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THE EFFECTS OF ROAD STRESS ON THE PERFORMANCE OF GALL-FORMING
INSECTS AND THEIR HOST-PLANTS

JOCELYN MARTEL, B.Sc., M.Sc.

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

Doctor of Philosophy

Department of Biology
Carleton University
Ottawa, Ontario
22 October 1993

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The undersigned hereby recommend to
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"The effects of road stress on the performance
of gall-forming insects and their host-plants"

submitted by
JOCELYN MARTEL, B.Sc., M.Sc.

in partial fulfilment of the requirements
for the degree of Doctor of Philosophy

Chair, Department of Biology

Thesis Supervisor

External Examiner

Carleton University

'C'est la société qui doit être au service de l'école et non l'inverse'

Albert Jacquard

ABSTRACT

The goldenrod-stem gall insect system comprises two gall-formers, Eurosta solidaginis (Fitch) (Diptera: Tephritidae) and Epiblema scudderiana (Clem.) (Lepidoptera: Tortricidae), and a perennial herb, Solidago altissima L. (Asteraceae). This system is common in disturbed and stressful environments such as road margins. This represents a good opportunity to study the indirect effects of environmental stress on the performance of gall-forming insects, which are poorly documented in the literature. In this study, it was hypothesized that the stressful conditions prevailing in roadside environments should affect the host-plant and the gall-former negatively, given that the needs of the gall tissue differ little from those of other plant tissues and assuming that gall-inducers require a fully grown gall to complete their development.

A survey of populations that were already established near and far from heavily used roads indicates that the survivorship and the biomass of gall-forming insects were not affected by road stress, even though the growth of the host-plant was significantly reduced. A two-year transplant experiment has uncovered the short-term and individual effects of the two major kinds of road pollutants on gall-formers, i.e. air pollutants and de-icing salt, which were found to be either neutral or positive. Finally, a greenhouse experiment where the indirect effects of high soil salinity were investigated indicated that the performance of gall-forming insects may be affected negatively if the host-plant is grown in a harsh environment.

These results show that gall-forming insects do not differ from other guilds of insect herbivores in terms of the variety of their responses to stressed plants. Also, this study tends to confirm that gall-forming insects are less sensitive to plant stress compared to sap feeders or leaf-feeders. Perhaps the gall, especially the outer layers of tissue, act as

a buffer against the drastic changes that may occur in the rest of the host-plant growing in a stressful environment. Therefore, further research is needed to measure the nutritive status of isolated tissues located at various distances from the gall chamber.

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The National Capital Commission and the Ontario Ministry of Transportation authorized sampling and experiments to be conducted along Highway 417 and Conroy Road. A private owner allowed me to do sampling on a site located on the margin of Highway 31. The Natural Sciences and Engineering Research Council provided financial support for this project (research grant to H. Damman). I received an Ontario Graduate Scholarship during two years.

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GENERAL INTRODUCTION

In addition to herbivory, plants must often cope with stressful abiotic factors. These factors can be natural such as a drought or light deficiency or they can be related to human activity such as any kind of pollutants. Plants under stress can generally survive and reproduce, however their development or growth form may change. Such changes may include variations in growth rate as well as altered biomass allocation, biochemistry and physiology, etc. All those stressed-induced changes in the plants may in turn affect insect herbivores, since insect performance will depend on the suitability of their host in terms of growth and nutritional status.

Several hypotheses have been proposed to explain the response of insect herbivores to plants under stress. The plant stress hypothesis is frequently cited in the literature. This hypothesis, which postulates that plants under abiotic stress become more suitable as food for herbivorous insects, was put forth by White (1984) and complemented by Rhoades (1979). The better suitability of stressed host-plants for herbivores is thought to be caused by an altered nutritional status, i.e. higher concentrations of nitrogenous compounds (White 1984; Brodbeck & Strong 1987; Cockfield 1988), where nitrogen is a key nutrient for insect herbivores (Mattson 1980), combined with reduced defenses (Rhoades 1979). White (1984) based his hypothesis on the assumption that a larger amount of free amino acids is released in the tissues of stressed plants and these chemical compounds are more readily available for insects than proteins in stressed plants. On the other hand, Rhoades (1979) proposed that stressed plants were more vulnerable because they are less able to synthesize defensive chemicals. More recently, Price (1991) suggested the plant vigor hypothesis, which states that the more vigorous (fast-growing) plants or plant tissues should be preferred by internally feeding insects that are less mobile and thus more dependent on a particular plant tissue. Vigorous plants or plant tissues are more likely to

reach a large size or to regrow and thus may represent a better regulated resource for insects closely associated with plant growth processes (Price 1991). This might be especially true for gall-formers, that depend entirely on the growth of the plant tissue in which they feed and develop.

Insect response to stressed host-plants has been the subject of a number of studies in the recent years. Reviews of the topic showed that with the exception of sucking insects for which a positive response is generally observed, responses of insect herbivores to plants under abiotic stress are diverse, i.e. the response can be negative, neutral or positive (Heinrichs 1988; Larsson 1989; Riemer & Whittaker 1989). For example, Price and co-workers have shown that plant stress can influence gall-forming insects negatively or positively (Price & Clancy 1986; Clancy & Price 1987; Preszler & Price 1988; Waring & Price 1990). Thus, the higher concentrations of nutrients found in many stressed plants cannot always predict the response of insect herbivores, especially in harsh environments where plant growth can be severely affected.

The gall-forming habit represents the most intimate relation between a plant and an insect herbivore. Gall-forming insects spend their larval and pupal stages encased in the gall tissue and they feed inside the gall on a thin layer of tissue that is very rich in nutrients (Bronner 1977; Rohfritsch & Shorthouse 1982). The gall is characterized by histological features that are unique to each species of gall-former (Rohfritsch & Shorthouse 1982). Gall traits are genetically controlled by both the host-plant and the gall-inducer (McCrea & Abrahamson 1987). There is no advantage for a plant to be attacked by a gall-former since the energy spent for the formation and maintenance of the gall tissue is taken from the energy that would be normally allocated the normal tissues. Gall tissues may act as a physiological sink or redirector, i.e. a partial block of nutrients and nutrients taken from the

other plant tissues (McCrea *et al.* 1985). This in turn has an effect on plant fitness (Hartnett & Abrahamson 1979; Stinner & Abrahamson 1979).

It has been proposed that gall-forming insects should be ranked as the least sensitive insect herbivores in terms of their response to stressed host-plants, although this hypothetical ranking was based on only a few experimental studies involving stressed trees (Larsson 1989). Larsson (1989) suggested that cambium-, leaf-, and sap-feeding insects were more susceptible to stressed plants compared to gall-forming insects. Gall-formers depend more strongly on the quality of the host-plant compared to the other feeding guilds, however they may have a certain control on the development of the gall tissue, regardless of the stress-induced changes in the host-plant.

Clancy & Price (1987) found that galls developed more slowly on plants that were watered infrequently and received no fertilizer. Also, population density and survivorship decreased for insects forming galls on plants under water stress (Price & Clancy 1986; Preszler & Price 1988). Those results tend to support the plant vigor hypothesis. However, Waring & Price (1990) found that gall-formers associated with meristematic branch terminals were more abundant on water-stressed plants because of a bushier architecture.

The plant stress and plant vigor hypotheses are not mutually exclusive. Rather, their applicability to plant stress-insect interactions seems to depend on the kind and intensity of stress exerted on the plant as well as on the response of plants to stress, as indicated by the diverse responses of insect herbivores (other than gall-formers) to plant stress (e.g. Riemer & Whittaker 1989). Thus, both hypotheses could be applied to a situation where stress reduces plant vigor. Fernandes & Price (1991, 1992) found that species richness and population size of gall-formers were higher in hygrothermally stressed

habitats, which is thought to be caused by a lower attack rate by parasites and fungal pathogens in dry environments. On the other hand, the effects of stressed plants on gall-formers have never been tested in habitats other than deserts and steep elevational gradients and even though gall-formers are presumably well adapted to harsh environments (Waring 1986; Price *et al.* 1987; Fernandes & Price 1991), their response to plants exposed to a combination of abiotic stresses that might affect both their growth and nutritional status remains an unresolved matter for the species that occur in other environments.

Roadside environments represent a good example of an urban modified habitat where several abiotic factors may affect plant performance simultaneously and perhaps affect gall-formers in turn. I will use the term "road stress" to refer to all abiotic factors associated with proximity to the road edge and capable of inducing a change in the plant growth or nutritional status. The most common air-borne pollutants found along roads are nitrogen oxides, dust and lead. Other potentially injurious pollutants directly associated with vehicle traffic or corrosion include cadmium, chromium, nickel, polycyclic aromatic hydrocarbons, carbon oxides and photochemical oxidants (Taylor 1984; Thompson *et al.* 1984; Larsson 1985; Pertoldi Marletta *et al.* 1986, 1989; Gilbert 1989; Yang *et al.* 1991). Besides the air-borne pollutants, there is a significant input of de-icing chemicals into the roadside system through runoff water since road de-icing is practiced during several months in our latitudes.

Nitrogen oxides may affect plants in a diversity of ways. They may represent an additional source of nitrogen for roadside plants. Fluxes and metabolism of nitrogenous compounds such as amino acids in the plant can be affected by this kind of gaseous pollutant (Rowland *et al.* 1988). Plants can absorb NO_2 present in the soil or in the air near roads and convert this to nitrite and nitrate before further utilization in plant metabolism

(Smith & Mayfield 1978; Yoneyama *et al.* 1979, 1980). However, nitrite can be accumulated in the plant tissues, which can harm plants (Zeevaart 1976; Malhotra & Khan 1984). Other negative effects of nitrogen oxides on plants include physical injury and reduced photosynthesis (see review in Malhotra & Khan 1984). On the other hand, stimulation of nitrate reductase in plants exposed to NO_2 may cause an accumulation of amino acids and proteins in their tissues (Zeevaart 1974; Yoneyama & Sasakawa 1979). Several studies have shown an increase in nitrogenous compounds in plants growing on road margins (Bolsinger & Fluckiger 1987; Spencer & Port 1988; Spencer *et al.* 1988).

In general, road dust may physically damage the leaves and block the stomata (Farmer 1993). Increased absorption of sunlight (Eller 1977), increased leaf temperature (see review in Farmer 1993), and reduced photosynthesis (Thompson *et al.* 1984) may result from dust falling onto plants. The effects of dust near roads on soil quality are unknown, although a long term accumulation of dust particles could probably affect such soil characteristics as texture, profile, and moisture.

All size classes of lead particles emitted in exhaust gases are transferred to the different compartments of ecosystems via sedimentation, impaction, precipitation or inhalation (Smith 1976). Lead was used widely as an "anti-knock" additive in gasoline until the 1980's but present regulations have limited lead in "unleaded" gasoline to a low concentration (Lavallée & Fedoruk 1989). Long-term accumulation of lead in soils and plants decreases asymptotically with the distance from the road edge (Martin & Coughtrey 1982; Favretto *et al.* 1986; Leonzio & Pisani 1987; Ho & Tai 1988) and more than 50% of lead is removed at a distance of 50 m (Smith 1976; Martin & Coughtrey 1982). Lead concentrations of soils reach the highest levels in the upper layers and represent a long-term accumulation (Ho & Tai 1988). Therefore, it can be used as an index of the history and

level of exposure of a particular roadside system to air-borne pollutants. The lead halides present in car emissions are converted rapidly to insoluble compounds in soils (Smith 1976; Fergusson 1990). Concentrations of exchangeable forms of lead are always low and, as a result, soil lead may be only marginally available to plants (Adriano 1986; Fergusson 1990). Much of the lead contamination of vegetation is present as a superficial and removable deposit of particulates on the leaves (Motto *et al.* 1970; Koeppe 1981; Martin and Coughtrey 1982). There is disagreement over whether lead is taken in through the leaves or the roots. Nevertheless, the phytotoxicity of lead is relatively low compared with other trace elements. Therefore, lead accumulations near pollution sources probably have little direct effects on plants grown in these areas (Koeppe 1981; Adriano 1986) but can serve as an effective marker.

Large amounts of sodium chloride are used to clear roads in winter and they are released in the environment mostly when the weather warms up and the snow ploughed from the road surface melts in the spring (Jones *et al.* 1986; Thompson *et al.* 1986a). Also, levels of sodium and chloride ions in roadside soils increase with the length of time on which salting has been practiced, especially in soils rich in clay (Hutchinson & Olson 1967; Liem *et al.* 1985). The accumulation of salt may alter the structure of the soil to reduce aeration and water availability (Brandt 1973; Jones *et al.* 1986). An addition of salt in the soil lowers its osmotic potential and then may cause an osmotic stress (Levitt 1980). In general, non-halophilic plants exposed to salinity grow more slowly (Greenway & Munns 1980; Munns & Termaat 1986). The maintenance of a low concentration of sodium in plant cells compared to the soil may require an expenditure of energy that would otherwise be available for growth processes (Levitt 1980). The slower growth of salt-stressed plants may also be caused by a suppression of nutrients due to an intake of sodium and chloride in competition with the other ions (Levitt 1980). Salt accumulation may

change drastically the ratio of sodium ions to key macronutrients such as potassium ions, and non-halophytes do not necessarily have the physiological ability to absorb sufficient amounts of potassium from a medium containing a large excess of sodium (Epstein 1972). Toxic levels of chlorine and sodium in the tissues may inhibit growth and cause dieback of the plant (Greenway & Munns 1980; Jones *et al.* 1986). Nitrate uptake and reduction as well as protein synthesis are all traits that can be affected by salts (Aspinall 1986; Munns and Termaat 1986). Higher tissue concentrations of N-based or C-based organic solutes may also result from salt-stress-induced physiological changes in non-halophilic plants (Greenway & Munns 1980).

The presence of a road may also affect the physical environment. For instance, the wind velocity may vary according to the the traffic load. The temperature near the road may be increased by the presence of the pavement during the hottest days of the summer. Finally, road maintenance operations and vehicle traffic can modify the soil characteristics at a short distance from the road edge (e.g. soil particle size and compaction). However, the effects of changes in the physical environment of roadsides on plants and insects have always been obscured by the effects of the pollutants listed above (e.g. Braun & Fluckiger 1984a). Therefore, this study will focus on the air pollutants and de-icing salt, where the term "air pollutants" refers to the combination of all air-borne pollutants mentioned above since the effects of individual pollutants are hardly separable while the term "de-icing salt" refers to the runoff water-borne sodium chloride because NaCl represent the vast majority of the chemicals used to clear the roads.

Evidence that road stress affects plant-insect interactions comes from several studies on aphid populations along highways (Fluckiger *et al.* 1978; Bolsinger & Fluckiger 1984, 1987, 1989; Braun & Fluckiger 1984a, 1984b, 1985; Spencer & Port 1988). Unusually

severe aphid infestations have been seen on plants growing on roadsides. A more rapid development of aphids was associated with a higher content of nitrogenous compounds and carbohydrates in the sap of the host-plants (Braun & Fluckiger 1985; Bolsinger & Fluckiger 1987, 1989; Spencer & Port 1988). The main promoting factors are thought to be NO_x and de-icing salts (Port & Thompson 1980; Bolsinger & Fluckiger 1987; Spencer & Port 1988).

Port & Thompson (1980) have reported outbreaks of defoliating insects on trees and shrubs growing on the margins of major roads. High nitrogen levels due to NO_x pollution were again the main stimulating factor. However, insects other than aphids are not always favored by the environmental conditions prevailing in roadside habitats. Przybylski (1979) noted a reduction in the number of species of arthropods in meadows and orchards located near highways.

This study will focus on the "goldenrod-stem gall insect" system, which is fairly common in old fields of northeastern North America. The tall goldenrod, Solidago altissima L., is the main host of a diverse insect fauna, which includes two gall-forming insect species, Eurosta solidaginis Fitch and Epiblema scudderiana (Clem.). S. altissima is also an opportunistic plant species that can be easily found along roads and thus represents a good opportunity to study the effects of plant stress on gall-forming insects where the stressing agents will not be only represented by humidity and temperature.

Study species

Many studies have been conducted to describe and understand the "goldenrod-stem gall insect" system (Weis & Abrahamson 1986 and references therein). Among these studies, a few mention in their site description that roadside populations were used (Weis &

Abrahamson 1986; McCrea & Abrahamson 1987; Abrahamson *et al.* 1988; Anderson *et al.* 1989). However, the perturbation that road stress might represent for the system has never been considered.

The tall goldenrod, *Solidago altissima* L. (Asteraceae), is a clonal perennial herb that is native to northeastern North America (Werner *et al.* 1980; Melville & Morton 1982). By means of vigorous rhizomes this plant can propagate quickly and dominate old fields for long periods (Tramer 1975; Newell & Tramer 1978; Melville & Morton 1982). Clonal growth occurs outward from a central area and the result is a cluster of stems (Melville & Morton 1982). The daughter ramets are located 0.5 to 60 cm from their parent ramet (Cain 1990a, b) and most rhizome connections break down after one year (McCrea *et al.* 1985). *S. altissima* has a broad site tolerance because of its ability to reallocate nutrients between different plant parts or among ramets of a clone via rhizomatous connections (Hartnett & Bazzaz 1983; Potvin & Werner 1984; Abrahamson & McCrea 1985), though it grows best in sites where soil moisture is never severely limited during the growing season (Potvin & Werner 1983). The tall goldenrod starts growing early in May, flowering occurs in mid-August and by the end of October seeds are fully developed and a basal rosette of new shoots is already formed for the next year.

Larvae of *Epiblema scudderiana* (Clem.) (Lepidoptera: Tortricidae) induce an elliptical gall on the stem of several species of goldenrods, although it is most frequent on *S. altissima* (Meaden 1956; Miller 1976). The gall is relatively rudimentary and as with the galls induced by other species of Lepidoptera (Dreger-Jauffret & Shorthouse 1992), the tissue differentiation in this case is minimal. The larva feeds on the inner layer of tissue within the stem and this feeding activity seems to be concentrated in the upper part of the gall; the regrowth of the inner tissue is probably stimulated by the insect (personal

observation). Adults of E. scudderiana emerge in mid-June in the Ottawa area (personal observation), which is the same as in central New York (Meaden 1956). The females deposit eggs both on leaves and stems, on the middle portion of the host plant. Larvae hatch 4-5 days after oviposition and immediately begin to feed in the shoot tip. After approximately one week they move down a few centimeters and bore into the stem where they induce the formation of an oblong gall with an irregular surface. The gall attains its full size in three to four weeks (diameter: 10 mm; length: 25-30 mm). Toward the end of the summer, the larvae prepare emergence holes for the next spring.

Larvae of the ball gall fly, Eurosta solidaginis (Fitch) (Diptera: Tephritidae), induce a spherical gall on goldenrod stems (Uhler 1951). The gall can be divided in four concentric tissue zones, the richer tissue lining the insect's central chamber (Weis et al. 1989). The inner nutritive-pith tissue is very thin compared to the bulk of the gall that consists of large and poor cells (parenchyma). E. solidaginis has been recorded from a variety of host plants but it performs best and is most commonly found on S. altissima (Uhler 1951; Miller 1959; Abrahamson et al. 1989; Lichter et al. 1990). Adult emergence in the Ottawa area occurs in late May - early June (personal observation), which is similar to central New York (Uhler 1951). The females oviposit on the terminal buds. By the middle of June most of the eggs have hatched and the larvae stimulate the formation of gall tissue until it attains its maximal size (diameter: 22-25 mm) during the first days of July (Uhler 1951; McCrea et al. 1985). Larval growth and preparation of an exit tunnel are completed during the second half of October (Uhler 1951).

Larvae of Trirhabda borealis Blake (Coleoptera: Chrysomelidae) hatch from overwintered eggs in early May and feed on young goldenrod leaves (Messina 1982). Then, the larvae leave the plants and pupate in the soil in early June. Adults of T. borealis

appear in late June and chew the young leaves of goldenrods. The mating period starts 1 to 2 weeks after emergence and ends when the beetles die in September or October. The eggs are laid in the soil litter prior to overwintering.

Overwintered adults of Microrhopala vittata (F.) (Coleoptera: Chrysomelidae) appear in late April (Messina & Root 1980). Mating and oviposition occur immediately and the eggs are laid on goldenrod leaves. Emerging larvae bore into the leaves and form blotch-type mines. A mine may be occupied by several larvae. The adults emerge in August and September after pupating within the mines.

Objectives and hypothesis

In this study, I will use goldenrods and their insects to address the question:

Does road stress influence the growth of roadside plants and will the performance of stem-gall insects feeding on these plants be in turn affected?

The hypothetical answer to that question is that the performance of both gall-forming insects and their host-plant should be affected by the stressful conditions prevailing in roadside environments. It is assumed that the nutrients required for the growth of gall tissue differ little from the needs of normal tissues and that gall-inhabiting insects need a fully grown gall to complete their development.

To test that hypothesis, I took three approaches that are often used in ecological research: field observations (Chapter 1), a field experiment (Chapter 2), and a lab experiment (Chapter 3). In Chapter 1, the performance near and far from the road edge of two gall-forming species and their host-plant (S. altissima) is compared, using populations that were already established along two major roads (Chapter 1). In such a descriptive

study, however, the effects of all road pollutants are combined and they may result from a long term exposure of the system to road stress. The effects of seasonal variations in roadside conditions on E. solidaginis and S. altissima are reported in Chapter 2. An attempt to separate spatially and temporally the effects of the two major kinds of road pollutants (air pollutants and de-icing salt) is made in a two-year transplant experiment. This was conducted on the margin of a heavily used highway, where there was a certain control on the short term exposure of the system as well as on the soil characteristic and plant genetic components. Finally, to see how a gall-forming insect such as E. scudderiana can perform in a harsh environment, its host-plant (S. altissima) has been exposed to potentially lethal concentrations of a pollutant typical of roadside environments (NaCl). Therefore, Chapter 3 is based on data gathered from a greenhouse experiment that investigates the indirect effects of high soil salinity on the performance of E. scudderiana, where most variables were under control.

CHAPTER 1

THE PERFORMANCE OF TWO GALL-FORMERS ON THE STEMS OF SOLIDAGO ALTISSIMA L. IN ROADSIDE ENVIRONMENTS

Introduction

Roadside plants and insects live along a gradient of pollution that can affect their interactions with each other. For example, aphid populations commonly increase along highways (see Riemer & Whittaker 1989 for a review), whereas road pollution may cause an impoverishment of the roadside insect-community as a whole (Przybylski 1979). De-icing salt (namely NaCl) and air pollutants (nitrogen and carbon oxides, lead, etc.) comprise the two major sources of pollution (Gilbert 1989). An accumulation of salt and heavy metals in the soil has been noticed for roads with a long history (e.g. Hofstra & Smith 1984; Ho & Tai 1988).

The effects of miscellaneous pollutants on plant-insect interactions have been the subject of a number of studies (see Riemer & Whittaker 1989 and Heliovaara & Vaisanen 1993 for reviews). Pollutants may affect insects through changes in the interactions between herbivores and their natural enemies (i.e. a positive effect on the former and/or a negative impact on the latter) or changes in the suitability of the host-plants (growth, nutritional status, etc.). Although the impact of pollution on sap feeders and various kinds of defoliators is well documented in the literature, the same impact on the interactions between gall-forming insects and their host-plant is poorly known. In a study on the accumulation of heavy metals in blueberry plants and insect gall tissues near a smelter, Bagatto & Shorthouse (1991) found the highest concentrations of copper and nickel in any plant organ to be in the gall tissue. However, this may not hold for all heavy metals (see

Heliovaara *et al.* 1987). Also, the effects of these pollutants on the performance of the gall-former were not investigated.

Gall-forming insects may be better protected against direct contamination since they live within the plant. However, they might be more susceptible to the stress-induced effects of pollutants because of their sedentary feeding habits, as opposed to externally feeding insect herbivores. Gall tissues act as metabolic sinks (Abrahamson & Weis 1987) and their needs differ little from those of normal plant tissues in terms of growth requirements (e.g. Arya *et al.* 1962). Reduced plant growth caused by environmental stress might therefore affect gall-formers negatively (Waring 1986), although in some cases stress-induced changes in plant architecture have positive effects (Waring & Price 1990).

The aim of the present study is to examine the effects of road-stress-induced changes in the host-plant on the performance of two insect species that induce galls on the stems of *Solidago altissima* L. (Asteraceae), i.e. *Eurosta solidaginis* (Fitch) (Diptera: Tephritidae) and *Epiblema scudderiana* (Clemens) (Lepidoptera: Tortricidae). The hypothesis to be tested is that the performance of both gall-forming insects and their host-plant is lowered by the stressful conditions prevailing in roadside environments. Data on plant morphology as well as the biomass and survivorship of gall-formers were gathered from two sites in 1991 and 1992.

Materials and Methods

Two roadside fields located in the Ottawa metropolitan area were selected for the presence of goldenrods and gall-formers. The two sites were located along Highway 31 and Conroy Road. These are suburban commuter roads and the annual average daily traffic in 1991 was 10 050 vehicles for H31 (Ontario Ministry of Transportation 1992) and 1 780

vehicles for Conroy Rd (Regional Municipality of Ottawa-Carleton, unpublished data). Two distances from the road were used as the treatments in this study: 10 m and 60 m. Ten meters was the closest vegetation margin set by road maintenance operations, while 60 m was far enough from the road to minimize the presence of pollutants. For instance, concentrations of salts and heavy metals in the soil attain normal levels by 20-30 m from the road edge (Smith 1976; Jones *et al.* 1986).

The soil was sampled randomly along two transects (10 m and 60 m) at each site in May and September of 1991. Twelve samples were collected along each transect. The samples were taken at a depth of 5-12 cm, weighed and air-dried. Soil moisture was determined by subtracting the weight of the air-dried samples from the weight of the fresh samples. The soil samples were then ground and passed through a 0.4 mm nylon sieve prior to chemical analyses. Cations were extracted with 1M ammonium acetate solution (pH = 7.0), whereas heavy metals were extracted with 0.005M diethylene triamine pentaacetic acid (DTPA) solution (pH = 7.3). The soil extraction procedures are described in details by Sheldrick (1984). Potassium, sodium, and lead concentrations were determined with an atomic absorption spectrophotometer (Perkin-Elmer model 703). Atomic absorption standard solutions were used (Aldrich Chemical Co., Milwaukee, Wisconsin).

The ratio of potassium ions to sodium ions was used as an index of the cationic balance of the soil since an excessive amount of sodium may reduce the absorption of a more important nutrient such as potassium (Epstein 1972). Lead was used as an index of the history and level of exposure of the selected sites to air pollution. It was measured in the soil sampled in May 1991.

Goldenrod ramets infested with stem gall-formers were selected randomly along the two transects (10 m and 60 m) at each site in the fall of 1991 and 1992. Sampling was terminated when approximately 100 galls of a given species were found along each transect. Ramet and gall heights were measured in the field. Gall height was used as an indirect measurement of ramet height when gall initiation was taking place (June for *E. solidaginis* and July for *E. scudderiana*). The diameter at the largest point of the galls was measured with calipers to the nearest 0.1 mm and the galls were dissected in the laboratory to determine the fate of the gall-former. Gall-diameter data were analysed only for the galls containing live *Eurosta* larvae. The mortality factors for *E. solidaginis* were classified as: predation by *Mordellistena unicolor* LeConte (Coleoptera: Mordellidae), parasitism by individual species of parasitoid, i.e. *Eurytoma gigantea* Walsh and *E. obtusiventris* Gahan (Hymenoptera: Eurytomidae), and unexplained mortality. *Epiblema* galls were sampled in 1992 only. For *E. scudderiana*, the mortality factors were classified as unidentified parasitoid wasps and unexplained mortality. For both species of gall-formers the term "unexplained mortality" refers to any unidentifiable cause of early larval death (hypersensitive response of the host-plant, pathogens, etc.). The data concerning the fate of the gall-formers were analysed in two ways: 1) A comparison of the percentages of the larvae that survived versus all the larvae that died, i.e. lumping together all the mortality factors, and 2) A comparison between the diverse mortality factors in terms of percentages within the larvae that died. Gall-forming larvae were oven-dried (72 hours at 60°C) and weighed.

Goldenrod ramets without galls were randomly selected and measured at the H31 site in 1991 and in both sites in 1992 to see if there was an interaction between the effects of road stress and those of the presence of a gall on the growth of *S. altissima*. Finally, the proportion of ramets attacked by either *E. solidaginis* or *E. scudderiana* was measured in

both sites in 1992. Contiguous quadrats of 1.5 X 1.5 m were established along the transects described above. To see if the population density of gall-formers could be affected by the proximity of a road, the numbers of attacked and unattacked ramets were determined in the quadrats that comprised at least one S. altissima ramet.

Results

Soil characteristics differed between the two sites and between the two distances from the road edge. Soil moisture was higher near the road at the Highway 31 site whereas the soil sampled at the Conroy Rd site contained the same amount of water at both distances from the road edge (Table 1.1 and Fig. 1.1A). In contrast, the ratio of potassium ions to sodium ions in the soil was three times lower at 10 m compared to 60 m from the road edge at the Conroy Rd site whereas no such difference was observed for the other site (Table 1.1 and Fig. 1.1B). Lead concentrations in the soil sampled near the road were at least twice those found at 60m in both sites, although the differences were much higher at the Highway 31 site (Table 1.1 and Fig. 1.1C).

In general, goldenrods growing near the road were smaller than those growing 60 m away. All goldenrod ramets were shorter or tended to be so near the road at both sites in 1992 and at the Conroy Rd site in 1991 (Table 1.2; Figs. 1.2B and 1.3A, B). The presence of a gall of either species of gall-former affected ramet height in various ways (Table 1.2). At the Highway 31 site, goldenrod ramets growing 10 m from the road edge were smaller when attacked by Eurosta galls in 1991 (Table 1.2 and Fig. 1.2A), whereas in 1992 the ramets attacked by either species of gall-former were smaller than the unattacked ramets, regardless of the distance from the road edge (Table 1.2 and Fig. 1.2B). In 1992, the ramets infested with E. solidaginis were taller than the intact ramets at the Conroy Rd site (Table 1.2 and Fig. 1.3B). The height of the gall on the stem was lower 10 m from the

road edge for both species of gall-forming insects, both sites and both years, although the differences were much higher in 1992 (Table 1.3 and Fig. 1.4A-C).

Unlike the ramets, the galls were not smaller near the road for the majority of the samples. Galls formed by E. solidaginis larvae were smaller for both sites in 1992, as opposed to 1991 during which the size of Eurosta galls did not differ (Table 1.3 and Fig. 1.5A, B). Epiblema galls tended to have larger diameters near the road only at the Conroy Rd site in 1992 (Table 1.3 and Fig. 1.5C).

The biomass of the two species of gall-formers in the fall did not differ between the two distances from the road edge for both sites and both years. The survivorship of Eurosta larvae was lower or tended to be so near the road at both sites in 1992, whereas a larger proportion survived at 10 m from the road edge at the Conroy Rd site in 1991 (Tables 1.4, 1.5). More larvae of E. solidaginis were attacked by M. unicolor near the road at the Highway 31 site in 1992 whereas more were parasitized by E. gigantea at the other site also near the road during the same year (Tables 1.4, 1.5). Table 1.4 shows that the distance from the road edge did not affect the survivorship of Epiblema larvae. The proportions represented by the different mortality agents of Epiblema larvae did not depend on the distance from the road edge for both sites in 1992 (Table 1.4). Finally, the percentage of infestation for both species of gall-forming insects increased near the road (16.7% versus 11.2% for E. solidaginis and 16.4% vs 12.5% for E. scudderiana) at the Conroy Rd site in 1992 (Table 1.4).

Discussion

Soil analyses indicate that road pollutants were present at 10 m from the road edge, although there were some discrepancies between the two sites. De-icing salt is more likely

to be a problem for the plants growing near Conroy Rd since the mean ratio of potassium ions over the sodium ions is lower than 0.4 at 10 m from that road. Potassium is an essential macronutrient, whereas sodium is generally not required by plants, and non-halophytes such as *S. altissima* do not necessarily have the physiological ability to absorb sufficient amounts of potassium from a medium containing a large excess of sodium (Epstein 1972). However, the ratio mentioned above is far below the range found in a typical saline medium (Epstein 1972). Conversely, the mean concentration of lead in the soil sampled near Highway 31 was almost twice the same concentration measured near Conroy Rd, which confirms that the amount of pollutants present near a road can be correlated with traffic volume.

The presence of road pollutants has probably caused the reduction in the growth of plants observed near the road, although this lower growth might be a trait of tolerant ecotypes that evolved after an exposure to road pollutants for a long period (Pitelka & Kellogg 1979). However, a transplantation experiment has shown that road pollutants have an impact comparable to this study on the growth of *S. altissima* plants that were not already adapted to the roadside environment (Chapter 2). Thus, the reduced growth of ramets growing near the road in this study is not necessarily due only to genetical differences between road edge and far populations at both sites.

Differences in height between the plants growing near and far from the road edge do not apply only to the end of the growing season. The plants were shorter near the road when gall initiation was taking place for the two species of gall-formers, as shown by the gall height measurements. Thus, goldenrod ramets were shorter at 10 m from the road edge in June, July, and at the end of the growing season, given that the periods of gall initiation for *E. solidaginis* and *E. scudderiana* correspond the months of June and July

respectively . The greater differences noted at the Conroy Rd site might result from the higher moisture levels observed for that site, however *S. altissima* grows better at the wet end of the soil-moisture gradient (Potvin & Werner 1983, 1984). Conversely, the higher soil moisture near Highway 31 may counteract the effects of road stress. In general, the differences in ramet heights were smaller in 1991 compared to 1992. This may be related to the year-to-year variation in temperature. The summer of 1992 was cloudier, wetter and cooler than 1991 (Environment Canada 1993) and this might have been an additional stress for the plants growing near the road.

The presence of the gall itself may represent an additional stress. For instance, galls induced by *E. solidaginis* are known to act as physiological sinks (McCrea *et al.* 1985) and to reduce plant fitness (Hartnett & Abrahamson 1979; Stinner & Abrahamson 1979). However, the presence of a gall and the distance from the road edge interacted significantly in only one case, i.e. at the Highway 31 in 1991 (Table 1.2 and Fig. 1.2A). Therefore, it is hard to conclude that the presence of the gall tissue enhances the effects of road stress on plant performance.

In theory, a reduction in the growth of the whole plant should also affect the gall tissue since they need the same nutrients for growth (e.g. Arya *et al.* 1962). Gall diameter was smaller for the galls induced by *E. solidaginis* near the road in 1992 but in general the results are not consistent. Similarly, it is unclear whether the gall-forming insects were affected by road stress, because of the year-to-year and site-to-site incongruities observed for survivorship, mortality factors, and infestation. Nonetheless, the gall-forming insects that were still alive at the end of the growing season did not seem to be affected by road stress since the biomass of the fully grown larvae sampled near and far from the road did

not differ, which indicate that their development was not affected by any of the road-stress-induced changes in the host-plant.

Gall-forming insects are typically more abundant in harsh environments and may be better adapted than any other feeding guild to the conditions prevailing in those habitats (Price *et al.* 1987; Fernandes & Price 1991). More goldenrod ramets were attacked by both species of gall-formers near Conroy Rd in 1992, although ramet density was lower at 10 m from the road edge for that site (unpublished data). Therefore, this result may just represent a redistribution of the same number of insects on different numbers of ramets, even though this is contrary to the positive linear relationship between the densities of *Eurosta* gall and goldenrod ramet reported by Abrahamson *et al.* (1983).

In addition to the adaptive nature of galls, insects living in a gall might be better protected against stress-induced changes in host-plant traits since they feed on a peculiar tissue that is rich in nutrients and poor in secondary compounds (Bronner 1977; Larew 1982; Rohfritsch & Shorthouse 1982; Cornell 1983). Thus, it may take stronger or different stresses than those observed in roadside environments to generate significant changes in the performance of gall-forming insects. For example, the use of a distance from the road edge shorter than 10 m might produce an effect on gall-formers since the amount of pollutants increases dramatically towards the road (Wheeler & Rolfe 1979; Thompson *et al.* 1986b; Leonzio & Pisani 1987; Yang *et al.* 1991). Otherwise, nutrient and water stresses may have a negative impact on gall-formers in harsh environments, such as along a steep elevational gradient (Price & Clancy 1986; Waring 1986; Waring & Price 1990). Nevertheless, there is no doubt that road stress present at 10 m from the road edge affects goldenrods but it is probably not severe enough to affect gall-formers consistently. On the other hand, the various pollutants found in roadside environments may have

counteracting effects, which then could lead to a marginal overall effect on gall-forming insects.

Table 1.1 Effects of the sampling month (May and September) and the distance from the road edge (10 m and 60 m) on soil characteristics in 1991 (water and K⁺/Na⁺ data were analysed by ANOVAs whereas lead data were analysed by an approximate t-test¹; see Fig. 1.1)

Source of variation	Water (%) ²		K ⁺ /Na ⁺		Lead (PPM) ³	
	H31 (47) ⁴	Conroy (47)	H31 (46)	Conroy (47)	H31 (11)	Conroy (13)
Month	<0.001 ⁵	<0.001	0.256	0.847		
Distance	0.001	0.152	0.773	<0.001	0.003	0.057
Month X distance	0.126	0.288	0.624	0.575		

¹Unequal variances

²Data were arcsine-transformed

³Lead was only measured in soil sampled in May

⁴Total number of degrees of freedom

⁵P values

Table 1.2 Effects of the presence of a gall and the distance from the road edge (10 m and 60 m) on goldenrod ramet height at the end of the growing season (data were log-transformed and analysed by ANOVAs; see Figs. 1.2 and 1.3)

Source of variation	Highway 31		Conroy Rd	
	1991	1992	1991	1992
	(254) ¹	(512)	(193)	(511)
Gall	0.200 ²	0.011		0.084
Distance	0.074	<0.001	0.094	<0.001
Gall X distance	0.040	0.664		0.863

¹Total number of degrees of freedom

²P values

Table 1.3 Comparison of gall and insect traits at 10 m and 60 m from the road edge (data were analysed by t-tests¹; see Figs. 1.4 and 1.5)

Variable	Highway 31				Conroy Rd			
	1991		1992		1991		1992	
	df	P	df	P	df	P	df	P
Gall height								
<u>E. solidaginis</u>	199	<0.001	199	<0.001	202	0.031	165	<0.001
<u>E. scudderiana</u>			97	<0.001			160	<0.001
Gall diameter								
<u>E. solidaginis</u>	103	0.095	111	0.003	112	0.250	55	<0.001
<u>E. scudderiana</u>			64	0.171			91	0.065
Larval dry mass								
<u>E. solidaginis</u>	99	0.619	105	0.798	112	0.165	85	0.115
<u>E. scudderiana</u>			60	0.684			87	0.116

¹Approximate t-test was used when the variances were unequal

Table 1.4 Comparison of survivorship, mortality factors, and percentage of infestation of goldenrod ramets by gall-forming insects at two distances from the road edge (data were analysed by Fisher's exact tests)

Variable	Highway 31				Conroy Rd			
	1991		1992		1991		1992	
	n	P	n	P	n	P	n	P
Survivorship								
<u>E. solidaginis</u>	201	0.324	201	0.075	204	0.049	167	<0.001
<u>E. scudderiana</u>			112	0.703			181	0.136
Mortality factors								
<u>E. solidaginis</u>	96	0.289	68	0.008	90	0.121	76	0.002
<u>E. scudderiana</u>			46	1.000			84	0.155
Infestation								
<u>E. solidaginis</u>			1056	0.945			802	0.004
<u>E. scudderiana</u>			1056	0.270			802	0.038

Table 1.5 Survivorship and mortality factors of E. solidaginis at two distances from the road edge (numbers are percentages)

Fate of the gall-former	Highway 31				Conroy Rd			
	1991		1992		1991		1992	
	10 m	60 m	10 m	60 m	10 m	60 m	10 m	60 m
Gall-former alive	48.5	56.0	60.2	72.5	63.0	49.0	40.7	69.1
Predation by <u>M. unicolor</u>	13.9	6.0	10.7	0.0	4.0	9.6	4.7	0.0
Parasitism by <u>E. gigantea</u>	15.8	17.0	16.5	16.3	8.0	17.3	34.9	7.4
Parasitism by <u>E. obtusiventris</u>	0.0	0.0	0.0	0.0	0.0	1.9	0.0	0.0
<u>E. scudderiana</u> ¹	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unexplained mortality	20.8	21.0	12.6	11.2	25.0	22.1	20.0	23.5

¹In one case, there was evidence that a larva of E. scudderiana caused the death of E. solidaginis directly by chewing its way through the gall formed by that species

Figure 1.1 Soil characteristics at two distances from the road edge in 1991. (A) Moisture (May+September). (B) K^+/Na^+ (May+September). (C) Lead (May).

Geometric means and 95% confidence intervals for (A); means and standard errors for (B) and (C). Statistical results are given in Table 1.1

Figure 1.2 Height of goldenrod ramets without galls and those attacked by *E. solidaginis* or *E. scudderiana* at two distances from Highway 31. (A) 1991. (B) 1992. Geometric means and 95% confidence intervals. Statistical results are given in Table 1.2.

Figure 1.3 Height of goldenrod ramets without galls and those attacked by *E. solidaginis* or *E. scudderiana* at two distances from Co.roy Rd. (A) 1991. (B) 1992. Geometric means and 95% confidence intervals. Statistical results are given in Table 1.2.

Figure 1.4 Height of galls on goldenrod stems at two distances from the road edge. (A) *E. solidaginis* - 1991. (B) *E. solidaginis* - 1992. (C) *E. scudderiana* - 1992.

Means and standard errors. Statistical results are given in Table 3.

Figure 1.5 Gall diameter at two distances from the road edge. (A) *E. solidaginis* - 1991. (B) *E. solidaginis* - 1992. (C) *E. scudderiana* - 1992. Means and standard errors. Statistical results are given in Table 3.

CHAPTER 2

SEASONAL VARIATIONS IN ROADSIDE CONDITIONS AND THE PERFORMANCE OF A GALL-FORMING INSECT AND ITS FOOD PLANT

Introduction

Several aspects of insect-plant interactions in roadside environments have been documented in the literature in the past 20 years. Long-term effects of road pollutants include mainly the bioaccumulation of heavy metals, especially lead, in plants and insect herbivores (e.g. Beyer & Moore 1980). Outbreaks of aphids and defoliating Lepidoptera near roads have been related to the short-term effects of air pollutants and de-icing salt on the host-plant's nutritional status (Fluckiger *et al.* 1978; Port & Thompson 1980; Braun & Fluckiger 1984b, 1985; Bolsinger & Fluckiger 1987; Spencer & Port 1988; Spencer *et al.* 1988). All the experiments that showed a causal relationship between insect outbreaks and road stress were done with aphids, which feed exclusively on plant sap and have short life cycles.

The effects of air pollutants and de-icing salt may depend on the life history of both insect herbivores and their host-plants. The tall goldenrod, *Solidago altissima* L. (Asteraceae), and its gall-forming herbivore, *Eurosta solidaginis* (Fitch) (Diptera: Tephritidae), occur commonly in roadside environments of northeastern North America. The high concentrations of air pollutants near the road are more likely to affect a perennial herb such as *S. altissima* during the summer when the aboveground tissues are present. On the other hand, de-icing salt will affect goldenrods more strongly when the contaminated snow ploughed onto the side of the road melts in the spring (Jones *et al.*

1986; Thompson *et al.* 1986a). Although some authors have suggested that air pollutants and de-icing salt might interact to favour insect herbivores (Spencer & Port 1988; Spencer *et al.* 1988), such synergism between the two kinds of pollutants has not been demonstrated clearly .

E. solidaginis is a sedentary and internally feeding insect that is a poor disperser and has a year-long life cycle (Uhler 1951). A study of populations of *E. solidaginis* and *S. altissima* that were already established along roads has shown that the gall-forming insects were not affected by road stress, even though the plants were smaller near the road throughout the growing season (Chapter 1).

This paper describes a field transplant experiment testing the effects of seasonal variations in roadside conditions on the performance of a gall-forming insect and its host-plant, a perennial herb. I hypothesized that a temporal separation of the two major classes of road pollutants would reveal the effects of each class, i.e. air pollutants during the summer and de-icing salt in the winter/spring period. This experiment was also designed to test for any interaction (antagonism or synergism) between air pollutants and de-icing salt.

Materials and methods

The plants used for the experiment were grown from seeds collected from clones of *S. altissima* that were susceptible to *E. solidaginis*. The seeds were not collected from roadside plants to avoid using genotypes adapted to road stress. Goldenrods were grown in nursery pots (6 L) during the summer of 1990, in a potting mix that contained equal amounts of perlite, peat moss, and top soil (sandy loam). The potted plants were moved to the experimental site in September 1990. The experiment was carried out at a site located

along Highway 417, a major commuter route in the Ottawa metropolitan area (Ontario, Canada). The annual average daily traffic for that road was 25 000 vehicles in 1991 (Ontario Ministry of Transportation 1992).

The design of the transplant experiment is depicted in Fig.2.1. Three "blocks" of 40 pots were set up and one of these blocks is illustrated in Fig. 2.1. Two distances from the road were used: 10 m, which represented the closest vegetation margin set by road maintenance operations, and 60 m, which was far enough from the road to minimize the presence of pollutants. The timing of exposure to road pollutants defined the four treatments of the experiment, i.e. 1) plants that were always far from the road ("control"), 2) plants that were always near the road ("air+salt"), 3) plants that were near the road in winter and far in the summer ("salt"), and 4) plants that were far from the road in winter and near in the summer ("air"). The "salt" treatment plants were at 10 m from the road from November 1990 to April 1991 and from November 1991 to April 1992. At the end of April of 1991 and 1992, the "salt" treatment plants were moved back to 60 m from the road and replaced by the "air" treatment plants, which were near the road from May to October of 1991 and 1992, and so on. The other potted plants were removed and put back in their holes to control for the effects of handling. The pots had holes at the bottom and they were embedded in the ground so that there was contact with the surrounding soil.

To supplement the natural populations at the experimental site, galls of *E. solidaginis* were added to the pots in May (3/pot in 1991 and 5/pot in 1992). The galls were all from the same site in the Ottawa area so that the proportion of galls producing adults would be the same for all treatments. In the fall of 1991 and 1992, the height of gall-bearing ramets and of the gall were measured. Gall height is an indirect measurement of ramet height when gall initiation was taking place, i.e. June for *E. solidaginis*. The

diameter at the largest point of the galls was measured to the nearest 0.1 mm with calipers and the galls were dissected in the laboratory to determine the fate of the gall-former. Gall diameter data were analysed only for the galls containing live Eurosta larvae. The mortality factors were classified as predation by Mordellistena unicolor Lec. (Coleoptera: Mordellidae), parasitism by Eurytoma gigantea Walsh (Hymenoptera: Eurytomidae), and unexplained mortality. The data concerning the fate of the gall-formers were analysed in two ways: 1) A comparison of the percentages of the larvae that survived versus all the larvae that died, i.e. lumping together all the mortality factors, and 2) A comparison between the diverse mortality factors in terms of percentages within the larvae that died. The gall-forming larvae were oven-dried (72 hours at 60°C) and weighed.

At the end of the experiment (September 1992), a subsample of 6 pots per treatment was randomly selected for a comparison of biomass allocation. The various plant tissues were sorted, oven-dried (12 hours at 60°C) and weighed.

Results

The population density and the fate of the gall-forming insect were not affected by the road stress exerted on S. altissima. The proportion of goldenrod ramets attacked by E. solidaginis did not differ among the treatments (Table 2.1). Also, the percentages of the gall-formers that survived the larval stage did not depend on the treatment for both years (Tables 2.1 and 2.2). However, the results for the individual mortality factors in 1991 and 1992 are not consistent. The mortality factors depended on the treatments in 1991 only (Tables 2.1 and 2.2).

Unlike their abundance and survivorship, the biomass of Eurosta larvae was affected by the treatments. The dry mass was higher for the larvae growing on plants

exposed to road stress (data of 1991 and 1992 were combined), regardless of the exposure period (Fig. 2.2). The size of the galls induced by *E. solidaginis* did not differ among the treatments for both years (data for 1991 and 1992 combined; 1-way ANOVA; $n = 54$, $P = 0.763$). Both gall and ramet heights were affected by the treatments. In 1991 and 1992, the galls were lower on the plants that were near the road during the growing season (Fig. 2.3A). Similarly, goldenrod ramets attacked by *E. solidaginis* were smaller or tended to be so for both years, whether they were kept near the road throughout the duration of the experiment or they were kept near the road only from May to October (Fig. 2.3B). However, the differences observed for both gall and ramet heights were greater in 1992 than in 1991.

At the end of the transplant experiment, the biomass allocation of *S. altissima* differed among the treatments. Shoot biomass was affected by the treatments whereas no such result was found for root biomass (Fig. 2.4). Plants that had grown near the road in 1992 allocated less to aboveground tissues, regardless of the time of exposure to road stress (Fig. 2.4). The root/shoot ratio tended to be affected by the treatments, i.e. the ratio of the "control" plants tended to be lower than for the other plants (Fig. 2.4).

Discussion

Unlike the observations made on "natural populations" of *E. solidaginis* attacking *S. altissima* in roadside environments (Chapter 1), stressed plants growing near Highway 417 did affect the biomass of the fully grown gall-forming larvae. The traffic volume is much higher on Highway 417 than on the roads used in the descriptive study, i.e. 25 000 compared to 10 000 and less than 2 000 vehicles/day (Chapter 1). The amount of pollutants is positively correlated with traffic volume (Motto *et al.* 1970; Wheeler & Rolfe 1979; Yang *et al.* 1991; Chapter 1). Therefore, the levels of pollutants along Highway 417

must have been high enough to affect both the host-plant and the gall-forming insect, although the survivorship of *Eurosta* larvae did not differ between the treatments.

The greater biomass of gall-forming larvae grown on goldenrods growing near the road indicates that these plants may represent a better host in terms of food quality. However, the proportions of goldenrod ramets attacked by *E. solidaginis* did not differ between the treatments. Also, the results show that shoot growth was reduced by road air pollutants throughout the growing season whereas *E. solidaginis* is known to prefer fast-growing ramets (Anderson *et al.* 1989; Horner & Abrahamson 1992). Data on gall diameter indicate that contrary to the shoot, growth of the gall tissue was not affected by road stress, which might indicate that shoot growth was not an important factor for gall initiation and growth and that smaller stressed plants allocated a larger proportion of resources to the unaffected gall tissue.

The nutritional status of the host-plant might provide a better explanation for the increase in biomass of insects grown on stressed plants. A number of studies have shown that concentrations of nitrogenous compounds and carbohydrates in the sap of plants growing near roads were increased because of the presence of air pollutants (Fluckiger *et al.* 1978; Port & Thompson 1980; Braun & Fluckiger 1984b, 1985; Bolsinger & Fluckiger 1987; Spencer & Port 1988; Spencer *et al.* 1988). Also, a greenhouse experiment has shown that salt stress may increase nitrogen concentrations in the stem gall tissue induced by another species of gall-former on *S. altissima* (Chapter 3).

The transplant experiment also showed that air pollution has a more dramatic impact on the gall-former than salt and that the effects of air pollutants might have been tempered by de-icing salt (Fig. 2.2). This pattern tends to hold for the host-plant's root/shoot ratio as well (Fig. 2.4). Perhaps the increasing concentrations of chlorine and sodium in plant

tissues caused by de-icing salt tempered the beneficial effects of increasing levels of nutrients due to the presence of air pollutants. Salt is known to have a negative impact on the growth of *S. altissima*, especially on the roots (Chapter 3).

Nevertheless, these results contradict those found by other researchers (Spencer & Port 1988; Spencer *et al.* 1988) who found a positive interaction between the effects of air pollutants and de-icing salt on roadside aphid populations. These authors also suggested that the presence of salt in the soil might enhance the plant intake of absorbed nitrogen oxides, a conclusion not supported by empirical data. Rather, the transplant experiment show that the effects of air pollutants and de-icing salt have on a gall-forming insect and its perennial host-plant are less than additive.

Table 2.1 Comparison of *E. solidaginis* survivorship and mortality factors, and percentage of infestation of goldenrod ramets by *E. solidaginis*, in the four treatments¹ of the transplantation experiment (data were analysed by Fisher's exact tests)

Variable	n	P values		n	1992
		1991			
Infestation				1014	0.102
Survivorship	74	0.242		61	0.212
Mortality factors	41	0.029		40	0.643

¹Control, air pollution (near the road in summer), salt (near the road in winter), air + salt

Table 2.2 Survivorship and mortality factors of E. solidaginis in the four treatments of the transplant experiment (numbers are percentages)

Fate of the gall-former	1991				1992			
	Control	Air	Salt	Air+Salt	Control	Air	Salt	Air+Salt
Gall-former alive	46.2	60.9	37.5	31.8	45.5	9.1	35.0	33.3
Predation by <u>M. unicolor</u>	7.7	0.0	12.5	0.0	0.0	0.0	5.0	0.0
Parasitism by <u>E. gigantea</u>	23.1	21.7	6.3	50.0	13.6	45.5	30.0	33.3
Unexplained mortality	23.1	17.4	43.8	18.2	40.9	45.5	30.0	33.3

Figure 2.1 Design of the transplant experiment where the circles represent the potted plants embedded in the soil; some pots were moved back and forth in April and November so that these plants were only exposed to either summer (air pollution) or winter (de-icing salt) road stresses. Note that all pots of the "salt" and "air" treatments were transferred in spring and fall, although only three are marked with arrows in this figure

Figure 2.2 Biomass of *E. solidaginis* larvae in the four treatments of the transplant experiment. Means and standard errors. Data of 1991 and 1992 were combined and were analysed by 1-way ANOVA; $n = 53$, $P = 0.012$

Figure 2.3 Height of the galls formed by *E. solidaginis* (A) and of goldenrod ramets (B) at the end of the growing season in the four treatments of the transplant experiment. Means and standard errors. Data were analysed by 1-way ANOVA; $n = 73$, $P = 0.020$ in 1991 and $n = 61$, $P < 0.001$ in 1992 (A); $n = 73$, $P = 0.061$ in 1991 and $n = 61$, $P < 0.001$ in 1992 (B)

Figure 2.4 Root and shoot biomasses of *S. altissima* at the end of the transplant experiment (means and standard errors); the average root/shoot ratios (geometric means) are given below the root biomass bars. Data were analysed by 1-way ANOVAs (ratios were log-transformed); $n = 24$, $P_{\text{root}} = 0.689$, $P_{\text{shoot}} = 0.015$, $P_{\text{ratio}} = 0.087$

CHAPTER 3

INDIRECT EFFECTS OF SOIL SALINITY ON A GALL-FORMING CATERPILLAR FEEDING ON A NON-HALOPHILIC HERB

Introduction

Several hypotheses predict the effects of plant stress on herbivorous insects. The plant vigor hypothesis applies particularly well to gall-forming insects (Price 1991). This hypothesis states that fast-growing plant tissues should better suit internally feeding insects that depend closely on plant growth processes. Therefore, the performance of a gall-forming insect will probably suffer from any stressing agent that reduces the growth of the host-plant.

Sodium chloride (NaCl) is currently used in northern countries as a de-icing chemical and is one of the most important pollutants of roadside environments. NaCl is released in large quantities when contaminated snow ploughed from the road surface starts to melt during the spring (Jones *et al.* 1986; Thompson *et al.* 1986a). Then, salt enters the soil and becomes available to the plants. A long-term accumulation of NaCl in the soil has also been noticed for roads with a long history of salting (Hutchinson & Olson 1967; Hofstra & Smith 1984), especially in soils rich in clay (Liem *et al.* 1985). High soil salinity may affect the structure of roadside plant communities in which non-salt-tolerant species may be displaced by coastal halophilic plants (Scott & Davison 1985).

The responses of non-halophilic plants to a saline environment may in turn have an impact on insect herbivores. Braun & Fluckiger (1984b) reported that the population densities of aphids were higher on salt-sprayed hawthorns, which had high amino acid and

sugar concentrations in their phloem. High concentrations of organic solutes (amino acids and carbohydrates) may represent a protection against osmotic imbalances for non-halophytes growing under extreme saline conditions (Greenway & Munns 1980). In another study the effect of salted plants on aphids was not significant but it was suggested that salt in the soil may enhance the availability of nitrogen originating from exhaust gases (Spencer & Port 1988; Spencer *et al.* 1988).

Outbreaks of defoliating Lepidoptera have also been observed on road edges (Port and Thompson 1980). However, the effects of high salt concentrations in plant tissues on caterpillars are poorly known. Larval feeding by the fall armyworm, *Spodoptera frugiperda* (J.E. Smith), was deterred by grass sprayed with sodium chloride (Leuck *et al.* 1974). The development of a leaf-mining caterpillar feeding on a salt marsh plant was not affected by the salinity conditions in an estuary, although high salinity might have had detrimental effects on larval growth in one location (Hemminga & van Soelen 1992). Conversely, negative effects of salted plants on a stem-boring beetle larva (Hemminga & van Soelen 1988) and a generalist leaf-chewing beetle (Stamp & Harmon 1991) have been found. Thus, insects feeding on plant tissues rather than sap may be more sensitive to salt in their food.

The sodium content of non-halophilic plants is generally low (Allen 1989) and this may represent a limiting factor for herbivores. Both grazing mammals and herbivorous insects need sodium in their diet. However, in contrast to vertebrate herbivores, insects feeding on plants have much more potassium than sodium ions in their body fluid (Boné 1946, 1947). Potassium is a key nutrient for phytophagous insects, whereas only trace amounts of sodium are required (Dadd 1977). Therefore, it may be more difficult for insect herbivores to maintain a balanced diet if the Na/K ion ratio in the host-plant tissue increases

dramatically. They may be more sensitive to the ratio than to absolute sodium concentrations.

The purpose of this research was to investigate the impact of high soil salinity on the biomass allocation and nutritional status of a non-salt-tolerant plant and a gall-forming caterpillar attacking that plant. The main working hypothesis was that salt stress should affect the gall-forming caterpillar strongly since the requirements for growth of the gall tissue differ little from the needs of normal tissues (e.g. Arya *et al.* 1962). Experiments were also carried out with a leaf-chewer and a leaf-miner to see if the impact of high salt content of plant tissues will be more noticeable on insects with different feeding styles, given that leaf-feeding insects are more directly exposed to salt-stress-induced changes in their food. Such ranking of insect feeding guilds in terms of their response to stressed host-plants has been proposed by Larsson (1989) who reviewed the performance of insects feeding on stressed woody plants.

Materials and Methods

The system

Larvae of Epiblema scudderiana (Clem.) (Lepidoptera: Tortricidae) induce an elliptical gall on the stems of Solidago spp. (Meaden 1956; Miller 1976). The gall-forming caterpillar feeds on the vascular tissues and may severely affect the connections between the lower and upper parts of the stem (Blum 1953). This results in a production of side branches by the host-plant. The larvae of Trirhabda borealis Blake (Coleoptera: Chrysomelidae) chew the young leaves of goldenrods (Messina 1982) whereas those of Microthopala vittata F. (Coleoptera: Chrysomelidae) bore into the leaves of Solidago spp. and form blotch-type mines (Messina & Root 1980). The tall goldenrod, Solidago

altissima L. (Asteraceae), is a non-salt-tolerant, clonal, and perennial herb that commonly invades and dominates patches of disturbed habitats, such as road edges, in eastern North America (Werner *et al.* 1980; Melville & Morton 1982).

Greenhouse experiment

Seedlings of S. altissima were grown from seeds collected in the Ottawa area. Road edges were avoided to prevent the use of plants that might be pre-adapted to a saline environment (Pitelka & Kellogg 1979). Seedlings were grown individually in a potting mix that contained equal amounts of top soil (sandy loam), peat moss, and perlite. For the first week of the experiment (mid-May), the plants were watered daily with 50 mL of 0, 8000, or 16000 PPM NaCl solutions (a preliminary experiment indicated that 16000 PPM was the highest concentration of salt that most seedlings of S. altissima could tolerate and as a comparison, runoff water from roads where salting is practiced may range from 300 to 3000 PPM of NaCl; Brandt 1973). Then, the plants were watered every 3 weeks with 75 mL of the solutions described above. The same amount of a commercially available fertilizer at 0.01 of the recommended concentration was given to each plant on the other weeks. The low concentrations of nutrients were used to simulate the nutrient status of roadside soil. Equal amounts of water were used to fulfill the needs of the plants between the treatments and the fertilizations.

Second-instar Epiblema larvae were collected in the field (early July) from S. altissima stems prior to gall formation. The larvae were then transferred to the experimental plants, leading to the successful establishment of 54 gall-forming larvae. At the end of the experiment (late September), the galls were dissected and the various plant tissues (root, stem, etc.) were sorted. All plant and animal tissues were oven-dried at 60°C and weighed. Stem and gall tissues were ground using a mill (mesh size = 0.4 mm) and then digested in

concentrated sulfuric acid. Mercury was used as the catalyst and lithium sulfate was added to increase the boiling temperature. One half of the digested product was used for the semi-micro-Kjeldahl nitrogen analysis (McKenzie & Wallace 1954). The other half was used for the determination of potassium and sodium concentrations with an atomic absorption spectrophotometer (Perkin-Elmer model 703). Atomic absorption standard solutions were used (Aldrich Chemical Co., Milwaukee, Wisconsin).

Concurrent experiments with leaf-feeders

Two other experiments were conducted with a subsample of the plants used by the gall-forming caterpillar. The objectives of these experiments were to see if indirect effects of soil salinity on insect herbivores would be noticeable before the transfer of the gall-forming larvae and if those effects would be similar for different feeding guilds. The feeding periods of the three insect species did not overlap.

Leaf sections sampled on the greenhouse plants were presented in petri dishes to starved (12 hours) third-instar larvae of T. borealis for a period of 2.5 hours. The leaf sections were inserted between two wet filter papers. The filter paper that was on top of the leaf was punched 3 times so that the larvae had only access to the equivalent of 3 leaf discs of equal size (diameter = 7 mm), without the presence of phenolics on the edges (Jones & Coleman 1988). The activity of the larvae (feeding, resting, moving) was noted every 15 minutes. The area chewed was then measured by tracing the leaf sections on paper and using the weight of the pieces of paper. Leaves were also oven-dried and analyzed for total nitrogen, potassium and sodium (see above for details).

Second-instar larvae of M. vittata were collected in the field (mid-June) from S. altissima leaves and then transferred to the greenhouse plants, leading to the successful

establishment of 34 leaf-mining larvae. This experiment ended with the emergence of the adults one month later. The beetles were oven-dried and weighed. The mined leaves were cut off from the the plants. The area mined was measured as described above.

Results

The gall-forming caterpillar

The size of the fully grown gall and gall-former differ in the three treatments. *E. scudderiana* larvae attained smaller sizes when fed on goldenrods treated with the highest salt concentration (Fig. 3.1), although all larvae completed their development, i.e. second to last instar. Similarly, galls induced on *S. altissima* treated with the 16000 PPM NaCl solution were smaller than those on other plants (Fig. 3.2A). On the other hand, the percentage of the total biomass of the host-plant represented by the gall increased with the amount of salt added to the soil (Fig. 3.2B), meaning that on high-salt plants the gall tissue represented a much greater proportion of the plant biomass.

The nutritional status of the plant tissues was affected significantly by soil salinity, (Fig. 3.3). Nitrogen concentrations found in the gall tissue were at least twice the concentrations of the stem tissue (Fig. 3.3A). However, the effects of the treatments on nitrogen levels in the gall and the stem were similar. Sodium/potassium ratios in both gall and stem tissues increased with the amount of salt added, with sodium ion concentrations attaining levels almost equal to those of potassium in the stem tissue in the 16000 PPM NaCl treatment (Fig. 3.3B). However, Na^+/K^+ ratios tend to attain higher values in the stem compared to the gall as the soil salinity increases. The mean ratio of sodium to potassium in the stem (0.740) of goldenrods watered with the 16000 PPM NaCl solution was almost three times higher than the same mean ratio measured in the gall (0.262).

Finally, both the root and the shoot were affected negatively by the salt treatments, but root/shoot ratios indicate that the root was more severely affected by salt (Fig. 3.4).

Leaf-chewing and leaf-mining beetle larvae

High soil salinity indirectly affected feeding by the leaf-chewer. Larvae of T. borealis spent less time feeding on the leaves of goldenrods treated with salt (Fig. 3.5A). Total leaf area chewed followed the same trend but did not differ significantly between the treatments (Fig. 3.5B). For this experiment all leaves contained the same amounts of total nitrogen (Fig. 3.6A). The ratio of sodium ions over potassium ions increased with the amount of salt added to the soil (Fig. 3.6B).

Feeding by the leaf-miner was also affected by salt-stressed host-plants. Microthopala larvae feeding on S. altissima treated with 8000 and 16000 PPM NaCl solutions mined smaller areas than those feeding on the control plants (Fig. 3.7A). On the other hand, the biomass of emerging adults did not differ significantly between the treatments (Fig. 3.7B). Also, the proportion of leaf-mining larvae that completed their development (up to the adult stage) was not affected by the treatments (Fisher's exact test, $P = 0.246$).

Discussion

There is no doubt that high soil salinity strongly reduces the growth of S. altissima, affecting especially root development. High internal Cl^- or Na^+ concentrations reduce whole plant growth, and salt-stressed plants typically contain more organic solutes, including nitrogenous compounds (Greenway & Munns 1980). This may explain the increase in the total nitrogen concentrations found in plants grown in a soil watered with a 16000 PPM NaCl solution. On the other hand, it is less clear that poor plant performance

in turn influences gall growth. The highest concentration of salt added to the soil had a negative impact on gall growth and biomass of Epiblema larvae. However, the results indicated that salt-stressed plants allocated a larger proportion of resources to galls. Also, gall-forming larvae in all treatments completed their development. These results suggest that the gall may be buffered from stress and its inhabitant may benefit from this situation.

The gall tissue may act as a metabolic sink or a redirector, i.e. a passive and partial block to the normal flow of resources or an active translocation of nutrients from other parts of the plant (e.g. Jankiewicz *et al.* 1970; McCrea *et al.* 1985). The Na^+/K^+ ratio increased less rapidly in the gall tissue than in the stem tissue. Also, the nitrogen concentrations found in the gall tissue were twice those found in the stem tissue, independently of the treatments. In this case the gall seems to act as a sink for nitrogen, which might benefit the gall-former (Mattson 1980). Also, the gall tissue retains only a fraction of the excess sodium contained in the ungalled tissue (stem). Thus, even though gall-forming insects are immobile and cannot look for a better source of food, the results show that the gall may protect its inhabitant from drastic changes in the mineral nutrient balance of the host-plant. Analyses of the mineral salt content of gall and normal tissues under stress are not well documented in the literature. Brewer *et al.* (1987) and Skuhravy *et al.* (1980) have shown that there is substantial variation in concentrations of sodium in gall vs leaf (normal) tissues and there seems to be no general trend in decreased or increased levels of most chemicals.

A comparison with leaf-feeders tends to confirm that the gall may represent an effective protection against high salinity. A feeding test showed that leaves of high salt-grown goldenrod deterred feeding by the leaf-chewing larvae of Trirhabda. Na^+/K^+ ratios in the leaf tissue indicate that insects feeding on salt-stressed plants would need to assimilate more sodium to fulfill their requirements in potassium, a key nutrient. Stamp &

Harmon (1991) reported negative effects of high salt concentrations on fecundity and survivorship of a leaf-chewing adult beetle and a deterring effect of NaCl on a caterpillar was detected in another experiment using sprayed leaves (Leuck *et al.* 1974). Leaf-mining Microthopala larvae needed a smaller quantity of tissue to complete their development. However, the biomass of the emerging adults tended to be lower when the larvae were fed on salt stressed plants; the differences were not significant probably because of the small sample size since several larvae never made it to the adult stage.

Although the results show that both gall-tissue growth and gall-former fitness may be affected indirectly by soil salinity, the extremely high concentrations of NaCl that were necessary to produce those effects may be lethal for S. altissima (unpublished data). Thus, unless the host-plant dies, it seems that larvae of E. scudderiana can nearly always produce a gall that will provide all they need to complete their development (food, protection, etc.). Both biomass allocation and nutritional status of S. altissima were altered but the impact of high soil salinity on the gall-former was proportionally lowered. This contrasts with insects that have other feeding styles such as leaf-miners and chewers that seem to be more sensitive to salt-stressed plants.

Figure 3.1 Biomass of fifth instar *E. scudderiana* larvae that formed a gall on *S. altissima* grown at 3 salinity conditions. Means and standard errors. Data were analysed by a Kruskal-Wallis test; $P = 0.031$

Figure 3.2 Gall biomass (A) and proportion of total plant biomass allocated to the galls (B) formed by *E. scudderiana* on *S. altissima* grown at 3 salinity conditions. Percentages were arcsine-transformed. Means and standard errors (A); geometric means and 95% confidence intervals (B). Data were analysed by 1-way ANOVA; $P = 0.009$ (A); $P < 0.001$ (B)

Figure 3.3 Total nitrogen (A) and Na^+/K^+ ratio (B) in the gall and stem tissues of *S.*

altissima grown at 3 salinity conditions. Percentages were arcsine-transformed. Geometric means and 95% confidence intervals (A); means and standard errors (B). Data were analysed by 2-way ANOVA; $P_{\text{sal.}} < 0.001$, $P_{\text{tis.}} < 0.001$, $P_{\text{int.}} = 0.884$ (A); $P_{\text{sal.}} < 0.001$, $P_{\text{tis.}} = 0.064$, $P_{\text{int.}} = 0.119$ (B)

Figure 3.4 Root and shoot biomasses of *S. altissima* grown at 3 salinity conditions; mean root/shoot ratio is given below the root biomasses bars. Means and standard errors. Data were analysed by 1-way ANOVA; $P_{\text{root}} < 0.001$; $P_{\text{shoot}} < 0.001$; $P_{\text{ratio}} < 0.001$

Figure 3.5 Time spent feeding (A) and surface area chewed (B) by third-instar larvae of *T. borealis* on leaves of *S. altissima* grown at 3 salinity conditions. Percentages were arcsine-transformed. Geometric means and 95% confidence intervals (A); means and standard errors (B). Data were analysed by 1-way ANOVA; $P = 0.046$ (A); $P = 0.306$ (B)

Figure 3.6 Total nitrogen (A) and Na^+/K^+ ratio (B) in the leaves of *S. altissima* grown at 3 salinity conditions. Percentages were arcsine-transformed. Geometric means and 95% confidence intervals (A); means and standard errors (B). Data were analysed by 1-way ANOVA; $P = 0.875$ (A); $P = 0.002$ (B)

Figure 3.7 Surface area mined by larvae of *M. vittata* (A) and adult biomass (B) when fed on *S. altissima* grown at 3 salinity conditions. Means and standard errors. Data were analysed by 1-way ANOVA; $P = 0.001$ (A); $P = 0.261$ (B)

GENERAL DISCUSSION

Depending on the kind of stress exerted on plants or on the insect feeding guild, insect responses to stressed host-plants will not necessarily support the positive response predicted by the plant stress hypothesis (Larsson 1989) or the negative response predicted by the plant vigor hypothesis (Waring & Price 1990; Price 1991). This study showed that gall-formers do not differ from the other guilds in this matter, i.e. the effects of plant stress on the performance of gall-forming insects can be neutral (Chapter 1), positive (Chapter 2), or negative (Chapter 3). However, this study also showed that in general road stress did not affect gall-formers strongly. There was no evidence of a significant and consistent impact of road stress on the larval biomass, survivorship and population density of gall-formers as well as the growth of the gall tissue

As discussed in Chapter 1, a long-term exposure of roadside plants to road stress reduced their growth but this in turn did not affect the gall tissue and the gall-forming insect. This might be explained by the fact that the "goldenrod-stem gall insect" system has adapted to road stress, given that the "natural populations" surveyed in this study have been established in a roadside environment for a long period. The different results obtained in the transplant experiment using "unadapted" plants (Chapter 2) confirms that the exposure period can make a difference for the gall-formers.

Unlike previous studies on the abundance and survivorship of gall-formers attacking hygrothermally stressed plants, there is no evidence in this study that gall-forming prefer the more vigorous plants, whether the plants were exposed to road stress for a long or a short period (Chapters 1 and 2). By using the tolerance limit of *S. altissima* to salt stress, however, I reproduced an environment where plant growth was so reduced that

both the gall tissue and gall-former were negatively affected (Chapter 3). Thus, the plant vigor hypothesis seems to apply particularly well to gall-formers in harsh environments.

The extreme experimental conditions required to induce a negative impact on the gall tissue and gall-forming insects in the greenhouse tend to confirm that this feeding guild should be ranked as one of the least sensitive to increasing host-plant stress level (Larsson 1989). By comparison the high concentrations of salt in plant tissues deterred leaf-chewing and leaf-mining insects. Other than the mode of feeding itself, the presence of the gall tissue might explain why gall-formers seem to be buffered against the unfavorable stressed-induced changes in the host-plant. We already know that the gall tissue is a drain for energy and nutrients. However, it is unclear that this tissue may act as a selective barrier against the chemical constituents that could affect the gall-former negatively.

Larsson (1989) hypothesized that the response of gall-forming insects to stressed plants can be only neutral or negative (it is noteworthy that this hypothesis was based only on a few studies), which is not supported by this study. By using potted plants I managed to uncover the short-term and individual positive effects of air pollutants and de-icing salt on the biomass of gall-forming larvae, although the effects of the two major classes of pollutants were less than additive (Chapter 2). Thus, as opposed to the host-plant, the performance of the gall-former was not lowered by the stressful conditions prevailing in roadside environments. Also, gall initiation was taking place on slow-growing plants near the road and there was no evidence of a preference for non-stressed plants (Chapters 1 and 2). In contrast with the findings of Abrahamson *et al.* (1989) and Horner & Abrahamson (1992), the goldenrods growing near the road may not be less suitable for the gall-formers, regardless of their growth rate. Moreover, the growth of the gall tissue was not affected consistently by road stress (Chapters 1 and 2), which suggests that more resources were

allocated to the gall. A proportionally lower effect of stress on the growth of the gall tissue and the gall-former compared to the plant has clearly been shown in the greenhouse experiment (Chapter 3) and has never been reported elsewhere. It suggests that as long as the host-plant and the gall-former are not killed the growth of the gall tissue will proceed up to a size determined by plant and insect genotypes, regardless of stress.

Further research is needed to determine if the nutritional status of the gall nutritive tissue is affected by stress. However, this kind of research, according to my own experience, is more time consuming and could involve the utilization of an expensive equipment, such as a scanning electron microscope combined with a x-ray micro-analyzer. This equipment would make possible the measurement of mineral nutrients in very small amounts of fresh plant tissue such as represented by the gall nutritive tissue. Even though the chemical analysis of whole galls or of a combination of several tissues may serve in a comparison with normal tissues, it does not provide a direct measurement of the nutritional quality of the food consumed by gall-forming insects.

The possible "buffering" action of the gall against plant stress is another interesting feature that should be investigated more thoroughly. As a comparison, secondary compounds are often concentrated in the outer tissue layers of galls and they may play a role in the protection against natural enemies (Larew 1982; Cornell 1983). By measuring the nutrient concentrations in tissues located at various distances from the gall chamber, we could determine if the outer tissue layers of the gall represent a selective barrier against drastic changes in the nutritional status of the gall-inducer's food source when the host-plant is stressed (see Chapter 3). This might be possible by using the equipment mentioned above or by injecting markers in the system (e.g. marked pollutants).

The directions for further research mentioned above intend to have a better understanding of the adaptive nature of galls and to test the nutrition hypothesis. This hypothesis, which suggests that living in a gall tissue is advantageous for an insect because it is a better food source compared to the corresponding unmodified plant tissue, is one of the several hypotheses for the adaptive nature of galls that have been discussed in the literature (Cornell 1983; Price et al. 1987). However, the attempts to test these theories experimentally are scarce.

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