be monitored, wasp responses could be correlated directly with larval activity.

I would follow up on the observation that females prefer taller *S. altissima* in the cage, but are found on lower galls in the field. Gall and plant heights could be measured when chosen by wasps and at the end of the summer to determine whether females select low galls or are inhibiting growth. Because gall-flies are more successful in ovipositing on taller plants (Walton *et al.*, 1990), it seems unlikely that wasps would preferentially search shorter plants. However, if parasitism reduces, or does not alter the cost to the plant (Stinner and Abrahamson, 1979) it also seems unlikely that they would inhibit growth more than the gall-fly. Ideally, this should be tested in the field. It would be of value to test any of the conclusions of this paper with field experiments. This, however, may not be practical due to the small size of the wasps.

To determine if the decision to stay or leave *S. altissima* is based on plant cues other than the presence of the host, an experiment similar to the plant-species experiment should be done, using resistant and susceptible clones of *S. altissima*. Such discrimination would provide more weight to the suggestion that the plant could encourage the wasp.

In assessing the interrelationship between plant, gall-fly and wasp, it would be valuable to know the impact of individual enemies on the cost of herbivory to the plant. If it could be established that *E. obtusiventris* individually reduces the cost of herbivory, then an investigation into the manipulation of the wasp by the plant should be considered. During this study, I collected seed heads from

galled plants and attempted to correlate the reproductive success of the plants with the contents of the galls. Low levels of *E. obtusiventris* emergence made this comparison insignificant. I recommend repeating such a study, as well as an in depth study of resource allocation in parasitized and unparasitized galls. Such a study would require a site with a larger proportion of *E. obtusiventris* than I had available.

Finally, it would be interesting to examine the interactions between the various natural enemies of the gall-fly. *E. obtusiventris* seems to avoid bird predation, but is susceptible to *E. gigantea*. Its interaction with *Mordellistena unicolor* or *Schizoprymnus* is not known. Few authors mention *Schizoprymnus*, which I found to be to be common at many of the sites used in this study. As the role of natural enemies must be considered in any plant-herbivore interaction (Price, 1980), similarly, the behavior of *E. obtusiventris* towards its host, cannot be understood without a full understanding of the interaction of all the natural enemies of the ball gall-fly.

CONCLUSIONS

E. obtusiventris utilizes a series of directed and undirected steps in searching out its host, the ball gall-fly. *S. altissima* are distinguished from other goldenrod and non-goldenrod species. Discrimination occurs before landing and after landing on a plant. Cues used in pre-alighting discrimination were not determined, but could be chemical or physical traits of the plant. The antennae are probably the most important sensory organ in post-alighting plant species discrimination, but it was not determined if discrimination was based on physical or chemical cues. Individual *S. altissima* are selected at random. Wasps are equally likely to land on plants without host larvae as those with a host. After landing some females appear to detect the host itself, probably through vibrotaxis, and oviposit into it on the first attempt. Ovipositor insertions fell into two distinct types. Short (<1 min) insertions were concluded to be sensory while long (>2 min) insertions represented actual oviposition. The majority of females do not appear to detect the presence or absence of a host and systematically search the top 1/3 of the plant using short insertions. The antennae were also used constantly and probably play an important role in host detection. While this search behavior causes females to spend a lot of time searching plants without hosts, it decreases the probability of failing to detect hosts when present. Further research is required to determine the exact nature of cues used by *E. obtusiventris* in host detection, and to examine the wasps' response to different genotypes and characters of *S. altissima* for possible discrimination among plants by other criteria than host presence or absence.

REFERENCES

- Abrahamson, W.G. and K.D. McCrea. 1986. Nutrient and biomass allocation in *Solidago altissima* : effects of two stem gallmakers, fertilization and ramet isolation. *Oecologia*, 68:174-180.
- Abrahamson, W.G., J.F. Sattler, K.D. McCrea and A.E. Weis. 1989. Variation in selection pressures on the goldenrod gall fly and the competitive interactions of its natural enemies. *Oecologia*, 79:15-22.
- Abrahamson, W.G., P.O. Armbruster and G.D. Maddox. 1983. Numerical relationships of the *Solidago altissima* stem gall insect-parasitoid guild food chain. *Oecologia*, 58:351-357.
- Abrahamson, W.G., S.S. Anderson and K.D. McCrea. 1988. Effects of manipulation of plant carbon nutrient balance on tall goldenrod resistance to a gallmaking herbivore. *Oecologia*, 77:302-306.
- Anderson, S.S., K.D. McCrea, W.G. Abrahamson and L.M. Hartzel. 1989. Host genotype choice by the ball gallmaker *Eurosta solidaginis* (Diptera: Tephritidae). *Ecology*, 70:1048-1054.
- Arthur, A.P. 1962. Influence of host stress on abundance of *Itoplectis conquisitor* (Say) (Hymenoptera:Ichneumonidae), a polyphagous parasite of the European pine shoot moth, *Rhyacionia buoliana* (Schiff.) (Lepidoptera:Olethreutidae). *Can. Entomol.*, 94:337-347.
- Bugbee, R.E. 1967. Revision of chalcid wasps of the genus *Eurytoma* in America north of Mexico. *Proc. U.S. Natl. Mus.*, 118:433-545.
- Cane, J.H. and F.E. Kurczewski. 1976. Mortality factors affecting *Eurosta solidaginis* (Diptera:Tephritidae). *J. New York Entomol. Soc.*, 84:275-282.
- Casas, J. 1989. Foraging behavior of a leafminer parasitoid in the field. *Ecolol. Entomol.*, 14:257-265.
- Clancy, K.M. and P.W. Price. 1987. Rapid herbivore growth enhances enemy attack: sublethal plant defenses remain a paradox. *Ecology*, 68:733-737.
- Confer, J.L. and P. Paicos. 1985. Downy woodpecker predation at goldenrod galls. *J. Field Ornithol.*, 56:56-64.
- Craig, T.P., J.K. Itami and P.W. Price. 1989. A strong relationship between oviposition preference and larval performance in a shoot-galling sawfly. *Ecology*, 70:1691-1699.

- Damman, H. 1987. Leaf quality and enemy avoidance by the larvae of a pyralid moth. *Ecology*, 68:88-97.
- Elzen, G.W., H.J. Williams and S.B. Vinson. 1983. Response by the parasitoid *Campoletis sonorensis* (Hymenoptera:Ichneumonidae) to chemicals (synomones) in plants: Implications for host habitat location. *Environ. Entomol.*, 12:1872-1876.
- Gahan, A.B. 1934. Description of a new species of *Eurytoma* (Hymenoptera: Chalcidoidea). *Entomol. News*, 45:116-118.
- Hartnett, D.C. and W.G. Abrahamson. 1979. The effects of stem gall insects on life history patterns in *Solidago canadensis*. *Ecology*, 60:910-917.
- Hawkins, B.A. 1988. Do galls protect endophytic herbivores from parasitoids? A comparison of galling and non-galling Diptera. *Ecol. Entomol.*, 13:473-477.
- Hughes, G.F. 1934. Two chalcid parasites of the goldenrod gall-fly, *Eurosta solidaginis* (Hymenoptera:Chalcidoidea; Diptera:Trypetidae, et al.). *Entomol. News*, 45:119-122.
- Jaenike, J. 1978. On optimal oviposition behavior in phytophagous insects. *Theor. Pop. Biol.*, 14:350-356.
- Judd, W.W. 1953. Insects reared from goldenrod galls caused by *Eurosta solidaginis* Fitch (Diptera:Trypetidae) in southern Ontario. *Can. Entomol.*, 85:295-296.
- Lawrence, P.O. 1981. Host vibration - A cue to host location by the parasite, *Biosteres longicaudatus*. *Oecologia*, 48:249-251.
- Maddox, G.D. and R.B. Root. 1987. Resistance to 16 diverse species of herbivorous insects within a population of goldenrod, *Solidago altissima* : genetic variation and heritability. *Oecologia*, 72:8-14.
- McCrea, K.D. and W.G. Abrahamson. 1987. Variation in herbivore infestation: historical vs. genetic factors. *Ecology*, 68:822-827.
- Miller, W.E. 1959. Natural history notes on the goldenrod ball gall fly, *Eurosta solidaginis* (Fitch), and on its parasites, *Eurytoma obtusiventris* Gahan and *E. gigantea* Walsh. *J. Tenn. Acad. Sci.*, 34:246-251.
- Milne, L.J. 1940. Autecology of the golden-rod gall fly. *Ecology*, 21:101-105.
- Moeller, R.K. and M.T. Thogerson. 1978. Predation by the downy woodpecker on the goldenrod gall fly larva. *Iowa Bird Life*, 48:131-136.

- Monteith, L.G. 1960. Influence of plants other than the food plants of their host on host-finding by tachinid parasites. *Can. Entomol.*, 92:641-652.
- Monteith, L.G. 1964. Influence of the health of the food plant of the host on host-finding by tachinid parasites. *Can. Entomol.*, 96:1477-1482.
- Moran, N. and W.D. Hamilton. 1980. Low nutritive quality as defence against herbivores. *J. Theor. Biol.*, 86:247-254.
- Papaj, D.R. 1986. Leaf buds, a factor in host selection by *Battus philenor* butterflies. *Ecol. Entomol.*, 11:301-307.
- Ping, C. 1915. Some inhabitants of the round gall of goldenrod. *J. Entomol. Zool.*, 7:161-177.
- Price, P.W., C.E. Bouton, P. Gross, B.A. McPherson, J.N. Thompson and A.E. Weis. 1980. Interactions among three trophic levels: Influence of plants on interactions between insect herbivores and natural enemies. *Ann. Rev. Ecol. Syst.*, 11:41-65.
- Price, P.W., G.W. Fernandes and G.L. Waring. 1987. Adaptive nature of insect galls. *Environ. Entomol.*, 16:15-24.
- Rausher, M.D., D.A. Mackay and M.C. Singer. 1981. Pre- and post-alighting host discrimination by *Euphydryas editha* butterflies: the behavioural mechanisms causing clumped distributions of egg clusters. *Anim. Behav.*, 29:1220-1228.
- Ritter, E. and C.E. Vasey. 1989. Sensory structures on the ovipositor of the ball gall fly *Eurosta solidaginis* (Fitch) (Diptera:Tephritidae). *J. New York Entomol. Soc.*, 97:341-346.
- Sato, Y. and N. Ohsaki. 1987. Host-habitat location by *Apanteles glomeratus* and effect of food-plant exposure on host parasitism. *Ecol. Entomol.*, 12:291-297.
- Schlichter, L. 1970. Winter predation by black-capped chickadees and downy woodpeckers on inhabitants of the goldenrod ball gall. *Can. Field Nat.*, 92:71-74.
- Semple, J.C. and G.S. Ringius. 1983. Goldenrods of Ontario. *Solidago* and *Euthamia*. University of Waterloo Biology Series. No. 26.
- Shahjahan, M. 1974. Erigon flowers as food and attractive odor source for *Peristenus pseudopaliipes*, a braconid parasitoid of the tarnished plant bug. *Environ. Entomol.*, 3:69-72.

- Sokal, R.R. and F.J. Rohlf. 1981. Biometry. Second edition. W.H. Freeman, San Francisco, California. pp. 721-725.
- Stinner, B.R. and W.G. Abrahamson. 1979. Energetics of the *Solidago canadensis* stem gall insect-parasitoid guild interaction. *Ecology*, 60:918-926.
- Stoltzfus, W.B. 1989. A non-gall forming *Eurosta solidaginis* (Diptera:Tephritidae) J. Iowa Acad. Sci., 96:50-51.
- Takabayashi, J. and S. Takahashi. 1989. Effects of host fecal pellet and synthetic kairomone on host-searching and postoviposition behavior of *Apanteles kariyai*, a parasitoid of *Pseudaletia separata*. *Entomol. Exp. Appl.*, 52:221-227.
- Thompson, J.N. 1986. Oviposition behavior and searching efficiency in a natural population of a braconid parasitoid. *J. Anim. Ecol.*, 55:351-360.
- Uhler, L.D. 1951. Biology and ecology of the goldenrod gall fly, *Eurosta solidaginis* (Fitch). *Cornell Univ. Agric. Exper. Stat. Mem.*, 300:1-51.
- Uhler, L.D. 1961. Mortality of the goldenrod gall fly, *Eurosta solidaginis* in the vicinity of Ithaca, New York. *Ecology*, 42:215-216.
- Vet, L.E.M., and J.J.M. van Alphen. 1985. A comparative functional approach to the host detection behavior of parasitic wasps. 1. A qualitative study on Eucilidae and Alysiinae. *Oikos*, 44:478-486.
- Vinson, S.B. 1975. Biochemical coevolution between parasitoids and their hosts. In: *Evolutionary Strategies of parasitic insects and mites*. P. Price (Ed.) Plenum Press. New York. 14-48.
- Vinson, S.B. 1976. Host selection by insect parasitoids. *Ann. Rev. Entomol.*, 21:109-133.
- Vinson, S.B. 1984. How parasitoids locate their hosts: A case of insect espionage. In: *Insect Communication*. T. Lewis (Ed.) Academic Press Inc. Ltd. London. pp 325-348.
- Walsh, B.D. 1870. On the group Eurytomides of the hymenopterous family Chalcidae. *Ann. Entomol. Botan.*, 2:297-301.
- Walton, R. 1988. Characteristics of galls of *Eurosta solidaginis* (Diptera: Tephritidae) and their relation to mortality of gall inhabitants. *Environ. Entomol.*, 17:654-659.

- Walton, R., A.E. Weis and J.P. Lichter. 1990. Oviposition behavior and response to plant height by *Eurosta solidaginis*, Fitch (Diptera:Tephritidae). *Ann. Entomol. Soc. Am.*, 83:509-514.
- Weis, A.E. and W.G. Abrahamson. 1985. Potential selective pressures by parasitoids on a plant-herbivore interaction. *Ecology*, 66:1261-1269.
- Weis, A.E. and W.L. Gorman. 1990. Measuring selection on reaction norms: an exploration of the *Eurosta-Solidago* system. *Evolution*, 44:820-831.
- Weis, A.E., W.G. Abrahamson and K.D. McCrea. 1985. Host gall size and oviposition success by the parasitoid *Eurytoma gigantea*. *Ecol. Entomol.*, 10:341-348.
- Zar, J.H. 1984. *Biostatistical Analysis*. 2nd Ed. Prentice Hall. Toronto. pp 170.

END

06-03-92

FIN