THE UNIVERSITY OF ALBERTA

BIOLOGICAL CONTROL OF WESTERN GALL RUST: USING *Epuraea obliquus* (COLEOPTERA: NITIDULIDAE) TO VECTOR A MYCOPARASITE

by

CAMERON ROBERT CURRIE 

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled: **Biological control of western gall rust: using *Epuraea obliquus* Hatch (Coleoptera: Nitidulidae) to vector a mycoparasite** submitted by **Cameron Robert Currie** in partial fulfillment of the requirements for the degree of **Master of Science** in **Entomology**.

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Date: July 28 1994

I dedicate this thesis to my parents, Leeann and Keith,

for their support, encouragement and love.

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ABSTRACT

The use of *Epuraea obliquus* Hatch (Coleoptera: Nitidulidae) to disseminate a mycoparasite for biological control of western gall rust was investigated near Hinton, Alberta.

The life cycle, phenology and abundance of the nitidulid beetle was investigated in 1992 and 1993. *Epuraea obliquus* has a very close association with the gall rust. Adults and the larvae feed on the spores produced by western gall rust. The phenology of this beetle is closely timed to sporulation of western gall rust. *Epuraea obliquus* is the most abundant invertebrate occurring on the gall rust in western Canada.

Dissemination of *Scytalidium uredinicola* Kuhlman *et al.* by *E. obliquus* was investigated in 1993. The beetle can act as an natural vector of the mycoparasite by transferring spores of the mycoparasite from infected galls to clean galls during migratory flights between galls. Also, the beetle can vector the mycoparasite by transferring viable spores to uninfected galls when they emerge from overwintering. Beetles become inoculated with these spores in the fall while feeding on galls.

It was determined that *S. uredinicola* is the best candidate for biological control of western gall rust. The mycoparasite is the most abundant mycoparasite in May and June, when most of the gall rust's spores are released. Also, the proportion of sporulating surface parasitized by mycoparasite increases as the gall ages. Most galls older than 8 years were completely inactivated by mycoparasite and thus provides little inoculum potential for western gall rust spread. The beetle is an excellent candidate for disseminating the mycoparasite because it is attracted directly to galls. Biological control of western gall rust by using *E. obliquus* to vector *S. uredinicola* is a very promising approach.

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1. Introduction

1.1 Controlling plant pathogens

Phytopathogenic fungi are important components of ecosystems, breaking down plant material in the early stages of nutrient re-cycling. However, they can have disastrous effects on valuable commodities if their populations become too large. Pest fungi are generally controlled through broad scale use of synthetic chemicals, such as fungicides. Although fungicides can reduce the impact of plant pathogens, they also kill beneficial fungi and microarthropods which are important for decomposing dead plant material and cycling nutrients (Moore and Walter 1988). Microarthropods may reduce the inoculum of soil pathogens by vectoring parasitic fungi and/or feeding on the plant diseases (Anas and Reeleder 1988). Since application of fungicides can disrupt natural soil dynamics, eliminate the natural control agents, and is generally harmful to the environment, their use should be minimized.

An alternative to using fungicides is development of biological control, which traditionally involves the use of natural enemies to reduce pest populations. Since the successful use of the vadalia beetle (*Rodolia cardinalis* Mulsant) to control the cottony-cushion scale, *Icerya purchasi* Maskell, in California in the late 1800's, biological control has progressed as a science. There are three main approaches to biological control using natural enemies: augmentation, or additions of native natural enemies; conservation and enhancement, or increasing the impact of native agents by manipulating habitat; and classical biological control, or introducing exotic biological control agents to control introduced pests.

Augmentation of natural enemies has been the primary focus in biological control of plant pathogens and has promise, since insects and fungi are often effective at naturally controlling plant pathogens (Papavizas 1973, Nelson 1982, Schneider 1982, Cook and Baker 1983, Beale *et al.* 1991, Fox *et al.* 1991, Whipps 1991). Classical biological control could be effective for exotic plant pathogens. For example, the most significant tree rust disease in the western United States is an exotic disease, white pine blister rust (*Cronartium ribicola* Fisch.), which has very few insects and mycoparasites associated with it (Nelson 1982). The problems associated with this pathogen have been explained in terms of low host resistance (Bingham *et al.* 1971, Hoff and MacDonald 1972). However, lack of natural enemies could also contribute to its ability to spread quickly and thus introduction of natural enemies could help to reduce the impact of this pathogen.

Although there are many examples of successful biological control of insect pests and weeds the use of biologicals to control plant pathogens has had very limited success despite its considerable potential (Snyder *et al.* 1976, Cook and Baker 1983, Sundheim and Tronsmo 1988, Upadhyay and Rai 1988, Whipps *et al.* 1988). The inability to develop successful biological control for plant pathogens is not a failure in identifying control agents, of which hundreds have been proposed (See Upadhyay and Rai 1988), but is a failure in implementation, largely due to insufficient understanding of the biology involved (Whipps *et al.* 1988, Lumsden 1992). For biological control of plant pathogens to progress, more studies on the biology and ecology of natural control agents are necessary.

Understanding the community processes occurring on plant pathogens can lead to development of new biological control approaches. One

possibility involves utilizing the natural interactions of insects and fungi to control pest populations. Insects, plants and fungi have evolved many close associations, including the mutualistic interactions involving bark beetles and destructive plant disease, such as Dutch elm disease and blue stain disease. Although many of the identified interactions of insects and plant pathogens are destructive to human food and/or fiber, such interactions are common in nature in most systems. For example, insects and fungi likely interact to naturally regulate populations of many plant pathogens, and utilizing these interactions could be an effective method of biological control.

Studying the diverse and abundant assemblage of invertebrates and fungi occurring on plant pathogens is also beneficial because these are interesting ecological communities. Studying community and population ecology occurring on plant pathogens is relatively easy because of their abundance, occurence as discrete units and their relatively small size.

1.2 Control of western gall rust

Western gall rust, *Endocronartium harknessii* J. P. Moore) Y. Hiratsuka, is a destructive forest pathogen of hard pines in western Canada (Ziller 1974, Hiratsuka *et al.* 1988). Because it is an autoecious rust (does not require an alternative host) this pine stem rust disease is able to spread quickly throughout pine stands. Upon successful infection it forms perennial galls on branches and stems of its host and every spring releases copious numbers of wind blown spores. Infection occurs only on the lead terminal of the tree or branch during elongation. Western gall rust can kill trees by forming galls on the main stem which end up girdling the bole of the tree. Branch galls reduce the yearly growth of the host.

Presently there are few successful methods for controlling western gall rust. Removal of infected branches and trees would be successful at

reducing this pathogen in intensively managed stands, such as in nurseries, but there is no method of control in larger scale forestry operations. In the future, development of rust resistant planting may decrease the impact of this pathogen in large scale forestry situations. If pre-commercial thinning is prescribed, then removal of heavily infected trees could reduce the impact of this pathogen (Bella 1985).

Another alternative is biological control of western gall rust. The abundance of mycoparasites (Tsuneda and Hiratsuka 1979, Tsuneda and Hiratsuka 1980, Tsuneda *et al.* 1980) and insects (Powell *et al.* 1972) on this pathogen suggests that biological control could be a successful control method. One promising biological control agent is the mycoparasite *Scytalidium uredinicola* Kuhlman *et al.* (Tsuneda *et al.* 1980). *S. uredinicola* is a very destructive mycoparasite which can destroy the basal cell region of the rust sori and rust hyphae (Tsuneda *et al.* 1980). It degenerates the surface of the wall layer and is believed to affect the permeability of the spore (Tsuneda 1983). This mycoparasite also has been identified on *Cronartium quercuum* f. sp. *fusiforme* in the western United States (Kuhlman 1981). It was shown to decrease spore production, germinability and delay spore release of this host (Kuhlman 1981). Augmentation of this mycoparasite could be an effective method for biological control of western gall rust. However, efficient application of this control agent is difficult and therefore field implementation has not been attempted.

Insects are very abundant on western gall rust (Powell *et al.* 1972). Several species feed on the fungus, including the nitidulid beetle *Epuraea obliquus* Hatch (Coleoptera: Nitidulidae). This lead Hiratsuka (1990) to propose an innovative new method of biological control of western gall rust. It involves using *E. obliquus,* which is attracted to western gall rust, to

disseminate the control agent, *S. uredinicola*. This could provide the efficient application necessary for using *S. uredinicola* for biological control of western gall rust.

1.3 Objectives of this thesis

The object of this work was to obtain a better understanding of the biology and ecological interactions of two organisms occurring on western gall rust. Specifically the biology and ecological interactions of the beetle, *Epuraea obliquus* Hatch (Coleoptera: Nitidulidae), and the mycoparasite, *Scytalidium uredinicola* Kuhlman *et al.* (Deuteromycotina: Hyphomycetes), were investigated. These two natural enemies were selected because they are the main control agents in the biological control approach proposed by Hiratsuka (1990), as discussed above. Better understanding of their biology and ecological interactions is necessary to further develop this biological control approach.

Because very little of the biology of *E. obliquus* is understood the life cycle, phenology and abundance of this nitidulid was investigated in Chapter Two. Also, the interactions of this beetle and mycoparasite were investigated. Specifically, in Chapter Three the hypothesis that *E. obliquus* is a natural vector of *S. uredinicola* was examined.

The potential of this method of biological control was investigated in Chapter Four. The most promising mycoparasite for biological control of western gall rust was determined by examining the timing and abundance of the common mycoparasites. Also, the progression of mycoparasites on the surface of galls was studied. The attraction of *E. obliquus* to the gall was investigated to determine how effective it would be in disseminating the mycoparasite. Finally, biological control of western gall rust is discussed.

In Chapter Five, the general benefits of using insects to disseminate mycoparasites for biological control of plant pathogens is discussed, with specific references to the use of *E. obliquus* and *S. uredinicola* for biological control of western gall rust. Future research needed before implementation of this biological control approach is proposed.

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2. Biology and life history of *Epuraea obliquus* Hatch (Coleoptera: Nitidulidae) on western gall rust

2.1 SYNOPSIS

The life cycle, phenology and abundance of *Epuraea obliquus* Hatch was studied near Hinton, Alberta. Most of the life cycle occurs on galls of *Endocronartium harknessii* (J. P. Moore) Y. Hiratsuka (western gall rust) infecting lodgepole pine (*Pinus contorta* Dougl. var. *latifolia* Engelm.), with both adults and larvae of the nitidulid feeding on the spores of the fungus. Individuals of this nitidulid beetle were found on the majority of galls sampled. Adults overwinter in the soil, and after they emerge in the spring, seeking out and colonize galls. Eggs are laid on the surface of galls, mainly under the periderm, and the larvae feed on the fungus and develop through three larval instars. Larvae in the last larval instar drop from the galls to pupate in the soil. After pupation adults leave the soil in the late summer and seek out galls to feed on. The phenology of *E. obliquus* is closely synchronized with the timing of rust sporulation and the impact of this nitidulid feeding appears to provide important natural control of western gall rust.

2.2 INTRODUCTION

Pine stem rusts, such as western gall rust, *Endocronartium harknessii* (J. P. Moore) Y. Hiratsuka, are a very destructive group of forest diseases (Baranyay and Stevenson 1964, Ziller 1974, Hiratsuka *et al.* 1988). It is increasingly apparent that they are controlled naturally to some extent by a

diverse assemblage of arthropods and hyperparasitic fungi (Powell 1971a, Powell 1971b, Powell *et al.* 1972, Wong 1972, Powell 1974, Tsuneda and Hiratsuka 1979, Tsuneda and Hiratsuka 1980, Tsuneda *et al.* 1980, Nelson 1982). Although many arthropods occurring on pine stem rusts in Western Canada (Powell 1971a, Powell 1971b, Powell *et al.* 1972), more information about their life history and biology is necessary to understand their possible impact as natural control agents.

A small nitidulid beetle, *Epuraea obliquus* Hatch, is one of the few true mycetobionts (Powell 1971a), feeding primarily on the spores produced by the fungus. This beetle was originally described by Hatch (1962) and the description was expanded by Parsons (1967). The abundance of *E. obliquus* on western gall rust (Powell *et al.* 1972) and the large impact of a hyperparasitic fungus, *Scytalidium uredinicola* Kuhlman *et al.* (Deuteromycotina: Hyphomycetes), on the gall (Tsuneda *et al.* 1980) led Hiratsuka (1990) to propose that *E. obliquus,* could be inoculated with *S. uredinicola,* and used in a biological control program to accurately disseminate the mycoparasite. However, little is known about the phenology and natural history of this species, or of other species in this genus. Therefore, I undertook a study of *E. obliquus* inhabiting western gall rust in lodgepole pine stands near Hinton, Alberta to assess the feasibility of the above mentioned approach to biological control.

2.3 MATERIALS AND METHODS

2.3.1 Beetle identity and life history

To confirm that the nitidulid beetle found on western gall rust near Hinton is indeed *E. obliquus,* specimens collected from galls in 1992 were keyed using Hatch (1962) and compared to Parsons' (1967) description of *E. obliquus.* A voucher specimen of *E. obliquus* identified by Parsons, from the collection of the Centre for Land and Biological Resources Research in Ottawa, was also compared to individuals collected in Hinton. Jean McNamara from the Centre for Land and Biological Resources Research in Ottawa also examined individuals from the study site to help determine the identity of material collected.

Measurements of head capsule width and labium width were made on larvae collected from western gall rust in 1992 to determine the number of larval instars. Frequency distributions of these variables were plotted and natural breaks in these distributions were assumed to represent differences between successive instars.

The biology and life history of *E. obliquus* was studied in the summers of 1992 and 1993 in naturally regenerating lodgepole pine stands approximately 25 km south of Hinton, Alberta. These stands were chosen because of their heavy incidence of western gall rust infection. The stand sampled in 1992 is a naturally regenerating stand harvested in 1960 and thinned in 1981, and the stand sampled in 1993 is naturally regenerating after harvest in 1973 and thinning in 1987.

Emergence traps were placed on the ground in the stand in early May of 1992 and mid-April of 1993 to test the hypothesis that adult beetles overwintered in the soil and to study the timing of post-winter emergence.

The traps were pyramid shaped with two opposite faces covered in mosquito netting and the other faces constructed of 1.8 cm thick plywood. An area of approximately 0.56 m^2 was covered by the base of each trap and emerging insects were funneled into a small, clear collecting jar at the top. The traps were placed close to trees (1-2 m) with many galls, to increase the probability of catching beetles. A total of 30 traps were set throughout the stand, with single traps being randomly placed in one of the four cardinal directions around the tree at each sampling location.

Sticky traps, hung by wire in front of galls, were also used in 1993 to confirm the timing of adult emergence and to study the movement of adults. The traps were made of circular plastic container lids (diameter of 11.5 cm) with a thin coating of automotive lubricating grease (Darina grease Ax, Shell Canada, Limited, Toronto) spread across the surface. Traps were checked three times per week from early May until mid-July, and bi-monthly during August. In mid-July of 1992, the soil under trees infected with western gall rust was searched for beetles that were pupating in the soil.

Information about the occurrence, phenology and abundance of the different life stages of *E. obliquus* on western gall rust was obtained by dissection of galls collected three times a week from early May until mid-July of both years. Ten galls were collected during each sampling date in 1992 and 5 galls were collected per date in 1993. In both years, living galls were selected arbitrarily while walking through the stand during each sampling period. Different age classes of galls were intentionally selected during each sampling period to obtain a good distribution of gall ages. Galls were frozen and later dissected using a probe and scalpel under a dissecting microscope. All individuals of *E. obliquus* mixed in the sporulating surface of western gall rust were counted and collected.

To determine the occurrence and timing of pupation in the soil, mature larvae were collected by drop traps, which were containers (top opening diameter of 11.5 cm and a depth of 20 cm) suspended under galls by wiring them to the branches. In 1992, 20 galls were sampled in this way, including 10 older (6-9 years old) and 10 younger galls (3-5 years old). In 1993, 24 galls were sampled using this procedure with 12 galls representing each age group mentioned above. Traps were checked three times a week.

2.3.2 Beetle Phenology and Abundance

Information from all sampling methods was used to place the phenology of *E. obliquus* on both calendar date and degree-day time scales. Daily minimum and maximum temperatures were collected at the study sites using a chart recording thermograph (Ryan Institute, Washington, USA) suspended 2m above the ground. The accumulation of degree-days was calculated from daily minima and maxima using Allen's (1976) modified sine procedure.

In the absence of independent data about development thresholds, degree-day accumulation was calculated for thresholds between 1°C and 12° C. Using a non-linear regression procedure and the model described by Dennis *et al.* (1986), parameter estimates were then made for each stage over all thresholds between 1-12°C. Values of R^2 were then compared to determine the most appropriate (i.e. best fit) developmental threshold for *E. obliquus*. Using the parameter predictions for this threshold, the predicted timing for *E. obliquus* eggs and the three larval instars were plotted on a degree-day scale. This displays the proportion of different stages at each degree date and thus is useful comparing phenologies among species and from year to year.

The phenology of western gall rust was determined and compared with the phenology of *E. obliquus*. Western gall rust activity was determined by assessing the degree of development of galls collected in the study. Galls were categorized into four developmental stages as follows: (1) galls which had not begun to sporulate, termed inactive; (2) galls which were beginning to sporulate but were not releasing any spores; (3) galls which had begun to release spores, with aecia's ruptured; and (4) galls which had finished both producing and releasing spores and were thus shutting down spore production until the next season.

Information from the gall collections was used to study the abundance of *E. obliquus* on western gall rust. The number of individuals in all stages was recorded during gall dissection. Galls were aged and classified with respect to the amount of the mycoparasite, *S. uredinicola*. In order to study the effects of gall age on the abundance of *E. obliquus,* the number of third instar larvae captured in drop traps was compared between younger (2-5 years old) and older galls (6-9 years old) using the 1993 data. To determine the relationship between gall age and resources available to *E. obliquus,* the length and width of the galls, a rough estimate of surface area, was compared among galls of the above age classes.

2.4 RESULTS

2.4.1 Beetle Identity and life history

The nitidulid beetle occurring on western gall rust around Hinton matches the description of *E. obliquus* given by Parsons (1967) and this species is known to occur on pine stem rusts in Western Canada (Powell *et al.* 1972) Examination of many specimens collected in 1992 and 1993

suggests that only a single nitidulid species occurs on western gall rust in the Hinton area.

Epuraea obliquus eggs were identified on galls based on both size and shape. The eggs were long (0.90±0.06 mm Mean±Standard deviation), tubular and cloudy white in color. Examination and rearing of newly hatched larvae confirmed that these were *E. obliquus* eggs.

There are three distinct groupings in a scatter plot of head capsule and labial width (Figure 2.1) showing that *E. obliquus* has three larval instars. The mean head capsule widths for first, second and third instars are 0.28± 0.016 mm (n=130), 0.41±0.019 mm (n=277), and 0.53±0.024 mm (n=407), respectively. Labial width for 1st, 2nd and 3rd instar was 0.16±0.008 mm, 0.22±0.010 mm and 0.27±0.011 mm.

Epuraea obliquus larvae are easily distinguished from other larvae occurring on western gall rust by their shape, size and color. Larvae are 1.6± 0.35 mm long as first instars, 2.7±0.33 mm long as second and 4.9±0.43 mm as third instars. They are white with dark stripes appearing across the dorsal surface of each abdominal segment. These stripes are not readily apparent in the first instar.

Epuraea obliquus is univoltine in Alberta with larval development completed on single galls of *E. harknessii* (Figure 2.2). Adults overwinter in the duff. They were captured in emergence traps mainly during the beginning of May (Figure 2.3, numbers indicate frequency of measurement) and were not found on galls in the winter or early spring (Figure 2.4). Adults were also captured in early May of 1993 on the sticky traps, as they apparently flew from overwintering sites in the soil to colonize the galls. Observation of adults feeding and examination of their frass revealed that they feed on the surface of western gall rust, consuming the aeciospores.

Adults were frequently observed feeding in *S. uredinicola* parasitized areas on galls. Year-old adults dropped from galls in mid to late June. Some of these adults were found alive in the drop traps, although it is not known if they overwinter a second time.

Eggs are laid on the surface of galls, in protected locations, often under leftover periderm which has not dropped off the gall. This habitat provides protection from predators and also keeps eggs from being blown or washed off of the surface. Observations of larval feeding and frass showed that they also feed on the spores of western gall rust. They may also feed on *S. uredinicola,* the mycoparasitic fungus of western gall rust.

Larvae dropped to the soil to pupate in late June and early July (Figure 2.5). Head capsule measurements of larvae captured in drop traps (0.54±0.010 mm) showed that they were third instar. With little effort more than 10 pupae and third instars larvae of *E. obliquus* were found in the soil in mid-July 1992. Larvae collected from galls, using Berlese funnels, pupated in petri dishes of soil without undergoing additional molts.

New adults returned to the galls in the later summer and were collected both from galls and on sticky traps during August 1993. New adults were observed feeding on the surface of western gall rust, consuming hyperparasitic fungi and the old sporulating tissue of the inactive gall. New adults leave galls during the fall and overwinter in the duff.

2.4.2 Beetle Phenology and Abundance

Most of the development of *E. obliquus* occurs in early May until early July and is tightly linked to the timing of western gall rust activity. Adults become active in early May (Figure 2.3), emerging from the duff to seek out galls just before they begin sporulation in mid-May (Figure 2.4 and 2.6). Eggs are found commonly on the surface of galls during the period of active

sporulation. Larvae develop on the surface of galls between mid-May and mid-July, during the main period of spore release. As the galls stops producing spores in late June, larvae begin dropping off to pupate in the soil. Third instar larvae, pupae and teneral adults were found in the soil in mid-July 1992 and larvae collected with Berlese funnels pupated during this time period as well. Adults were collected from galls and found on sticky traps in mid-August of 1993. These adult were observed feeding on mycoparasites, such as *S. uredinicola,* which were located on gall surfaces.

The beetle life cycle was placed on a degree-day time scale. Parameter estimates for different developmental thresholds differed little. R^2s for the different thresholds between 1°C and 12°C ranged from a low of 0.91 at 12°C and a high of 0.94 at 5°C (Table 2.1). The threshold of 5°C was selected to model the development of *E. obliquus* because it had the highest R^2 and also because selecting an intermediate threshold is of value. High thresholds reduce the resolution of the model (Lysyk 1989) and application of models based on lower values requires initiation of field studies earlier in the year which may be difficult or expensive (Volney and Cerezke 1992).

Parameter estimates from the preferred model, (Table 2.2), were used to depict the timing of life stages for *E. obliquus.* Under this scheme, eggs occurred from 0 to 225 degree-days (Figure 2.7), first instars occurred from 75 to 325 degree-days, second instars from 100 to 375 degree-days and third instars from 125 to 475 degree-days. Third instars dropped off of galls between 225 and 500 degree-days.

Epuraea obliquus is common on western gall rust in the Hinton area. Between May 12 and July 8 1992, 74.0% (n=200) of galls collected had at least one individual of this nitidulid. In 1993, 69.6% (n=125) of galls collected during the period of beetle activity had at least one individual.

There was no apparent relationship between gall age and occupancy by *E. obliquus* (Table 2.4). Most galls of all age groups were colonized by *E. obliquus*. However, more larvae dropped from older galls (6-9 years old, 43.7 ±0.72) than younger galls (3-5 years old, 19.9±1.11). Also, there was a strong correlation between gall age and gall length (Linear regression, R^2=0.650, n=248, p<0.0001) and width (Linear regression, R^2=0.680, n=248, p<0.0001). Older galls thus have greater surface area, providing more resources for beetle larvae. Since older galls produced more larve it appears that the beetle may be limited by the amount of resources, suggesting intraspecific competition is important.

2.5 DISCUSSION

Epuraea obliquus is closely associated with western gall rust sporulation. Most of the life cycle of the beetle occurs on the sorus of the sporulating galls, with both larvae and adults feeding primarily on the spores. Adults and larvae also feed on the mycoparasites of western gall rust, such as *S. uredinicola,* although it is not clear whether they ingest significant amounts. Pupation and overwintering by adults occurs in the soil close to trees that have galls. Pupation in the soil is likely adaptive because after the spores are released the gall rust is barren, providing little protection for vulnerable stages like the pupa. Snow cover provides some insulation and protection for adults overwintering in the soil.

The timing of the life cycle of *E. obliquus* closely corresponds to the timing of western gall rust activity. Peak larval abundance occurs in the middle of western gall rust reproductive activity. Third instar larvae drop from galls as galls have finished releasing spores. Western gall rust phenology

estimated in this study matches that observed previously in Alberta (Chang *et al.* 1989).

Epuraea obliquus colonized more than 65% of galls sampled in 1992 and 1993 near Hinton. This probably underestimates the true percentage of galls colonized because some of the galls which were not colonized were dead and others may have been colonized later in the season if they had not been sampled. This hypothesis is supported by drop trap sampling in which 23 of 24 galls were colonized. *Epuraea obliquus* is also found in large numbers on western gall rust. For example, galls six to nine years old produced an average of over 45 larvae.

The close association between *E. obliquus* and western gall rust suggests that this beetle specializes on western gall rust. However, *E. obliquus* has been identified on other pine stem rust diseases, such as comandra blister rust, (*Cronartium comandrae* Peck) and *Cronartium coleosporioides* Arth. occurring on lodgepole pine in Western Canada (Powell 1971a). Although I observed *E. obliquus* adults feeding on *C. coleosporioides* near Hinton, I did not find any larvae in several samples of this pine stem rust. The timing and location of these rusts is similar to western gall rust thus specialization on pine stem rusts is a plausible hypothesis. However, since the relative abundance of *E. obliquus* on these other pine stem rusts is small in comparision to western gall rust I suggest that this beetle only occurs incidently on these other rusts in this area.

Pine stem rusts provide a suitable habitat for a fungus feeding specialist. *Epuraea obliquus* seems to occupy this habitat in Western Canada and in the Northwestern United States. In the more mid-western states *Phalacropsis dispar* (Coleoptera: Phalacridae) occupies this habitat (Nelson 1982). Other *Epuraea sp.* may occupy this niche in other locations

as well, such as *E. lengi* which has been found abundantly on *Cronartium quercuum* f. *sp. fusiforme* on loblolly and slash pine in North and South Carolina (Kuhlman 1981).

Epuraea obliquus is probably an important component in the natural regulation of western gall rust populations. The beetle occurs abundantly on the gall rust while it is sporulating, feeding on the spores of the pathogenic fungus. Large areas of sporulating tissue were commonly consumed by larvae of *E. obliquus,* as evidence by the abundance of beetle frass. With as many as 50 larvae *E. obliquus* on the surface of a gall, large numbers of rust spores can be consumed. Infact, areas of the gall rust which have *E. obliquus* larvae often are prematurely barren of western gall rust spores, with only masses of spore filled frass remaining. The number of larvae is higher on larger galls (older galls), suggesting that the beetle population is regulated by intraspecific competition and therefore the gall rust is a limited resource.

Although many other arthropods are common on western gall rust in Western Canada, *E. obliquus* likely has the largest impact on the spread and thus abundance of western gall rust. Powell *et al.* (1972) also classified *Paracacoxenus gluttatus* Haryd and Wheller (Diptera: Drosophilidae) and *Diapterobates principatlis* Berlese (Acarina: Ceratozetidae) as mycetobionts on western gall rust but these were not commonly observed in the sporulating tissue in my study. The timing and abundance of *E. obliquus* on western gall rust makes it a very important component in the natural control of western gall rust. This beetle's close association with western gall rust makes it an excellent candidate for dissemination of *S. uredinicola* in a biological control program against western gall rust.

Table 2.1 Comparison of R^2 (Non-linear regression) for different developmental thresholds.

Threshold temperature (°C)	Hinton, Alberta 1993
1	0.934
2	0.933
3	0.933
4	0.929
5	0.934
6	0.929
7	0.925
8	0.925
9	0.924
10	0.921
11	0.919
12	0.910

Table 2.2 Parameter estimates, for all thresholds, for the development of *Epuraea obliquus* near Hinton, Alberta. (a_j are stage-specific parameter estimates and b^2 is a parameter proportional to development variability (Lysyk 1989))

Threshold Temp. (°C)	*a*1	*a*2	*a*3	*a*4	*a*5	b^2
1	225.83	300.59	379.49	462.41	484.52	3.84
2	203.03	268.54	338.91	417.34	430.16	3.57
3	183.41	239.84	304.33	372.25	387.04	3.21
4	163.44	214.92	271.01	332.74	347.95	2.54
5	145.97	191.80	243.04	294.32	307.40	2.55
6	129.63	168.89	215.19	260.67	272.51	2.19
7	115.14	152.56	189.42	228.84	239.76	1.68
8	103.91	135.53	167.97	201.86	211.47	1.49
9	91.51	117.64	148.71	177.00	183.77	1.23
10	82.01	103.09	128.15	153.95	158.70	1.14
11	71.75	90.03	111.10	131.89	136.12	1.00
12	61.64	77.06	94.95	112.73	116.49	1.00

Table 2.3 Parameter estimates for *Epuraea obliquus* development near Hinton, Alberta in 1993 at a 5°C threshold.

Parameter	Estimate	Asymptotic standard error	Asymptotic 95% CI Lower	Upper
$a1$	145.97	2.36	141.31	150.64
$a2$	191.80	2.86	186.15	197.45
$a3$	243.05	2.81	237.48	248.59
$a4$	294.33	3.57	287.28	301.37
$a5$	307.40	3.89	299.72	315.08
b^2	2.55	0.18	2.20	2.91

Table 2.4 Proportion of galls collected, for different age groups, which have at least one individual of *Epuraea obliquus*.

Gall age	Galls sampled in 1992	Galls sampled in 1993
≤3	0.74	0.59
4	0.78	0.68
5	0.77	0.68
6	0.64	0.81
7	0.77	0.93
8	0.72	0.46
9	0.75	0.60
≥10	0.73	0.64

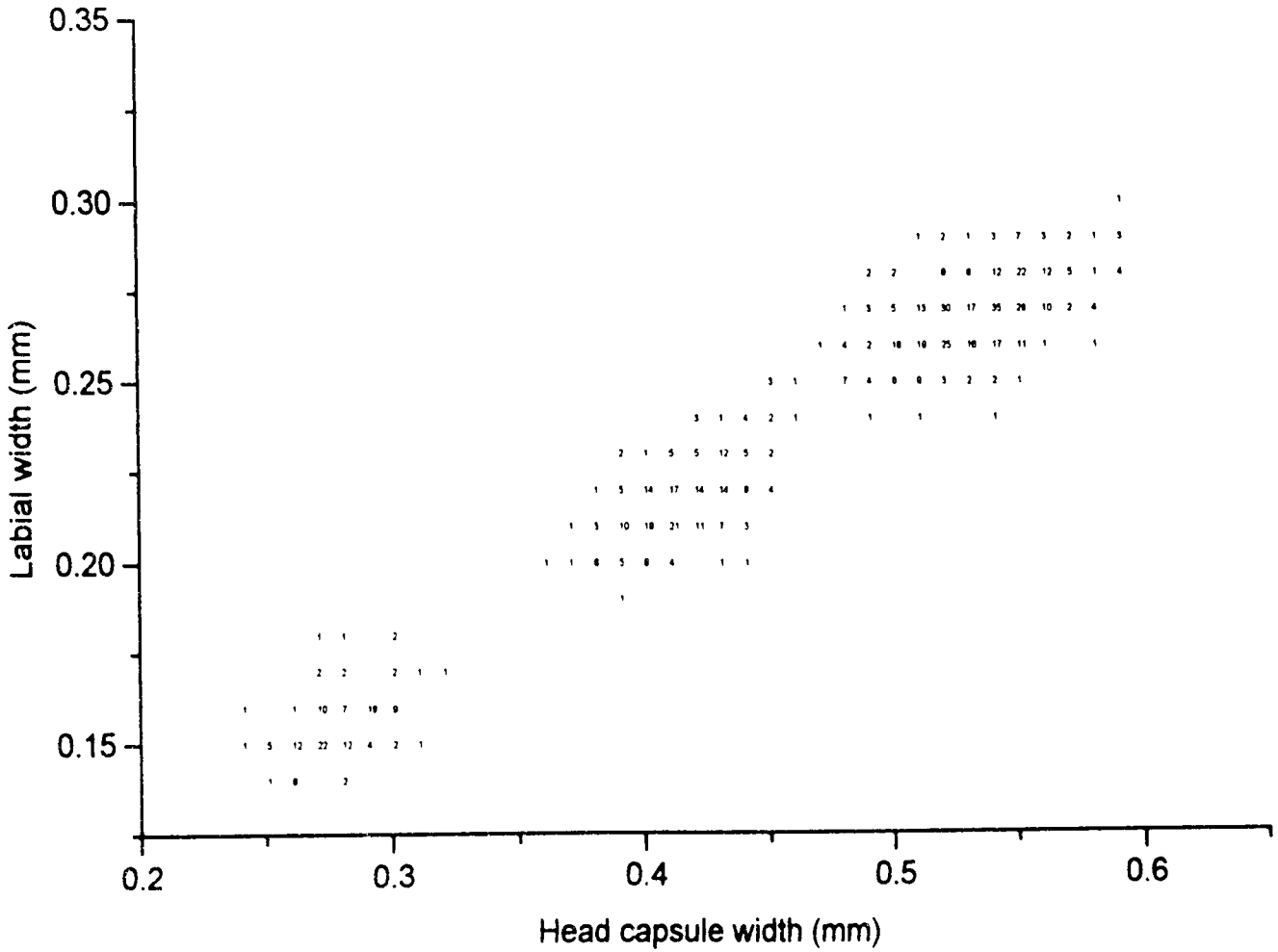

Figure 2.1 Scatter plot of *Epuraea obliquus* larval head capsule width by labial width. (n=727, Numbers represent the frequencies of measurement combinations)

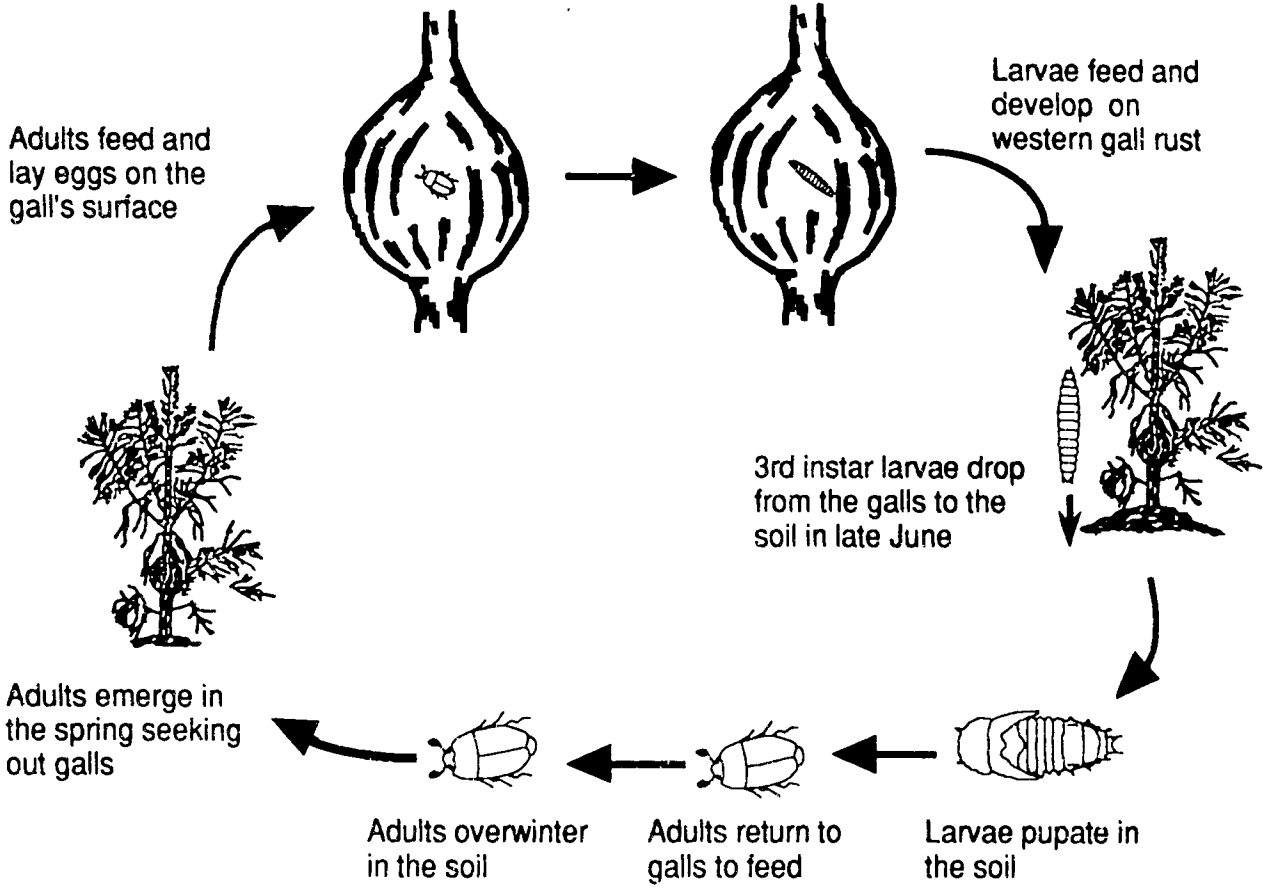

Figure 2.2 Life cycle of *Epuraea obliquus* on western gall rust near Hinton, Alberta.

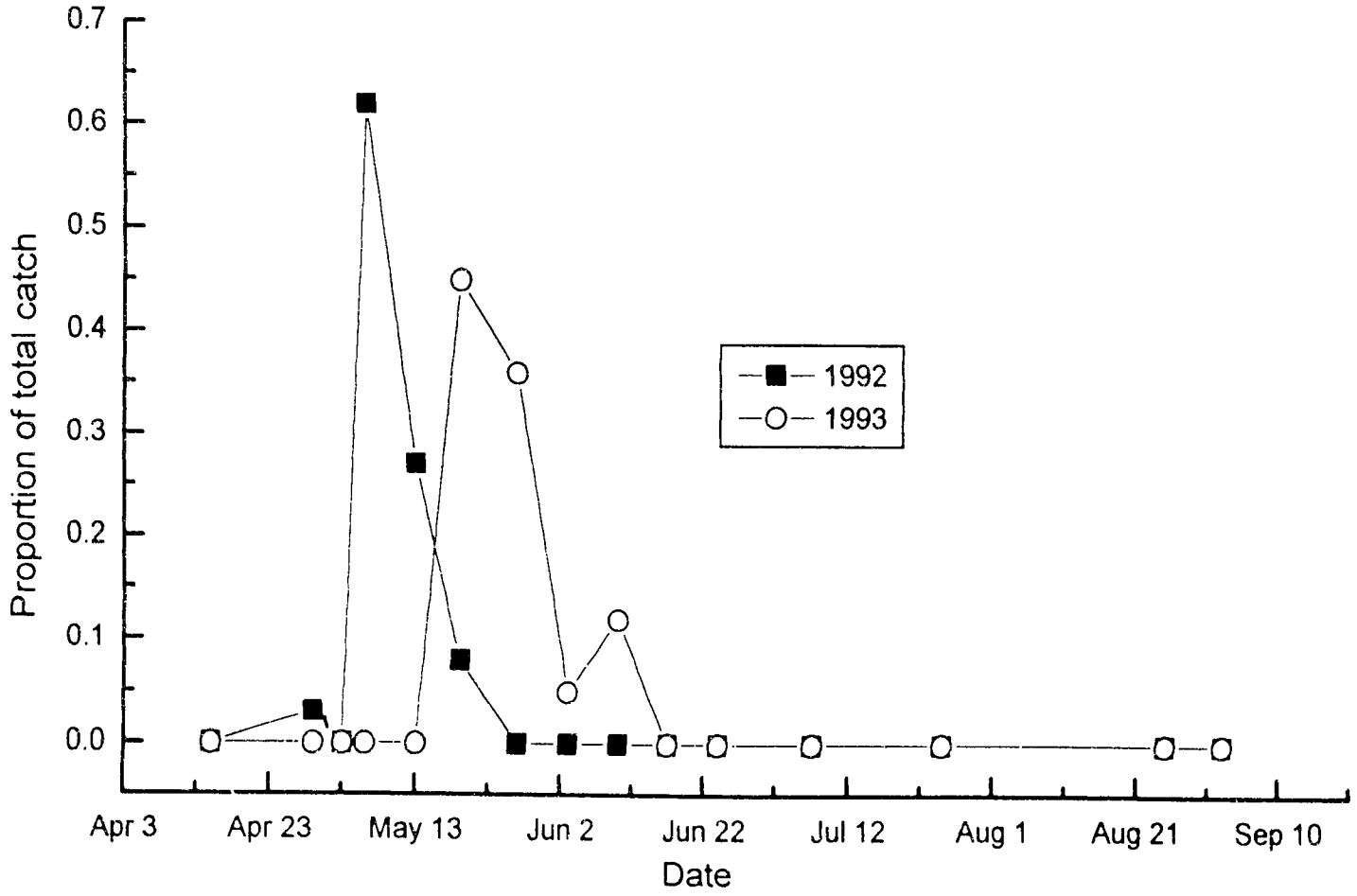

Figure 2.3 Catches of emerging adults of *Epuraea obliquus* near Hinton, Alberta. (n=38 for 1992 and n=37 for 1993)

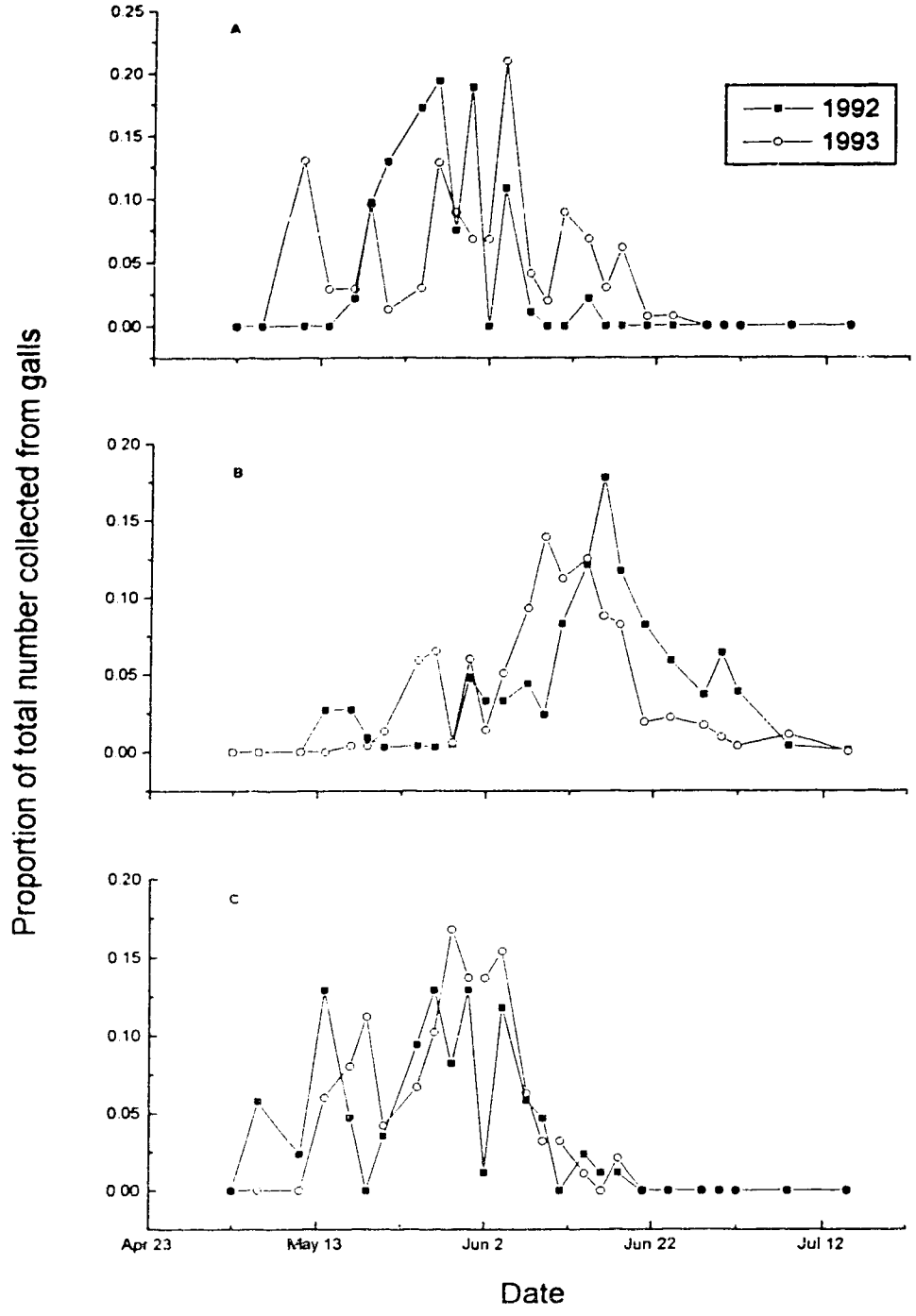

Figure 2.4 Phenology for *Epuraea obliquus* near Hinton, in 1992 and 1993 for a)Eggs, b)Larvae and c) Adults.

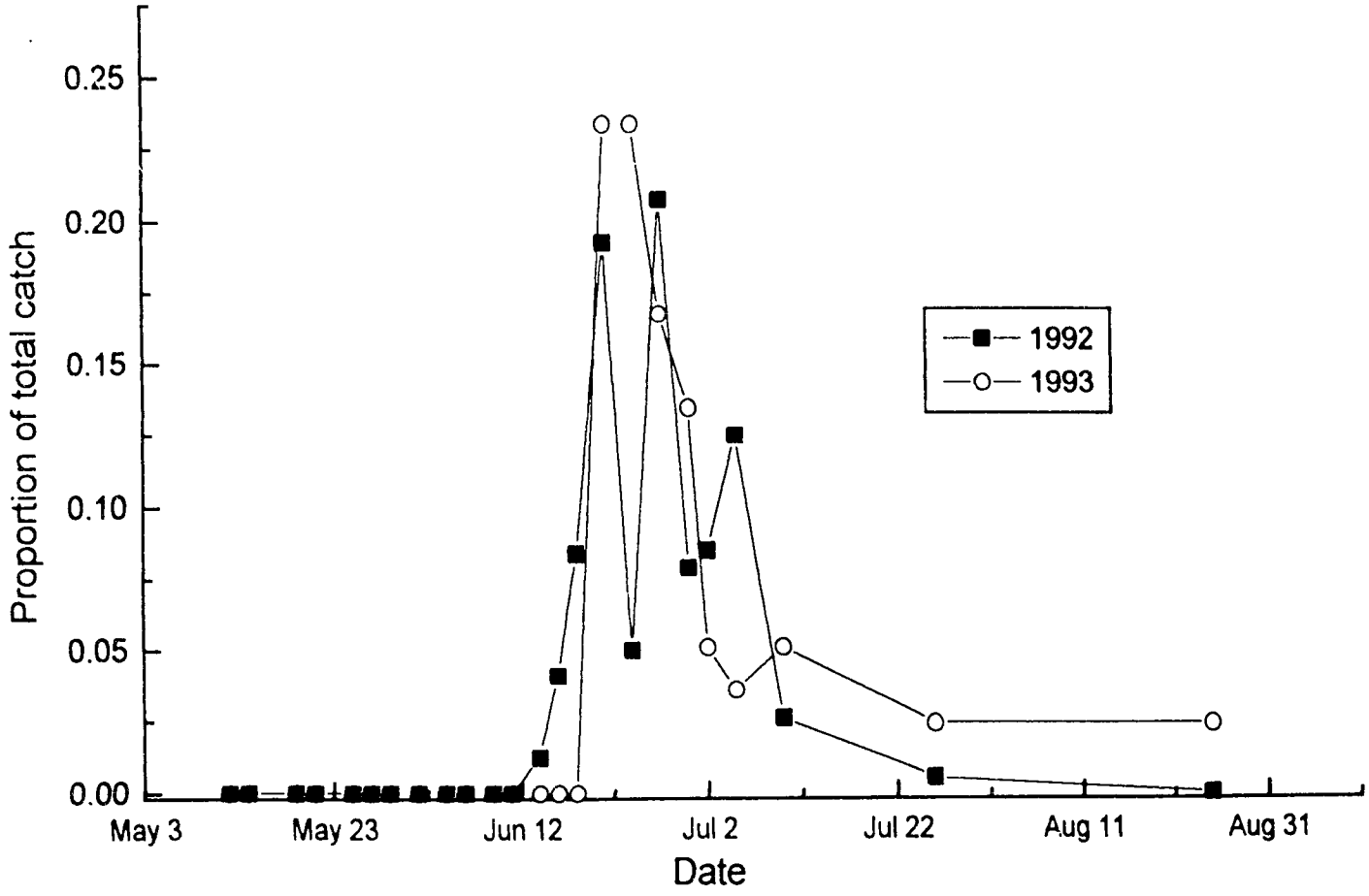

Figure 2.5 Timing of third instar *Epuraea obliquus* larvae dropping from western gall rust near Hinton. (n=333 for 1992 and n=777 for 1993)

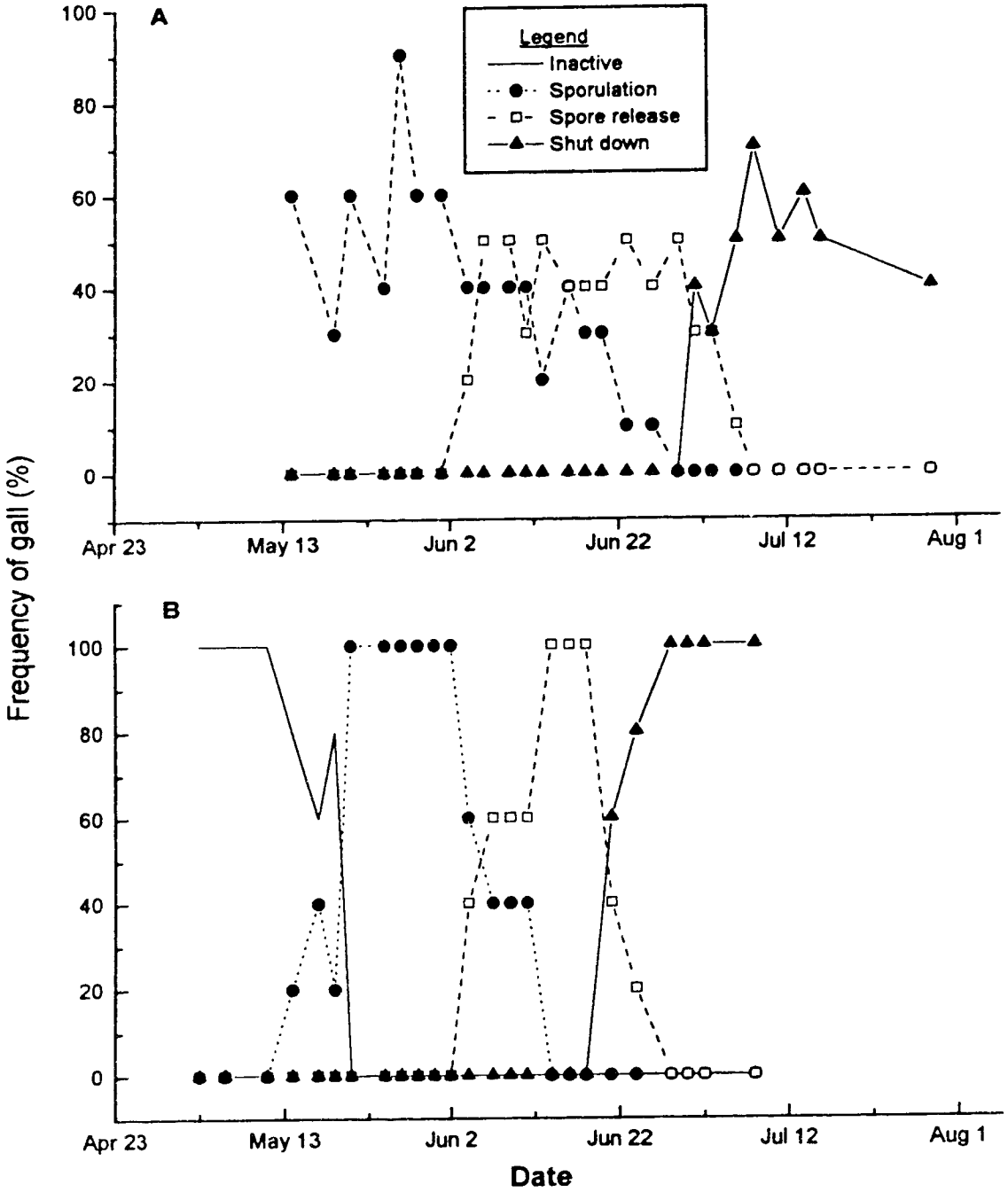

Figure 2.6 Timing of western gall rust activity in Hinton, in A) 1992 and B) 1993.

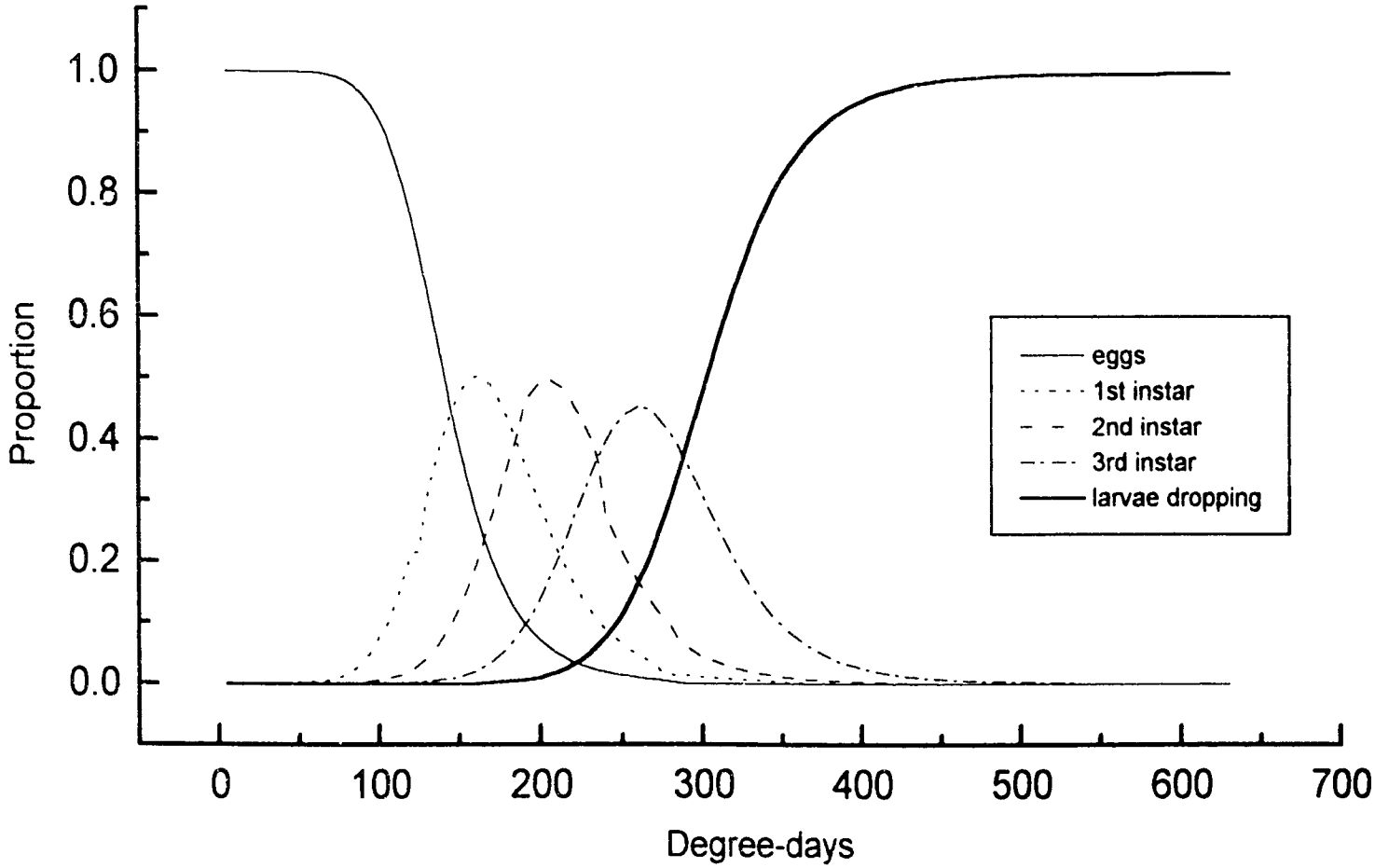

Figure 2.7 Predicted timing of different life stages for *Epuraea obliquus* near Hinton, Alberta in 1993 with a 5 degree threshold.

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3. Dissemination of the mycoparasite, *Scytalidium uredinicola* Kuhlman *et al.*, by *Epuraea obliquus* Hatch (Coleoptera: Nitidulidae)

3.1 SYNOPSIS

The role of *Epuraea obliquus* in disseminating *Scytalidium uredinicola,* a mycoparasite of western gall rust, *Endocronartium harknessii* (J. P. Moore) Y. Hiratsuka, was investigated in a lodgepole pine, *Pinus contorta* Dougl. var *latifolia* Engelm., stand near Hinton, Alberta. Spores of this mycoparasite were observed on the surface of adult beetles collected from western gall rust and adults migrate from one gall to another. Thus it is concluded that *E. obliquus* can transfer the mycoparasite from infected to clean galls. Viable spores of the mycoparasite were common on the body of overwintering beetles. Therefore, beetles emerging in the spring can disseminate *S. uredinicola* to previously uninfected galls. In field and greenhouse experiments, adults inoculated with spores of the mycoparasite caused significant numbers of *S. uredinicola* infections on western gall rust infected seedlings. *E. obliquus* is an important natural vector of *S. uredinicola* in western Canada.

3.2 INTRODUCTION

Although wind dispersal is often considered the most common method of fungal dispersal, the role of arthropods in fugal dissemination may have been underestimated (Malloch and Blackwell 1992). Most identified arthropod vectors of fungi carry destructive plant pathogens and our understanding of the arthropod-fungal relationship springs from examples of

economic importance. For example, nitidulid beetles act as vectors of destructive plant diseases, including oak wilt (Jewell 1956, Juzwik and French 1986), brown rot of stone fruits (Tate and Ogawa 1975) and pineapple disease of sugarcane (Chang and Jensen 1974).

Invertebrates also have been identified recently as vectors of some mycoparasites (Blackwell and Malloch 1989a, Blackwell and Malloch 1990, Blackwell *et al.* 1986), which are fungi which parasitize other fungi. Since the habitat of mycoparasites often consists of small ephemeral patches, limiting them both spatially and temporally, dissemination must be accurate and efficient. This makes dissemination by arthropods, which actively seek out the same habitats, a plausible method of dispersal. A better understanding of mycoparasite dispersal could be useful because many of these abundant organisms are effective natural enemies of plant pathogens and thus have potential in biological control.

Western gall rust is a destructive pathogen of hard pines in Western Canada (Ziller 1974, Hiratsuka *et al.* 1988) and harbors a diverse assemblage of both arthropods and mycoparasites (Powell *et al.* 1972, Tsuneda and Hiratsuka 1979, Tsuneda and Hiratsuka 1980, Tsuneda *et al.* 1980). Two common natural enemies of western gall rust are the mycoparasite, *Scytalidium uredinicola* Kuhlman *et al.*, and the nitidulid beetle, *Epuraea obliquus* Hatch. Since both species occupy the sporulating surface of western gall rust, they have a close spatial and temporal relationship (Chapter 2). This suggests that *E. obliquus* may serve as a vector for *S. uredinicola.*

The relationships between *E. obliquus* and *S. uredinicola* were investigated near Hinton, Alberta in 1993. In particular I studied the ability of

E. obliquus to carry spores, transfer spores and cause infection of the mycoparasite.

3.3 MATERIALS AND METHODS

This work was carried out in a naturally regenerating lodgepole pine stand approximately 25 km south of Hinton, Alberta in 1993. The stand was chosen because of its heavy incidence of western gall rust infection. This stand was harvested in 1973 and thinned in 1987.

To determine whether *E. obliquus* carries spores of *S. uredinicola,* beetles were collected from galls parasitized by the mycoparasite. Scanning electron microscopy was used to examine the beetles for spores of *S. uredinicola* which were identifiable by their cylindrical shape, characteristic indentations on their dorsal and ventral surfaces and their size (typically 2.1-2.9 μm x 2.3-3.5 μm) (Tsuneda *et al.* 1980). To confirm the viability of spores, beetles were placed on the surface of carrot agar for several hours. After two days, fungi grown in culture which appeared to be *S. uredinicola* were stained using lacto-phenol cotton blue and examined on slide mounts to confirm the identification.

I investigated whether *E. obliquus* could serve as a vector for *S. uredinicola* in two different ways. First, the mycoparasite could overwinter in the soil with the beetles and then be carried to galls by beetles as they emerge in the spring (Figure 3.1). Secondly, adults could move the fungus from infected galls to clean galls though inter-gall movement. If beetles emerging in the spring do not carry viable spores of *S. uredinicola* and if adult beetles do not move from gall to gall during the year, *E. obliquus* would have no apparent potential as a vector of *S. uredinicola*.

To determine if *S. uredinicola* successfully overwinters on the bodies of *E. obliquus*, adults were collected from the soil and examined. Twenty wooden emergence traps, each covering approximately 0.56 m^2 of soil, were placed on the ground close to trees infected with western gall rust. The traps were pyramid shaped with two opposite sides made of wood and two covered in mosquito netting. They funneled emerging insects into a small plastic collecting container at the top. To ensure these beetles had not already visited galls, potentially picking up spores of the mycoparasite, traps were placed in the stand on April 3, before the soil had thawed. *E. obliquus* adults collected in these emergence traps were placed on carrot agar and the presence of viable *S. uredinicola* spores was determined by subsequently examining the plates for the presence of the mycoparasite. Since *S. uredinicola* grows slowly in culture, this method is highly conservative. Therefore, a better estimate of the number of beetles carrying spores was obtained by examining these beetles using scanning electron microscopy.

The possibility of beetles transferring *S. uredinicola* from an infected to a clean gall was first investigated by determining if adults migrate from one gall to another. On April 3, before adult beetles had emerged from the soil, 62 galls were randomly selected and then caged by placing pollination bags over them and closing each end with duct tape. Bags were held away from the galls with frames made of plastic tubing. The emergence timing was monitored using emergence traps (as described above) checked three times a week. Three days after final beetle emergence (May 24, 1993), bags were removed from 18 galls, allowing these galls to be colonized if adults were migrating from gall to gall. Eighteen galls were kept bagged for comparison. Bagged and open galls were left in the field for two weeks before being collected. The remaining bagged galls were divided into two groups of 13

and used as above to monitor beetle movement during the period of June 4-17. All galls were frozen shortly after collection and later dissected to determine the presence of adults and/or larvae of *E. obliquus*. The presence of larvae was monitored because it is further indication of adult visits.

The potential movement of beetles from gall to gall was also monitored using sticky traps. Twenty two sticky traps were placed in front of or behind galls, to capture *E. obliquus* adults. Traps made of circular plastic container lids (diameter of 11.5 cm) with automotive lubricating grease (Darina grease Ax, Shell Canada, Limited, Toronto) spread lightly across the surface were hung with wire from branches at a height ranging from 1-2 m. Twelve traps were placed in the east part of the site and ten were placed in the west. I assumed that adults captured by sticky traps after adults had finished emerging from the soil were individuals migrating from other galls.

Bagging studies were done in both the field and the greenhouse to confirm that *S. uredinicola* spores carried by *E. obliquus* could infect western gall rust. Seedlings, inoculated with western gall rust and reared in the greenhouse at the Northern Forestry Centre in Edmonton, were used to reduce the possibility of previous infection of *S. uredinicola*. Seedlings which were sporulating over significant amounts of their surface were selected and bagged with pollination bags to either keep beetles out or to retain beetles added to the bags.

In the field trials two beetles, a male and a female, which were allowed to feed on *S. uredinicola* for 24 hours were added to 12 bagged galls. Similarly handled beetles were also introduced onto twelve seedlings which had been held under colder temperatures in the greenhouse, and thus were delayed developmentally. This was done to determine if infection of *S.*

uredinicola was influenced by the stage of western gall rust aecia development. Another twelve caged galls had no beetles added.

The possibility of beetles traveling from an infected gall, creating an infection on an unifected gall was examined by placing 2 beetles collected from parasitized galls in the field, immediately into 12 caged seedlings. The rate of natural field infections was examined by placing 21 seedlings in the field with no bags, allowing natural colonization by *E. obliquus*. Once a week these galls were examined to determine what insects (potential vectors) were present on the galls. Seedlings were placed in the stand in early May and collected in early June.

Similar experiments were done in a greenhouse to provide better control of environmental conditions. Two beetles, which had been allowed to feed on *S. uredinicola* for 24 hours, were added to 15 bagged galls. Another 15 galls were bagged without beetles. The timing of this experiment closely corresponded to the timing of the field experiment.

Presence or absence of *S. uredinicola* was determined by gall dissection. The identification of mycoparasites on the galls was made by placing pieces of parasitized sorus on carrot agar and trying to isolate the fungi. Also examined by placing parasitized pieces on a microscope slide and using lacto-phenol cotton blue stain. Positive determination of the presence of *S. uredinicola* on the galls was made without knowledge of the treatment to prevent experimenter bias. Presence of other fungi, such as *C. gallicola*, was also noted to examine the potential of beetles transferring other fungi other than *S. uredinicola*.

3.4 RESULTS

Scanning electron microscopy revealed that beetles collected from western gall rust parasitized by *S. uredinicola* frequently had spores of the mycoparasite on their surface. Adults often were observed feeding in the location of *S. uredinicola* infections and thereby probably picked up spores of the mycoparasite. Spores were commonly found on the antennae and other areas of the beetle's head. They also were often found on the ventral surface of adult beetles (Figure 3.2). Spores seemed to be concentrated around sutures, setae and sulci, although spores were also located on flat open areas of the beetle's elytra.

Viable spores of *S. uredinicola* can overwinter successfully on the surface of overwintering *E. obliquus* adults although the frequency of this is unclear. Of the 30 emerging beetles placed on carrot agar only one produced a successful culture of the mycoparasite. This is probably an underestimate because most cultures were overrun with other fungi, such as *Penicillium sp.,* and the relatively slow growth of *S. uredinicola* in culture makes it difficult to isolate. Scanning electron microscopy of beetles collected by emergence traps also showed that spores of *S. uredinicola* are found on overwintering beetles. Of twelve beetles examined, six had significant numbers of spores on their body. Therefore, I conclude that *E. obliquus* can vector *S. uredinicola* by emerging in the spring with viable spores of the mycoparasite and carrying them to potentially uninfected galls. Adults likely obtained spores of the mycoparasite in the late summer when teneral adults travel to galls to feed (Chapter 2).

Bags opened after final emergence were colonized by *E. obliquus* (Figure 3.3). During the first time period, 8 of the 18 galls with bags removed during the first time period were colonized by adults as compared to one

bagged gall being colonized (Fisher's exact test, n=18, p=0.02). The presence of an adult in the caged bag indicates that these enclosures did not stop all beetles from finding their way into the bags. Beetles likely entered through the duct-taped ends of the enclosure since adults were commonly found stuck to the tape when these bagged galls were examined in the laboratory. The presence of larvae, indicating colonization by adults, which may no longer be present on the gall, was not significantly different between the open and caged galls during time period one.

During the second time period, significantly more open galls, 5 of the 13, were colonized by *E. obliquus* adults than the bagged galls, 0 of 13 (Fisher's exact test, n=13, p=0.05). Also, larvae were found on more of the open galls than the bagged galls (marginally significant, Fisher's exact test, n=13, p=0.09). Although no adults were located on the bagged galls during this time period one gall did have larvae. The larvae indicate that at least one adult colonized the gall but it may have escaped the bag or was not located when the bag and gall were examined in the laboratory.

Comparison of the number of galls colonized by adults, as indicated by their presence or that of larvae, in both time periods show that a significant number of galls were colonized once the bags were removed as compared to the control. Eighteen out of 31 galls were colonized by *E. obliquus,* while only 4 out of 31 of the control galls were colonized (Fisher's exact test, n=31, p=0.009).

Beetle movement from gall to gall was also detected by sticky trap captures (Figure 3.4). *E. obliquus* captured on sticky traps after final beetle emergence are individuals migrating from one gall to another. In 1993, final beetle emergence occurred on May 20 but many individuals were still caught on sticky traps after this date. The peak capture of individuals in both sticky

trap lines probably consisted mostly of emerging individuals, although some could have been individuals moving from gall to gall. However, individuals caught after these peaks (after final beetle emergence) must have been moving from one gall to another. Individuals, presumably moving from gall to gall, were caught as late as June 23, 1993 on sticky traps. Since the results of the bagging and sticky trap sampling both indicate that adults migrate from gall to gall, *E. obliquus* could act as a vector from *S. uredinicola* by transferring the spores from infected to uninfected galls.

Western gall rust infected seedlings placed in the field showed that contact with *E. obliquus* can cause infection by *S. uredinicola*. *S. uredinicola* was not detected on bagged seedlings (Figure 3.5). However, five of ten seedlings exposed to beetles allowed to feed on *S. uredinicola* developed infection of the mycoparasite (Fisher's exact test, n=10, p=0.02).

Seedlings held initially under colder environmental conditions in the laboratory with beetles added showed marginally significant amounts of infection (Fisher's exact test, n=12, p=0.07), and did not differ significantly from the faster developing galls. These results suggest there is no effect of gall development on infection.

Seedlings placed in the field without bags, showed marginally significant amounts of infection in comparison to the bagged control treatment (Fisher's exact test, n=21, p=0.07). *E. obliquus* adults were observed commonly on the surface of these galls in the field. No other invertebrates were frequently observed on these galls. Galls that had beetles collected from native galls introduced into bagged seedlings, did not develop significant infections. However, one seedling from this treatment was infected, suggesting that this method of vectoring is possible.

The seedlings placed in the field were also parasitized by other fungi. *Cladosporium gallicola* was found in significant amounts on two of the treatments (Figure 3.6). The fungus was found on 4 of the seedlings which had *S. uredinicola* feed beetles added and found on 10 of 21 of the open seedlings. *C. gallicola* was not found on galls in any other treatments. *Penicillium sp.* was also found on many of the galls.

Seedlings kept in the greenhouse to which beetles had been added had significant amounts of *S. uredinicola* infections (Fisher's exact test, n=15, p=0.001). No *C. gallicola* was found the on these galls but *Penicillium sp.* occurred frequently.

3.5 DISCUSSION

S. uredinicola is a mitotic fungus producing arthrocondia which parasitizes pine stem rusts such as western gall rust (Tsuneda *et al.* 1980) and fusiform rust, *Cronartium quercuum* f. sp. *fusiforme* (Kuhlman *et al.* 1976). This mycoparasite breaks down spores and the active rust sori of western gall rust (Tsuneda *et al.* 1980) making infected areas appear clumpy and stringy (personal observation). Infections begin early in the season (Chapter 3) and are found in the developing sorus under the periderm. The appearance, location and timing (as mentioned above) of this fungus suggest that it is not wind disseminated. Also, since the mycoparasite obtains its resources from the sporulating tissue of its host, it is benefited locally by preventing wind dispersal of the gall rust spores, prolonging the duration of available resources. Kuhlman (1981a) found that *S. uredinicola* delays the spore release of *C. fusiforme*. Since the prevention of wind dissemination by

the host likely prevents wind dissemination by the mycoparasite *S. uredinicola* must have an alternative method of dispersal.

There is ample evidence indicating a long co-evolution between fungi and invertebrates. This is well documented, for example, in mutualistic interactions in which insects have developed specialized structures, called mycangia, for disseminating fungi. This type of interaction has been identified to occur in at least three insect orders (Malloch and Blackwell 1992), suggesting that such co-adaptation is common. Fungi involved in such relationships also could evolve specialized spores for invertebrate dissemination. For example, Tsuneda (1987) found that the fungus *Dipodascus aggregatus* produces both wet-spored conidia, which are dispersed by insects, and dry-spores types, which are wind disseminated. *S. uredinicola* spores are small and round with indentations and readily adhere to the body of *E. obliquus,* suggesting that their structure is specialized for insect dissemination.

Powell *et al.* (1972) collected 78 species of invertebrates from western gall rust in western Canada. However, *E. obliquus* is the most abundant invertebrate species occurring on western gall rust near Hinton, with much of its life cycle occurring on the gall during sporulation (Chapter 2). Since *S. uredinicola* is also present on the sporulating tissue during the same period, it has a close association with *E. obliquus.* The beetle also feeds on *S. uredinicola* (Chapter 2), potentially picking up spores of the mycoparasite. The association between the nitidulid and the mycoparasite and the abundance of the beetle on western gall rust make it plausible that *E. obliquus* is the predominant natural vector for the mycoparasite.

E. obliquus vectors the mycoparasite, *S. uredinicola,* by transferring spores from infected galls to clean galls. Adults on the surface of western gall rust are commonly observed feeding on parasitized areas of western gall rust and spores were observed on these beetles using scanning electron microscopy. Adult beetles also actively migrate from one gall to another during the season, easily transferring the mycoparasite to previously uninfected galls. Adults inoculated with mycoparasite and placed into seedlings in the field and the greenhouse caused significant numbers of infections. Also, adults collected from other galls and placed on seedlings created one infection, and although not a statistically significant effect in this experiment, it indicates that this method of dissemination is possible. It is not clear if adults actively move among galls as part of their reproductive strategy or if they just fall from their initial gall and end up on a different gall as a consequence of subsequent host searching behavior. It is common to observe adults either falling or being blown from galls. Also, when adults are touched they feign death, a predator avoidance behavior, often letting go of the gall and dropping to the soil.

The beetle also disseminates the mycoparasite by transferring the spores to new galls as they emerge from overwintering in the soil. Overwintered beetles carry viable spores of the mycoparasite on their body. Newly emerged adults probably come in contact with the mycoparasite in the fall, when they seek out western gall rust to feed on *S. uredinicola* and other mycoparasites on the surface of galls before dropping to the soil to overwinter (Chapter 2).

The natural role of *E. obliquus* in vectoring the mycoparasite was also confirmed with seedlings placed in the field. Galls which were placed into a

lodgepole pine stand without being bagged were colonized abundantly by adult beetles. These seedlings had significant numbers of infections of the mycoparasite as compared to the bagged seedlings. No other invertebrates were found more than incidentally on these seedlings, suggesting that *E. obliquus* is the predominant vector.

It is likely that there is a common association of this mycoparasite with the genus *Epuraea.* Both the *S. uredinicola* and *E. obliquus* occur on other pine stem rust diseases in Western Canada. Also, this mycoparasite is found on pine stem rusts in other areas of North America where other species of *Epuraea* occur. Kuhlman (1981b) suggests that *E. lengi* could be a vector for *S. uredinicola* in South and North Carolina on *Cronartiurn quercuum* f. *sp. fusiforme* on loblolly and slash pine.

Dissemination of *S. uredinicola* is important on three different spatial scales. At the lowest scale, the mycoparasite is disseminated across the surface of an individual gall by mycelial growth. Western gall rust sori are not continous on the surface of galls, preventing this kind of spread. However, *E. obliquus* adults and larvae probably contribute to the spread of the mycoparasite across the surface of galls. Dissemination on two other spatial scales, between galls and between stands, is likely done exclusively with the assistance of a vector like *E. obliquus.*

Although wind dissemination is believed to be the most common method of fungal dispersal, this has been mostly shown in crop pathogens where there are massive sources of inoculum and very large target destinations (Malloch and Blackwell 1992). Releasing massive amounts of wind disseminated spores in these situation is a promising strategy since hosts are large and abundant. However, in situations where fungi have a

more limited target, such as fungi which parasitize other fungi, it is not as likely to be successful. The use of invertebrates is likely to be more successful because of the accurate dissemination. The relatively small spatial scale and ephemeral nature of fungal hosts likely selects for more accurate dissemination, as is possible with the assistance of insect vectors. Additionally, if the mycoparasite parasitizes a wind dispersed fungus it is unlikely that they could also be wind dispersed.

As previously discussed, mycoparasites should be selected to suppress wind dissemination of the host which would likely prevent their own wind dissemination. Therefore, arthropod dissemination is likely a common method of mycoparasite dispersal. For example, the species of the genus *Pyxidiophora* Bref & Tav. are believed to be mostly mycoparasites (Blackwell and Malloch 1989b) and are commonly associated with mites which aid in dispersal (Blackwell and Malloch 1990). This study has shown that *E. obliquus* is an important natural vector of the mycoparasite *S. uredinicola*. Such interesting relationships may be common in nature and likely play an important role in the natural control of many plant pathogens.

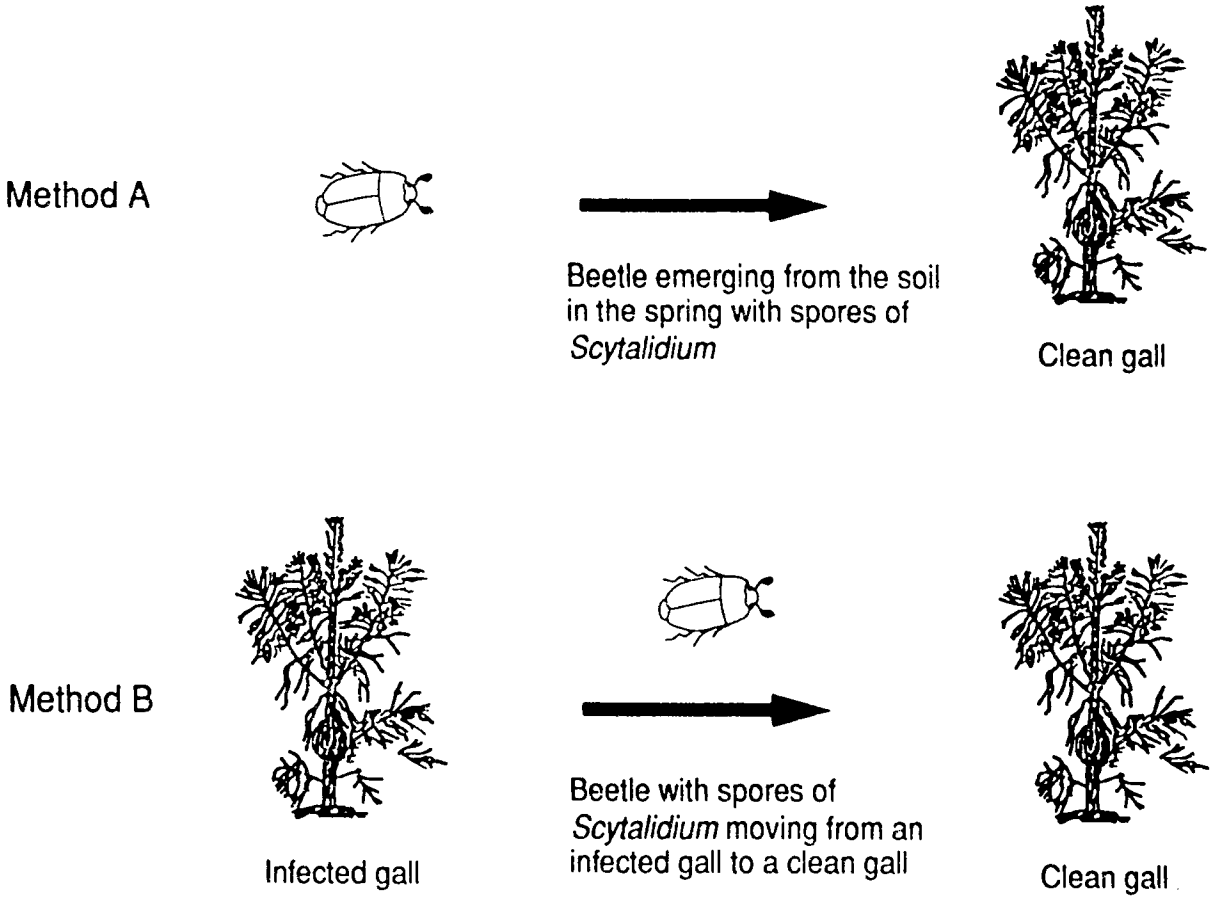

Figure 3.1 Possible methods of *Epuraea obliquus* vectoring *Scytalidium uredinicola*.

Figure 3.2 Spores of A) *Endocronartium harknessii* and B) *Scytalidium uredinicola* on the body of overwintering *Epuraea obliquus*.

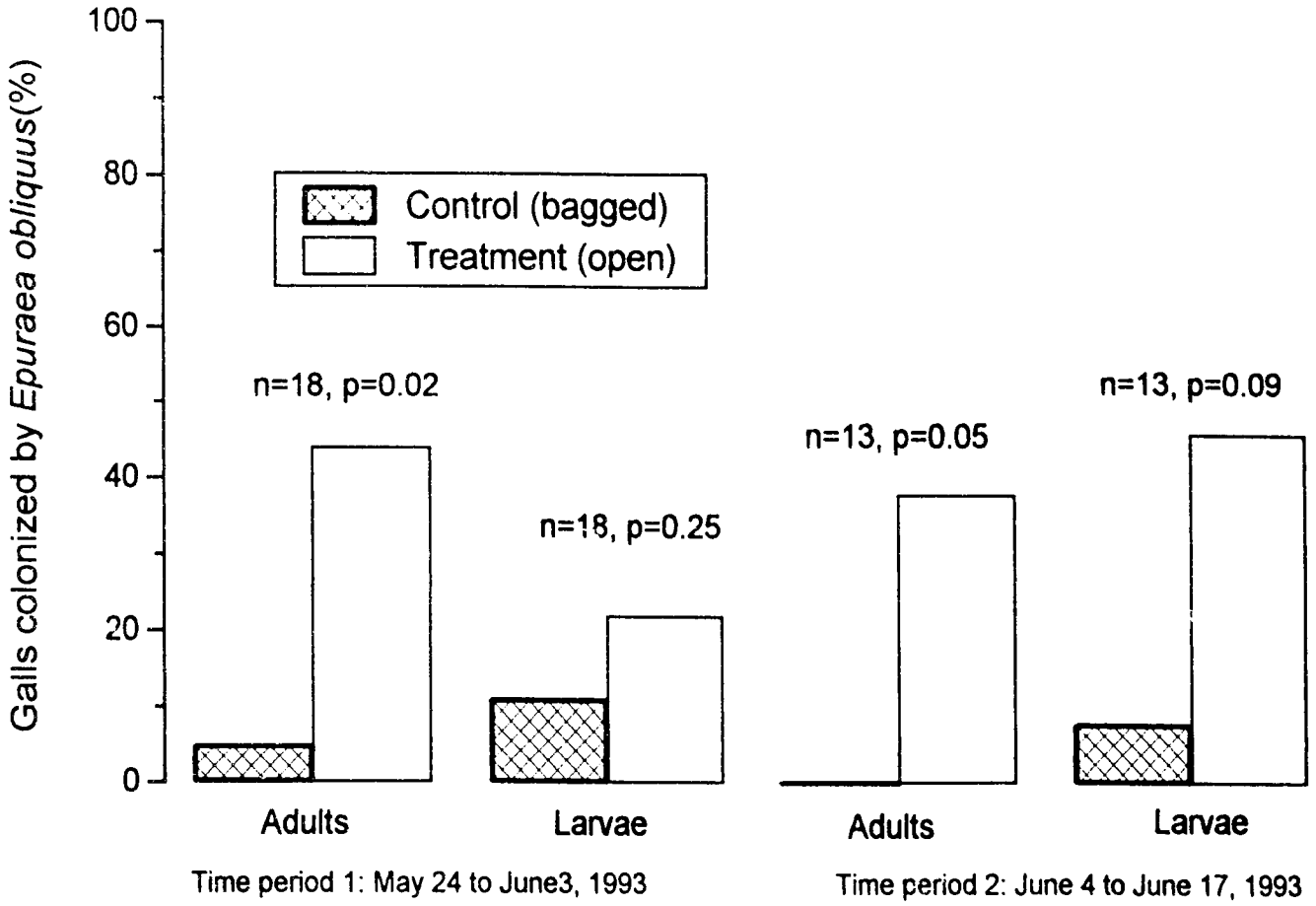

Figure 3.3 Movement of *Epuraea obliquus* from gall to gall near Hinton, 1993.

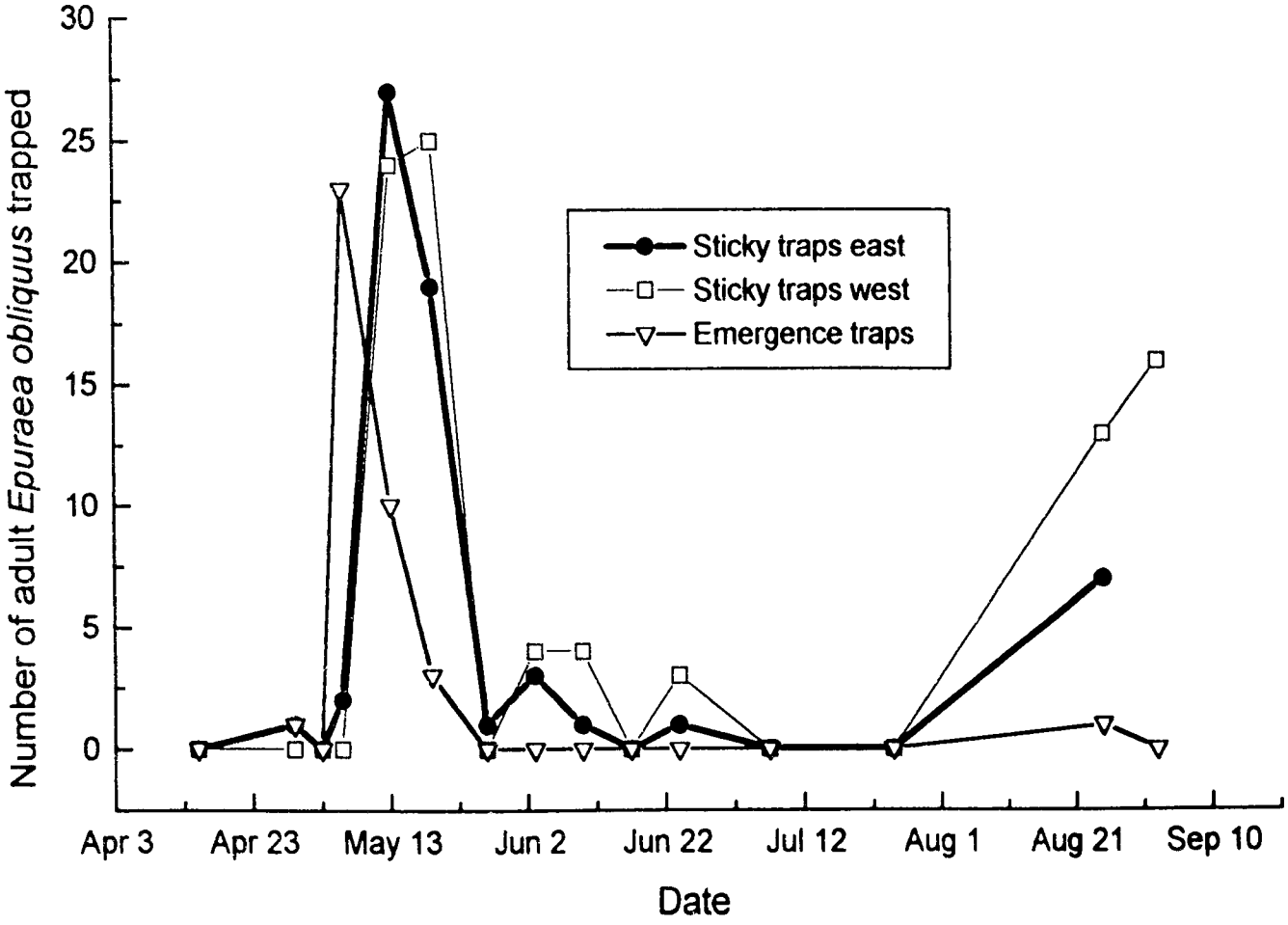

Figure 3.4 Emergence and sticky trap capture of *Epuraea obliquus* near Hinton, 1993.

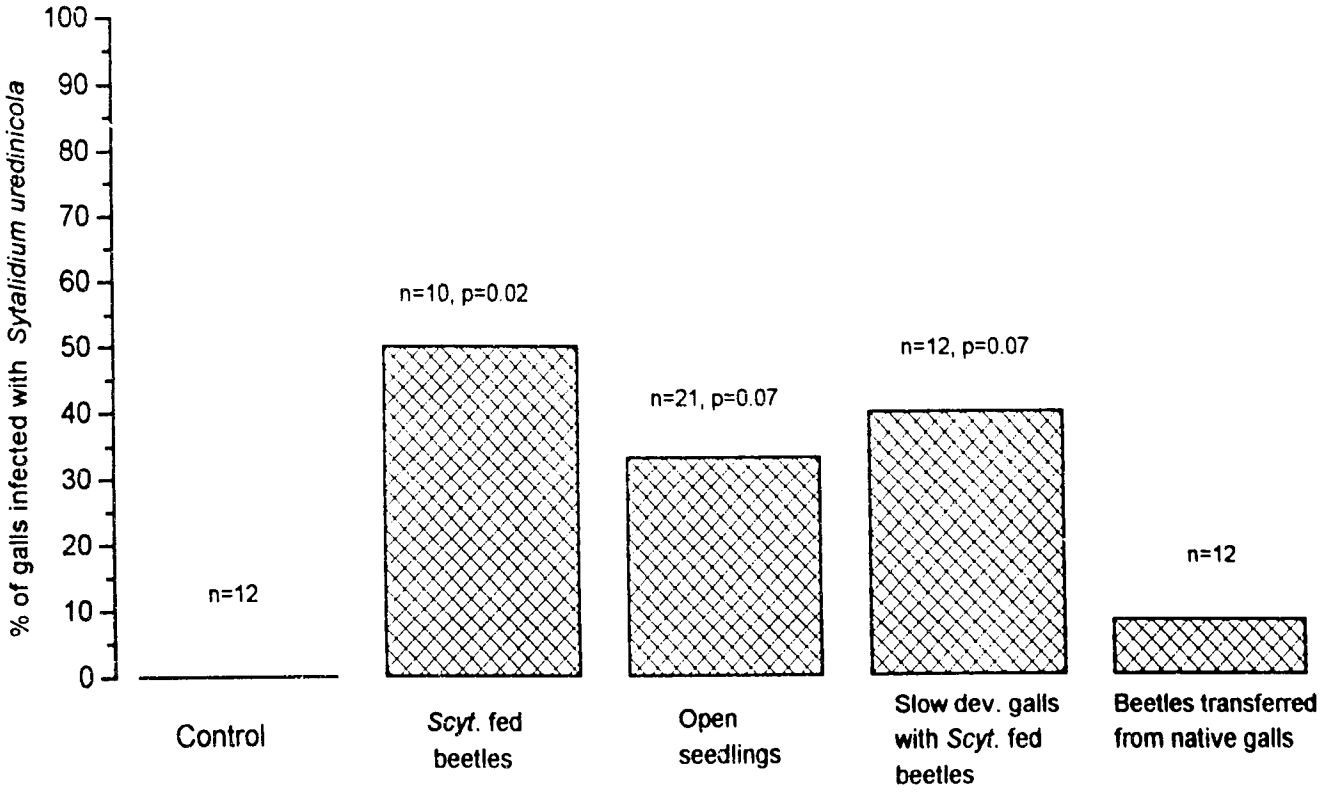

Figure 3.5 Transfer of *Scytalidium uredinicola* to seedlings infected with western gall rust by *Epuraea obliquus*.

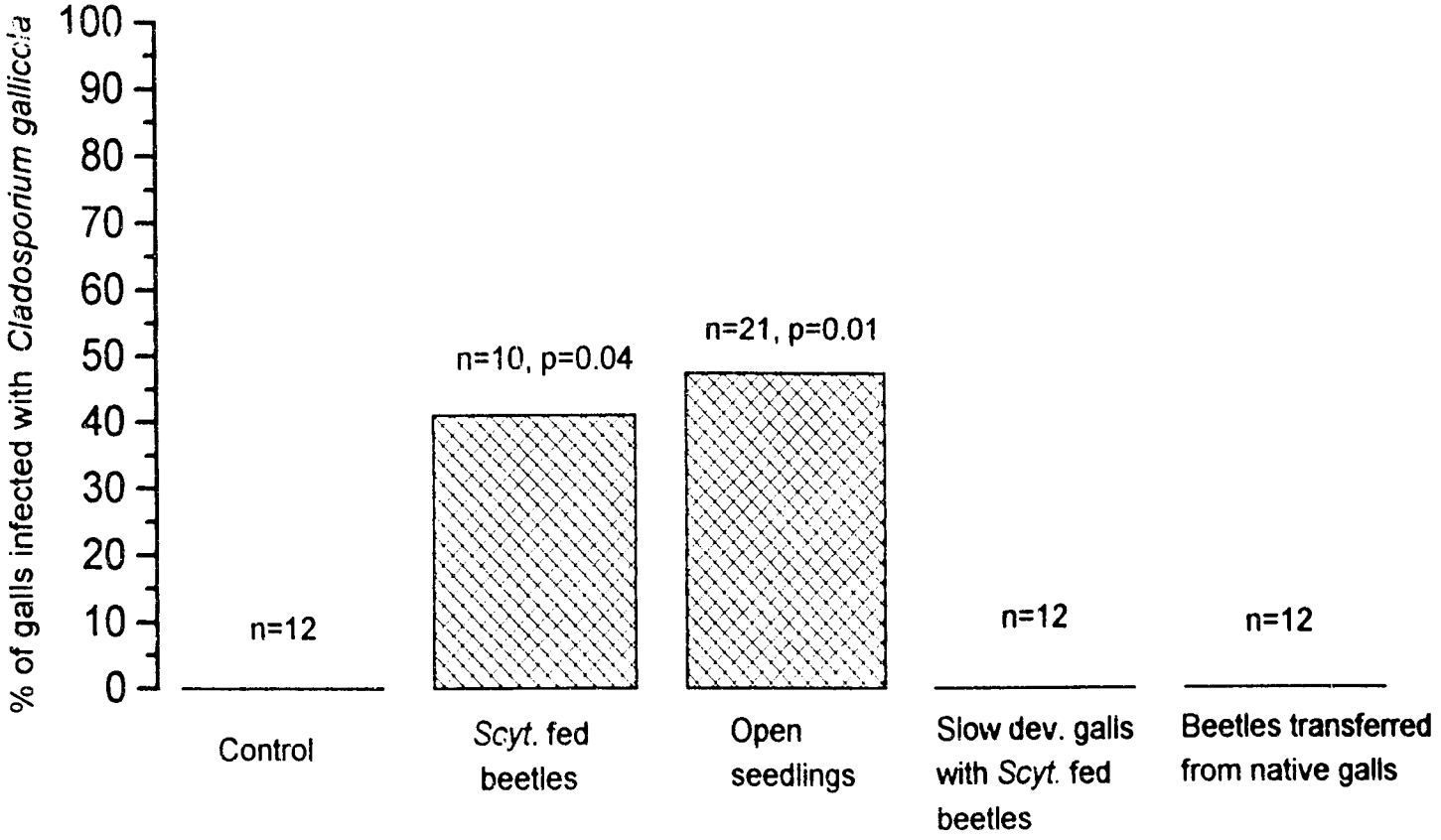

Figure 3.6 Transfer of *Cladosporium gallicola* to seedlings infected with western gall rust by *Epuraea obliquus*.

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4. Biological control of western gall rust: using *Epuraea obliquus* Hatch (Coleoptera: Nitidulidae) as a vector for a mycoparasite

4.1 SYNOPSIS

The potential for biological control of western gall rust, *Endocronartium harknessii* (J. P. Moore) Y. Hiratsuka, on lodgepole pine (*Pinus contorta* Dougl. var. *latifolia* Engelm.) by using a beetle, *Epuraea obliquus* Hatch, to vector a mycoparasite, *Scytalidium uredinicola* Kuhlman *et al.* (Deuteromycotina: Hyphomycetes), was investigated near Hinton, Alberta in 1992 and 1993. As galls age the proportion of sporulating surface parasitized by mycoparasites increases so that most galls ten years or older have more than 95% of their sporulating surface parasitized. The most common mycoparasite in the sporulating tissue of western gall rust was *S. uredinicola*. A mark recapture experiment and sticky trap sampling showed that *E. obliquus* is strongly attracted to western gall rust and therefore it is a promising candidate to disseminate the mycoparasite. The potential use of *E. obliquus* to disseminate *S. uredinicola* for biological control of western gall rust is discussed.

4.2 INTRODUCTION

Western gall rust, *Endocronartium harknessii* (J. P. Moore) Y. Hiratsuka, is an extremely destructive forest pathogen of hard pines in Western Canada (Ziller 1974, Hiratsuka *et al.* 1988). Many different approaches have been tried for control of western gall rust, including breeding for rust-resistant trees, improved tree management and the use of silvicultural controls, such as pruning infected branches (Hunt 1982, Offord *et al.* 1958). However, no control measure has been completely successful. Another possibility is biological control

involving the use of an aggressive mycoparasite to kill and/or reduce the reproductive output of western gall rust. Three agressive mycoparasites are commonly found on western gall rust in western Canada, *Scytalidium uredinicola* Kuhlman *et al.*, *Cladosporium gallicola* Sutton and *Monicillium nordinii* (Bourchier) W. Grams. and all have potential for controlling western gall rust (Tsuneda and Hiratsuka 1979, Tsuneda and Hiratsuka 1980, Tsuneda *et al.* 1980).

A major barrier to using mycoparasites for biological control is the difficulty in effectively delivering the control agent to the disease. Agents might be sprayed in valuable plantations but this would be expensive and ineffective on normal forestry scales because of the large area to be covered and the extreme precision required to hit the gall rust target. However, the nitidulid beetle, *Epuraea obliquus* Hatch, is a natural vector for the mycoparasite (Chapter 3) and could possibly be used to distribute *S. uredinicola* efficiently (Hiratsuka 1990).

To determine the impact of mycoparasites on the epidemiology of western gall rust, the progression of mycoparasitic infection on western gall rust was investigated at two sites near Hinton, Alberta. The timing and abundance of common mycoparasites were examined under natural conditions to determine the most effective mycoparasite for biological control. The attraction of *E. obliquus* to galls was also investigated to determine if it could be effective in disseminating the mycoparasite.

4.3 MATERIALS AND METHODS

This study was conducted in two naturally regenerated lodgepole pine stands approximately 25 km south of Hinton, Alberta in 1992 and 1993. These stands were chosen because of their heavy incidence of western gall rust

infection. The stand studied in 1992 was cut in 1960 and thinned in 1981 and the stand studied in 1993 was harvested in 1973 and thinned in 1987.

4.3.1 Impact of mycoparasites over time

To determine the impact of mycoparasites on the epidemiology of western gall rust, galls were collected three times a week from early May until mid-July (during western gall rust sporulation) of both years. Ten galls were collected per sampling date in 1992 and five galls were collected per collection date in 1993. Branch galls of different ages were collected while walking through the stands and these were frozen until their subsequent study in the laboratory. Galls on dead branches were not collected.

The age of each gall was determined. This can be done by aging the branch, beyond the gall since infection occurs on shoots in their first year of growth. Also, the sporulating surface parasitized by mycoparasite was estimated visually, placing each gall into one of four classes as follows: (1) less than 5% parasitism; (2) 5%-50% parasitism; (3) 50%-95% parasitism; and (4) greater than 95% parasitism. Galls with more than 95% of their sporulating surface parasitized were termed inactivated galls. Galls which had not begun to sporulate were not included in the analyses. Estimates did not differentiate the species of mycoparasite.

4.3.2 Mycoparasite abundance and timing

Galls sampled, as above, were examined to determine the presence and absence of *S. uredinicola, M. nordinii* and *C. gallicola* on the sporulating tissue of western gall rust. These mycoparasites were examined because they are the most promising for biological control of western gall rust. *M. nordinii* was rarely observed, so it was not examined in this study. The abundance of these two mycoparasites was determined by counting the number of galls sampled which

were infected with the mycoparasite. Then the percentage of collected galls parasitized by the mycoparasites was compared. The timing of each mycoparasite was determined by plotting the number of galls infected, by each mycoparasite, on each collection date.

4.3.3 Attraction of the beetle to western gall rust

In 1992, a mark recapture study was conducted to determine the potential attraction of adult beetles to western gall rust. Beetles were released in a naturally regenerated lodgepole pine stand harvested in 1981. This stand was selected because it had a relatively low density of gall rust and most galls were younger than 5 years. In half the stand, approximately 500 m x 200 m, thirty galls were flagged and the remaining galls were removed from the stand. The release occurred in the middle of this section of the stand.

One hundred and fifty adult beetles were collected from galls in other lodgepole pine stands in the area and marked using Fire-Orange Day-Glo pigment (Day-Glo Color, Cleveland, OH). The beetles were released in the middle of the stand on June 6, 1992. The thirty flagged galls were collected on June 20, 1992 and individually placed in plastic containers. Adult beetles were collected from the galls and examined under ultra-violet light to determine if they were marked with the fluorescent powder.

Attraction of *E. obliquus* to western gall rust was assessed further with sticky traps. Thirty-six sticky traps, made of circular plastic container lids (diameter of 11.5 cm) with automotive lubricating grease (Darina grease Ax, Shell Canada, Limited, Toronto) spread lightly across the surface were hung with wire from branches at a height ranging from 1-2 m. Twelve groups of sticky traps, with three traps per group, were set out in a completely randomized block design. One trap was placed either in front or behind a randomly selected gall. Another trap was then placed on the same tree but on a branch with no galls.

The third sticky trap was placed on a neighboring tree (within 5m) which had no visible infection of western gall rust. Trap groups (blocks) were placed in two rows, each having 6 groups placed approximately 50m apart. The rows were placed running east to west and were approximately 50m apart. The number of beetles captured was compared across trap location.

4.4 RESULTS

4.4.1 Impact of mycoparasite over time

Of 248 galls sampled in 1992, 60 (24.2%) were inactivated with more than 95% of sporulating surface parasitized. In 1993, 129 galls were collected and 19 (14.7%) of these galls were inactivated by mycoparasites. The amount of sporulating surface parasitized tended to increase with gall age in both 1992 and 1993 (Tables 4.1a & b). Fourteen out of 15 galls 10 years or older that were collected in 1992 were inactivated. In 1993, all galls sampled 11 years and older were inactivated.

4.4.2 Mycoparasite abundance and timing

S. uredinicola and *C. gallicola* were the most common mycoparasites on the sporulating surface of western gall rust. *M. nordinii* was not commonly observed on western gall rust during May and June in this study. *S. uredinicola* was more abundant than *C. gallicola* on galls collected from early May until early July in the stand studied in 1993. Over 75% of galls parasitized were infected with *S. uredinicola* as compared to less than 35% being infected with *C. gallicola*. Also, *S. uredinicola* was more common on the sporulating surface of western gall rust earlier in the season than *C. gallicola* (Figure 4.1).

4.4.3 Attraction of beetles to western gall rust

Beetles are strongly attracted to western gall rust. Five of fifteen *E. obliquus* adults collected from the 30 galls in the release study were marked with

the fluorescent powder. Thus 3.3% of the released beetles were recaptured on galls showing that *E. obliquus* will search out and colonize western gall rust.

E. obliquus adults are attracted directly to the surface of western gall rust. Of the *E. obliquus* captured, 69.2% were taken on sticky traps close to galls, 28.9% were captured on sticky traps on trees with galls, and only 1.9% of beetles captured were caught on sticky traps on trees with no western gall rust infections (Figure 4.2). Individuals were trapped on significantly more of the sticky traps placed on branches with galls as compared to traps on branches without galls but on trees with galls (Fisher's exact test, n=12, p=0.047) and traps on trees without galls (Fisher's exact test, n=12, p=0.00004). On average 3.3±0.44 (Mean±Standard error) individuals were collected from traps which were associated with western gall rust and 1.4±0.63 individuals per trap were collected from trees with galls. Only 0.35±0.15 individuals per trap were collected from trees with no infection of western gall rust.

4.5 DISCUSSION

In this study most galls sampled were infected with mycoparasites and the older galls tended to have more of their surface area parasitized. At both sites, the older galls were completely inactivated by mycoparasite and thus they provided little inoculum for further western gall rust infections. The increased mycoparasitism on the surface of older western gall rusts could be a result of a slow, progressive spread around the surface, requiring several years after an initial infection, or it could be that older galls attract more beetles, which actually bring in more mycoparasite (Chapter 2).

S. uredinicola was the most abundant mycoparasite occurring in the sporulating tissue of western gall rust. Over 75% of the galls sampled during May and June in the Hinton area had *S. uredinicola* infections. This corresponds

closely with Tsuneda *et al.* (1980) estimate of 80% of galls being infected with *S. uredinicola* in areas of Alberta. *C. gallicola* was not found to be as abundant in the sporulating tissue of western gall rust during these two months, occurring on less than 35% of the galls sampled. The difference in abundance may be due to earlier activity of *S. uredinicola*. *C. gallicola* is active later in the season, increasing in abundance in late June after most spores have been released and thus its impact on the spread of western gall rust is much less than *S. uredinicola*. *C. gallicola* is a much more aggressive fungus in culture than *S. uredinicola*. It is likely that for *S. uredinicola* to occur on western gall rust it must become active earlier to avoid competing with *C. gallicola*. *S. uredinicola* prolongs the sporulation of *Cronartium quercuum* f. sp. *fusiforme* (Kuhlman 1981). This may be beneficial to *C. gallicola* by increasing the duration that western gall rust spores are present for parasitism by *C. gallicola*.

The trend observed in this study, for increasing amounts of mycoparasite on the surface of western gall rust is believed to be mostly driven by *S. uredinicola* because this mycoparasite is the most abundant and occurs earlier in the season For the above reasons and because it significantly reduces the germinability of western gall rust spores (Tsuneda *et al.* 1980) it is concluded that *S. uredinicola* is the most promising mycoparasite for biological control of western gall rust. Kuhlman (1981) also found that *S. uredinicola* significantly reduces the spore production and germinability of *Cronartium quercuum* f. sp. *fusiforme* and it is likely that this mycoparasite is an excellent candidate in biological control of many different pine stem rust diseases.

The potential use of mycoparasites for biological control of pine stem rusts has been suggested several times (Byler *et al.* 1972, Tsuneda *et al.* 1980, Kuhlman 1981). Although it is clear that mycoparasites have a large impact on the inoculum potential of pine stem rusts (Byler *et al.* 1972, Hungerford 1977,

Tsuneda *et al.* 1980, Kuhlman 1981a, Nelson 1982), there have been no successful field implementations.

One of the major problems in using mycoparasites as control agents is their application. Spray programs over large forest areas would be very inefficient and costly. Hiratsuka (1990) proposed using *E. obliquus* for efficient application of *S. uredinicola.* The beetle is an excellent candidate in disseminating *S. uredinicola* because, as shown in this study, it specifically seeks out galls within stands and is attracted directly to the surface of the galls.

Other insects have been shown to be effective in disseminating mycoparasite in biological control programs. Peng *et al.* (1992) increased transmission of mycoparasite to the flowers of strawberries by inoculating bees (which frequently visit strawberry flowers) with mycoparasite as they came out of their hives. This was used to prevent the spread of gray mold, *Botrytis cinerea,* on strawberries. Bees have also been used as efficient vectors for bacterium, *Pseudomonas fluorescens* and *Erwinia herbicola,* antagonistic to fire blight, *Erwinia amylovora* (Thomson *et al.* 1992, Johnson *et al.* 1993).

Before beetles can be released into stands for biological control, it is necessary to determine that their activity does not promote western gall rust infections. It is important that the beetle is attracted directly to the surface of the galls so that the beetle does not also promote and spread western gall rust infection. Since my results show that beetles are attracted directly to galls, there should be minimal contact with lead terminals, which are tree tissue susceptible to infection. Few individuals were collected from sticky traps which were not beside galls or at least on trees with galls. If they just migrated haphazardly to galls more individuals should have been collected on the sticky traps which were not close to galls. Therefore it is unlikely that increasing the population of *E. obliquus* will promote western gall rust infections.

4.5.2 Biological control of western gall rust

Biological control of western gall rust by augmentating populations of *E. obliquus* to increase dissemination of *S. uredinicola* is a very promising approach. The beetle actively seeks out galls and is actually an important natural vector for the mycoparasite (Chapter 3), making it an excellent candidate for accurately disseminating the mycoparasite for biological control of this pathogen. This accurate dissemination of *S. uredinicola* would increase its density thus reducing the spread of western gall rust because it significantly reduces the western gall rust spore viability and decreases the number of spores produced (Tsuneda *et al.* 1980, Kuhlman 1981). Also, the copious feeding of *E. obliquus* adults and larvae on western gall rust spores (Chapter 2) may further decrease the number of spores released by the gall rust. The subsequent increase in abundance of this beetle would then promote quicker natural spread of the mycoparasite both within the stand and between stands.

Although this approach could be costly over large scale forestry and over the life of a lodgepole pine stand it should be only necessary to apply the control technique for a few years. Bella and Navratil (1988) have shown that the incidence of western gall rust in stands less than 12 years old is only about 5%, while it increases rapidly before leveling off at 20% at the age of 20. It may be possible to significantly reduce the number of galls in these stands by releasing the beetle and the mycoparasite sometime during the stand's 10th to 20th year.

Particular attention should be paid to high risk stands such as stands which were thinned with the largest trees indiscriminately retained (Bella 1985) and sites near heavily infected stands. Also, sites which are on east-facing slopes and at elevations between 1200 and 1400 m are considered higher risk (Bella and Navratil 1988). Releases could be conducted in these high risk sites.

Rearing of large numbers of beetles and producing massive amounts of mycoparasite could be done over the winter in the laboratory. Then at the first sign of sporulation, beetles which were fed the mycoparasite could be released into the stand. The number of beetles released would be dependent on the size of the stand and on the stand's risk of infection.

Table 4.1 Accumulating of mycoparasitism as galls age in A) 1992 and B) 1993. Note: sampling in 1992 and 1993 was done in different sites.

A)

	Percentage of gall with estimated amount of parasitism			
gall age	<5%	5%-50%	50%-95%	>95%
2	100	0	0	0
3	56.2	31.2	12.5	0
4	50	40.1	9.1	0
5	36.4	45.4	18.2	0
6	9.5	61.9	28.6	0
7	26.7	13.3	40	20
8	0	20	60	20
9	0	16.7	33.3	50
10	0	0	44	56
11	0	0	0	100
12	0	0	0	100
13	0	0	0	100

B)

	Percentage of galls with estimated amount of parasitism			
gall age	<5%	5%-50%	50%-95%	>95%
2	100	0	0	0
3	58.3	35.4	2.1	4.2
4	50	37.5	7.8	4.7
5	16.1	29.0	51.6	3.2
6	14.3	25	25	35.7
7	0	26.1	34.8	39.1
8	0	16.7	16.7	66.7
9	0	0	25	75
10	0	8.3	0	91.7
11	0	0	0	100
12	0	0	0	100

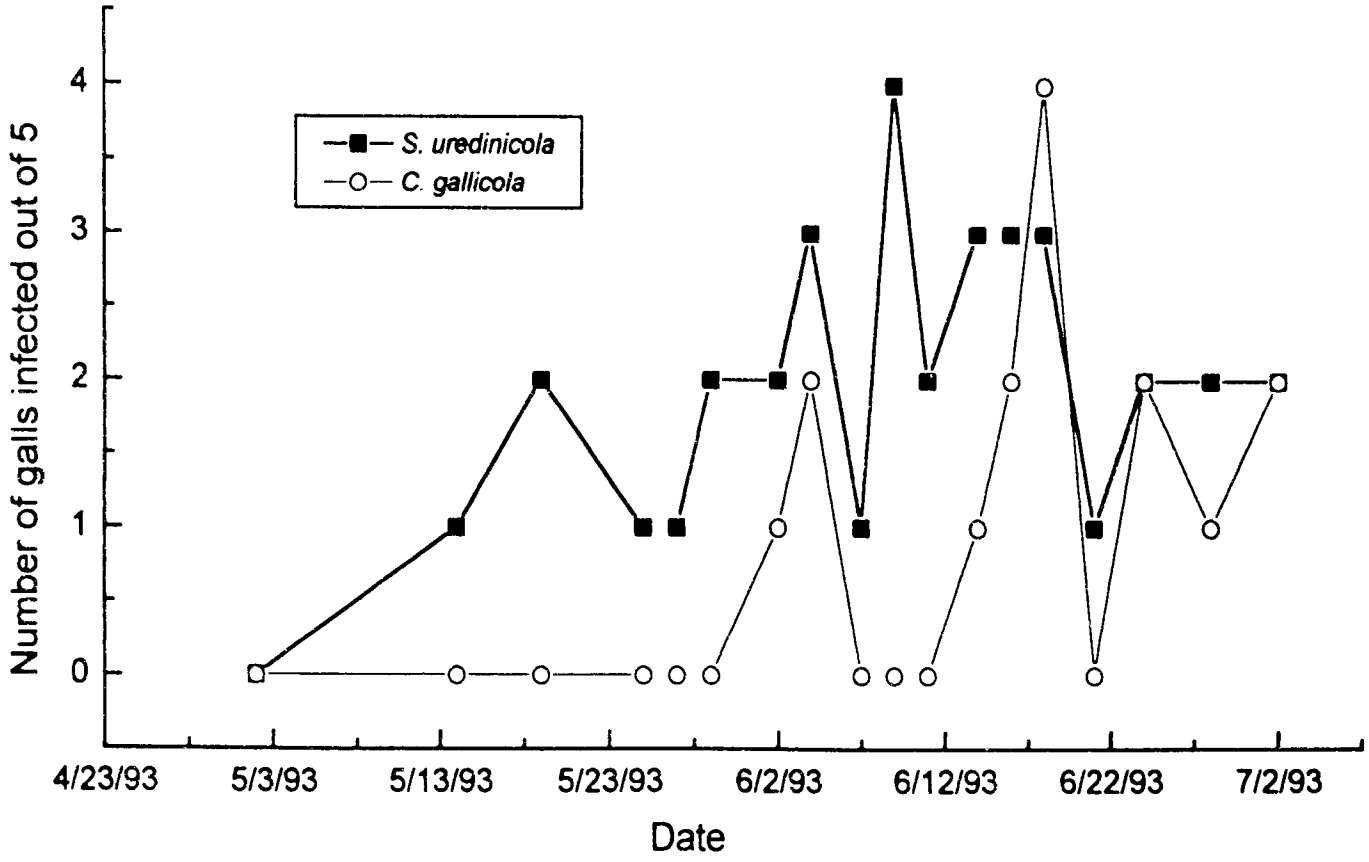

Figure 4.1 Timing of *Scytalidium uredinicola* and *Cladosporium gallicola* near Hinton, Alberta in 1993

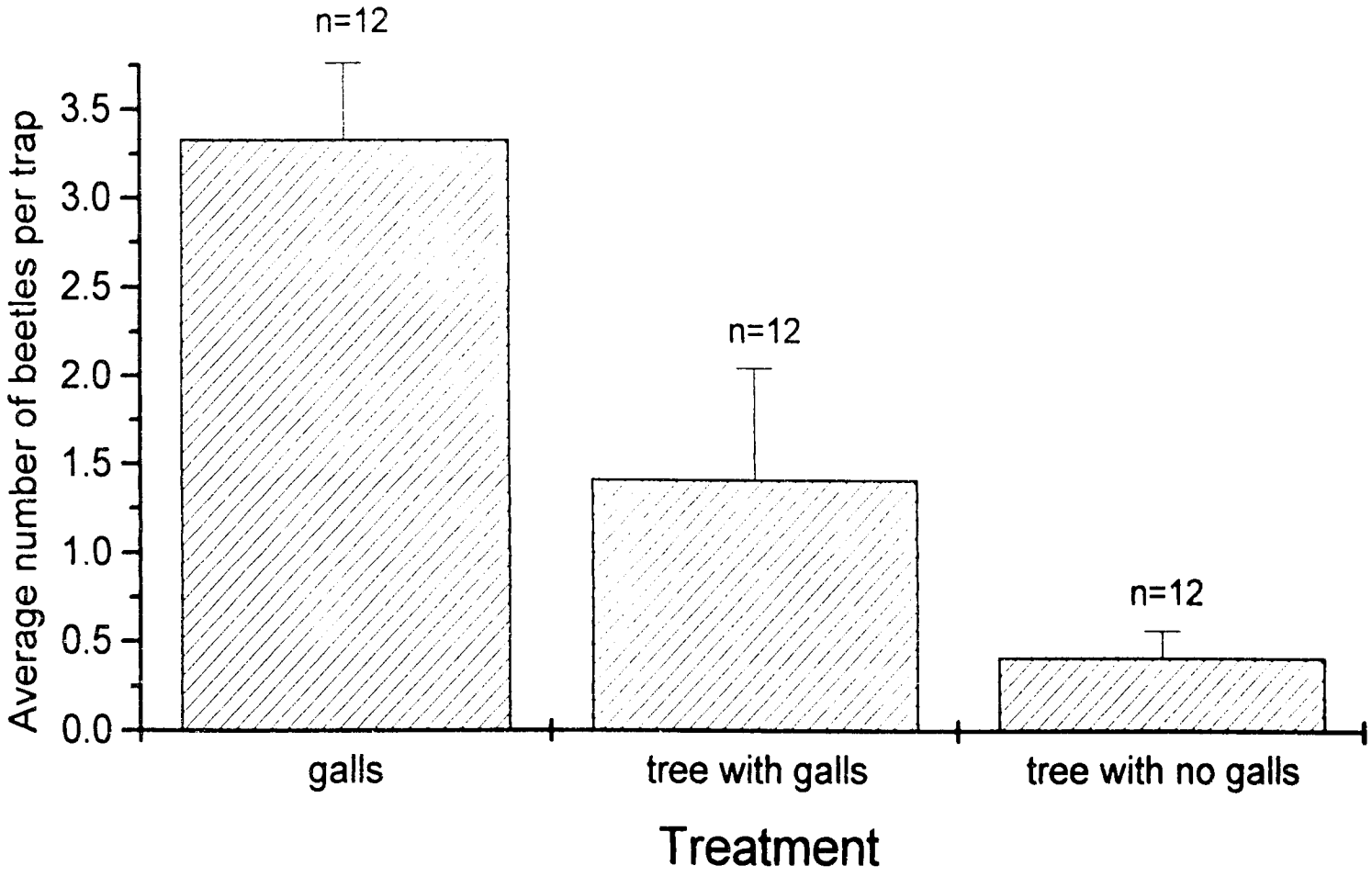

Figure 4.2 Attraction of *Epuraea obliquus* to western gall rust

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5. CONCLUSION

5.1 Biological control of plant pathogens

Research on biological control of plant pathogens has been primarily on augmenting mycoparasite populations (See Beale *et al.* 1991, Fox *et al.* 1991, Whipps 1991). However, despite the proposed use of hundreds of mycoparasites few have been successfully implemented (Sundheim and Tronsmo 1988, Snyder *et al.* 1976, Upadhyay and Rai 1988, Cook and Baker 1983, Whipps *et al.* 1988). Examination of these biological control agents has mainly been in the laboratory and field implementation attempted by artificial means (massive spray programs). This is likely the main reason for the unsuccessful control of plant pathogens with mycoparasites. In addition, none of this work has significantly helped to increase our understanding of the biology and ecology of mycoparasites (Whipps *et al.* 1988, Lumsden 1992).

The use of an insect to disseminate a mycoparasite, such as proposed by Hiratsuka (1990) for controlling western gall rust, should be an effective method of biological control because it utilizes the natural interaction of mycoparasites and invertebrates. This method of biological control could overcome many of the problems which have prevented successful augmentation of mycoparasites.

The first major problem with using mycoparasites for biological control is efficiently applying the control agent. Spraying mycoparasites in large management areas is costly and often difficult since pathogens are often sheltered by branches and leaves or located in the soil. For mycoparasites to provide effective control, more efficient means of application are necessary (Upadhyay and Rai 1988).

Insects which seek out plant pathogens could provide efficient dissemination of the mycoparasite. This utilizes natural interactions since invertebrates often act as natural vectors for mycoparasites. For example, microarthropods vector parasitic fungi of soil disease (Anas and Reeleder 1988) and mites vector mycoparasites of dung inhabiting fungi (Blackwell and Malloch 1989, Blackwell and Malloch 1990). In fact, it appears that insects are likely a common method of dispersal for many mycoparasites (Chapter 3). *Epuraea obliquus* would provide efficient dissemination of *S. uredinicola* since it is a natural vector of the mycoparasite (Chapter 3).

Successful use of mycoparasites requires either high rates of primary infection or greater potential for secondary infection. Not only will higher rates of primary infection be obtained by releasing a mycoparasite with its natural vector, but secondary spread of the mycoparasite also will be greatly promoted. Significantly more inoculum and insect vectors will be present to provide additional control through secondary infections. *Epuraea obliquus* can promote spread of *S. uredinicola* on three spatial scales (Chapter 3). It can move the mycoparasite across the surface of individual galls, it can disseminate the mycoparasite to new galls and it can even disseminate the mycoparasite to new stands.

Another problem with many mycoparasites is that their slow killing speed prevents adequate control (Whipps 1991). As previously discussed, there is an abundance of both insects and mycoparasites occurring on plant pathogens and it is likely that the combined effect of these agents is necessary to naturally control plant diseases. Releasing both an insect and a mycoparasite would provide better control than using a single agent because it provides two control agents. For example, adult and larval feeding by *E. obliquus* can reduce the spore inoculum of western gall rust

(Chapter 2) and the mycoparasite decreases the germinability of spores and breaks down the rust sori (Tsuneda *et al* 1980). Similarly, the combination of effects provided by using several agents is necessary for successful biological control of many weeds (Kremer and Spencer 1989, Emge *et al.* 1981, Charudattan 1986, Spencer 1987).

Wounds may be necessary for mycoparasites to successfully infect many pathogens. Anas and Reeleder (1988) found that sclerotia of *Sclerotinia sclerotiorum,* grazed by a fungus gnat in the soil, were colonized more by the mycoparasite, *Trichoderma viride.* An insect, used to disseminate the mycoparasite, could provide the entry wounds necessary for successful infection. This is most important for mycoparasitic infection of soil pathogens because these diseases are often associated with very tough sclerotia structures (Whipps 1991). Feeding by *E. obliquus* may be necessary for *S. uredinicola* to infect western gall rust. Beetles likely carry spores of the mycoparasite deep into the sorus of the gall where infection by the mycoparasite may be initiated.

Lastly, mycoparasitic infection of plant pathogens is influenced by environmental conditions. The temperature and humidity are very important in mycoparasitic infections of plant pathogens (Lumsden 1992). Charudattan (1990) suggests that small sticky spores, the kind selected for invertebrate dissemination, are less dependent on proper environmental conditions and therefore can be more effective in biological control. These types of spores would be efficiently disseminated by insects. *Scytalidium uredinicola* spores, likely selected for invertebrate dissemination (Chapter 3), may be less sensitive to environmental conditions that other mycoparasites. This would make biological control attempts with this fungus more successful.

Using insects and mycoparasites which are mutualists would be very effective approach to biological control. Better control is achieved by control agents which have mutualistic associations (Howarth 1985). For example, the use of *Mixoma* virus against rabbits in Australia was effective because mosquitoes (efficient vectors) were present (Moore 1989). *E. obliquus* and *S. uredinicola* may be mutualists. The beetle vectors the mycoparasite (Chapter 3) and preliminary investigation suggests that the fungus increases the duration that western gall rust is suitable to the beetle.

Insects have successfully disseminated mycoparasites to plant pathogens in several different systems. Peng *et al.* (1992) increased transmission of mycoparasite to the flowers of strawberries using bees to control *Botrytis cinerea* (gray mold). The bees, inoculated as they came out of their hives, provided efficient and constant application of the control agent. Bees are also efficient vectors for bacterium (*Pseudomonas fluorescens* and *Erwinia herbicola*) antagonistic to fire blight, *Erwinia amylovora* (Thomson *et a.* 1992, Johnson *et al.* 1993).

Insects have also been used to disseminate control agents for weeds. Quimby and Frick (1985) used a herbicide-coated larvae of the nutsedge moth and American waterhyacinth borer moth for improved control of these weeds. Also, insects released to control a weed have unintentionally promoted a pathogen, thus providing control (Peschken and Beecheer 1973; Wilson 1969). I have seen no reports of intentional release of a weed pathogen with an insect, however this would likely be a successful new biological approach for controlling weeds for many of the same reasons previously discussed, with regards to plant pathogens.

This method of biological control could be used in an integrated pest management program for plant pathogens. It could be successfully integrated with cultural practices in both agriculture and forestry. Crop rotation and tillage practices, which are effective in reducing many plant pathogens in agriculture, could be integrated with releases of mycoparasites with insects. Also in forestry, silvicultural practices such as adjusting stand densities to reduce the impact of plant pathogens, and precommercial thinning and pruning of disease trees could be successfully integrated with this approach. This would be very effective in biological control of western gall rust. Combining the control provided by removal of heavily infected trees during pre-commercial thinning (Bella 1985), development of rust resistant seedlots and releasing the nitidulid beetle with the mycoparasite in high risk stands could significantly reduce the impact of this pathogen.

The use of insects to disseminate mycoparasites could provide very effective control of plant pathogens. Augmentation of natural interactions of insects and fungi in nature would overcome many of the problems associated with present biological control programs. Insects would provide very accurate dissemination of the fungal propagules and promote secondary spread of the control agent. Also, the insect and mycoparasite would provide two control agents of the pathogen, increasing the impact. The insects also could provide wounds of entry for the mycoparasite and would effectively disseminate sticky spore producing fungi thus overcoming environmental limitations. This is a promising approach which utilizes a better understanding of the natural interactions of insects and mycoparasites.

5.2 Areas of future research

For biological control of plant pathogens to be successful a better understanding of the biology and ecological interactions of their natural enemies must be developed. The community of organisms occurring on plant pathogens must be examined and future work should not ignore the role of invertebrates in these systems.

Biological control of western gall rust by using *E. obliquus* to disseminate *S. uredinicola* is very promising and deserves further investigation. Several areas of research are necessary before this approach can be implemented. The biology of *S. uredinicola* is still poorly understood. For example, the life history of this fungus and its interactions with other fungi on western gall rust, such the mycoparasites *Cladosporium gallicola* and *Monocilium nordinii,* is not known. A understanding of the population biology of *S. uredinicola* is essential to understanding its potential in biological control of western gall rust. For example, why is this mycoparasite so rare on the young galls, and will augmentation of this natural enemy of western gall rust increase the occurrence of this mycoparasite on young galls? Also, how does this mycoparasite become established in young stands? Finally, releases of beetles inoculated with the mycoparasite into stands is necessary to determine the effectiveness of large scale releases.

Further study on using *E. obliquus* to disseminate *S. uredinicola* for biological control of western gall rust will likely lead to successful implementation of this control procedure. The use of an insect to disseminate a mycoparasite will likely also work in other biological control programs for plant pathogens.

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