Occurrence of Ca, Fe, K, Mg, N, Na, S, and P Within Rose-Stem Galls and Ungalled Wild Roses: Implications for the Nutrition Hypothesis of Gall Evolution

by

Mark G. St. John

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN BIOLOGY

SCHOOL OF GRADUATE STUDIES
LAURENTIAN UNIVERSITY
SUDBURY, ONTARIO
1998

Master of Science
Biology
1998

Laurentian University
Department of Biology
Sudbury, Ontario

Title: Occurrence of Ca, Fe, K, Mg, N, Na, S, and P Within Rose-Stem Galls and Ungalled Wild Roses: Implications for the Nutrition Hypothesis of Gall Evolution

Name: Mark G. St. John

Supervisor: J. D. Shorthouse, Ph. D.

Number of pages: 87

Abstract:

The relationship between host plant and gall inducer is perhaps the most specialized of all insect/plant interactions. Insect galls are believed to be feeding adaptations which provide gall inducers with a better source of nutrition than ungalled tissues. The gall is thought to act as a plant physiological sink, accumulating high concentrations of nutritious substances such as proteins, sugars, lipids and minerals. Cynipids in the genus *Diplolepis* Geoffroy have been well studied and much is known of their life history, interactions with parasitoids and inquilines, gall anatomy and developmental morphology, host shifts, distribution, and more, yet little is known about the nutritional physiology of their galls.

Stem-galls induced by *Diplolepis spinosa* (Ashmead) on *Rosa blanda* Ait. and *D. triforma* Shorthouse and Ritchie on *R. acicularis* Lindl., and ungalled host plant tissues were measured for levels of organic N and minerals (Ca, Fe, K, Mg, Na, P and S) during gall growth and maturation phases. Ungalled tissues, gall tissues, and tissues growing distally to the galls all had significantly different organic N and mineral compositions. Galls either had lower or similar mean concentrations of organic N and mineral nutrients compared to ungalled host tissues in 28 of 32 comparisons; higher concentrations were measured in galls in only four cases. The concentration of K was higher in galls of *D.*

spinosa than ungalled tissues while the concentration of Na in galls of *D. triforma* was also significantly higher than ungalled tissues. For more than half of the nutrients measured, the concentration found in tissues distal to the gall was greater than in the galls themselves, suggesting that host plants are not suppressing the amount of nutrients being brought to galls.

Concentrations for all nutrients measured in galls, except Na in maturation phase galls of *D. spinosa*, were not highly correlated with the number of gall inhabitants (inducer and parasitoids); however, amounts (μg) of these nutrients were highly correlated with the number of gall inhabitants. Larvae of gall inducers and parasitoids were not significantly different in their mineral compositions indicating that parasitoids do not kill their hosts until adequate levels of minerals are consumed. The rate of parasitism of *D. spinosa* and *D. triforma* had no effect on the concentrations or amounts of organic N or minerals within gall tissues.

Results of this study are discussed in light of the nutrition hypothesis of gall evolution. Similarities between galls and plant sinks are explored as is the effect of gall inducer parasitism on gall nutrient concentrations.

I dedicate this thesis to my parents, Suzanne and George St. John, who have supported me in countless ways, all of them unfaltering.

Acknowledgments

I am indebted to many people for the successful completion of this thesis. Foremost, I thank my supervisor, Dr. J. D. Shorthouse, for his support, guidance and patience. I would also like to thank my advisory committee members, Dr. Yves Alarie and Dr. Frank Mallory for their time and help. My external examiner, Dr. Beverly Hale is sincerely thanked for her contribution. I am also grateful to Dr. Giuseppe Bagatto for his advice which helped shape my thoughts.

I thank my colleagues and friends Scott Brooks, Sharon Campbell, Mike Malette, Louise Paquette and David Shorthouse for lending an ear and giving advice whenever it was needed. I am especially grateful to Siphone Boualavong for her encouragement and support through the good and the bad. I thank my Mother, Father and especially my Sister, Chantal, for being there all the way.

Dr. Graeme Spiers and Naj Elwear of the Ontario Geological Survey are thanked for their helpfulness, time and hard work in performing the ICP determinations. I thank Dr. Peitrezak of Agriculture Canada for allowing me to use his Kjeldahl analysis equipment and Don Quin for taking the time to show me how everything worked. I also thank Les Rajula of Falconbridge Limited for granting me permission to collect my samples from company property.

Financial support for this thesis was provided by grants from the Natural Sciences and Engineering Research Council of Canada, and the Laurentian University Research Fund awarded to Dr. J. D. Shorthouse and by a bursary and two graduate teaching assistantships provided by Laurentian University.

Table of Contents

List of Figures

List of Tables

I. General Introduction

The relationships between plants and insects have fascinated people since the beginning of recorded history. Today, understanding plant-insect relationships is of utmost importance as we try to comprehend the world around us and ensure the sustainability of natural and human-influenced ecosystems. Early farmers and horticulturists probably recognized the injury phytophagous insects imparted to plants, as well as the benefits of insect pollinators. More observant early peoples may have noticed that certain insects only feed on select plants and that different insects feed in strikingly diverse manners, some on leaves, some within the leaves or stems, while others fed from within bizarre-looking growths. Today we recognize these growths as insect galls and the attempt to understand them is as fascinating to modern scientists as perhaps it was to early peoples who cut open galls to gaze at the insects within.

The study of insect galls is known as cecidology and the roots of this science can be traced back to ancient times. Hippocrates (460 B.C.) was the first author to describe galls and he was aware of the insects within. Through his writings, we know that galls have been used for medicine and food for at least 2500 years. Many kinds of galls contain high levels of tannin from which tannic acid was derived for use as a bactericide and fungicide in wounds to accelerate healing. Early North American natives recognized the healing qualities of galls and would cut open large galls found on wild roses and place them on wounds (Shorthouse pers. comm.). Oak galls induced by cynipid wasps were harvested for their tannic acid used in the tanning and dying of fabrics and leather and for the manufacture of writing inks.

Although Hippocrates recognized that galls contained insects, the mechanism of gall formation has remained elusive to this day. Theophratus (327 - 286 B.C.) still believed that galls were 'normal' structures produced by the plant and others such as Pliny (23 - 79 AD) thought that galls found on oak leaves were fruits. Through the middle ages no major

advancements were made in our understanding of gall formation. Leuwenhoek (1632 - 1723) and Malpighi (1628 - 1694) were the first to associate gall formation with oviposition by an insect. Malpighi believed that galls resulted from an irritating substance originating from the female egg-laying apparatus, which is partially the case for galling sawflies. Two hundred years later, in the second half of the nineteenth century, Beijernick (1882) was the first to identify formation of some galls as the result of larval activity. Once this discovery was made, cecidology developed rapidly; hundreds of new species were described and numerous monographs were written. Most students of insect galls, following Beijernick, concentrated on cataloguing the rich diversity of gall inducers and their galls and indeed this process continues today. However, once the inducers were identified and could be distinguished from all others, biologists began looking at the anatomy and developmental events of the galls themselves, while others began unraveling the complex relationships occurring between the myriad of parasitoids and inquilines associated with larvae of the inducers.

Modern interest in galls appears to be growing as indicated by the plethora of books on the subject that have appeared within the past 20 years (Meyer and Maresquelle 1983, Ananthakrishnan 1984, Meyer 1987, Shorthouse and Rohfritsch 1992, Williams 1994, Price et al. 1994, Abrahamson and Weis 1997), along with hundreds of journal articles and numerous book chapters. Four international conferences on galls have been held in the last 20 years. All International Congresses of Entomology since 1980 have had symposia on galls and as well, papers on galls attract attention at many entomological and botanical meetings.

Two approaches to studying insect galls appear to have developed over the past 20 years. One approach is to concentrate on unraveling the complexities of galls themselves. Here the pursuit is to understand the life history strategies of select gallers, attempt to culture them in the laboratory, examine developmental morphology and anatomy of their galls, examine ways in which parasitoids and inquilines influence composition of

associated component communities and alter gall anatomy, and then to determine how the inducers influence the distribution of minerals and other compounds within the host plant (see references in above books). A second approach is to use gallers and their galls as research subjects in explaining basic principles of insect-plant relationships, community ecology and population dynamics. Both approaches provide useful information on gall biology, but regardless of the way in which galls are studied, many fundamental attributes remain elusive. For example, we still know little about the process of gall initiation. That is, no one has determined how gallers gain control of host plant development to produce functional galls. Furthermore, we know little about how closely related gallers are able to induce strikingly different, species-specific structures from the same host tissues on which eggs of each have been deposited simultaneously.

One reason why little progress has been made in deciphering events in the initiation of galls is that few gallers amenable to laboratory culture have been found. For researchers to use sophisticated techniques such as high performance liquid chromatography, scanning electron microscopy and inductively-coupled plasma-emission (ICP) spectrophotometry, a steady and consistent supply of gallers and gall tissues is required. Second, for non-gall biologists to become involved with gall studies, there must be assurance that the same species of inducer is being used throughout the study. For most genera of gallers, both problems remain impediments to advancement in cecidology at, for example, the molecular or biochemical level. A non-gall biologist wishing to undertake any sort of scientific inquiry using galls would need to choose from, and distinguish among approximately 13, 000 cecidogenous insects in 7 orders attacking hundreds of host plants belonging to all branches of the plant kingdom from gymnosperms to angiosperms, monocots and dicots (Dreger-Jauffret and Shorthouse 1992).

For interdisciplinary progress to be made in understanding gall biology, it is perhaps best to concentrate on one species of galler or a single genus of gallers. W. G. Abrahamson and associates of Bucknell University in the U. S. A. have made major

advances by using one species of inducer, the tephritid fly *Eurosta solidaginis* (Fitch) on goldenrod (*Solidago* sp.). Papers from this team have covered subjects such as speciation (Abrahamson et al. 1994), host specificity (Abrahamson et al. 1989, Craig et al. 1994), evolution (Weis and Abrahamson 1986), nutritional ecology (Abrahamson and Weis 1987), inducer-parasitoid relationships (Abrahamson et al. 1983, Weis and Abrahamson 1985, Weis et al. 1985) and others, all of which have been recently synthesized in one volume (Abrahamson and Weis 1997). Similarly, P. W. Price and associates at Northern Arizona University in the U. S. A. have made significant contributions by using gall-inducing sawflies as tools to understand basic principles of insect-plant interactions (Craig et al. 1986, Sacchi et al. 1988), life history strategies (Price and Craig 1984), community ecology (Price et al. 1980), population dynamics (Price and Clancy 1986) and others.

A common theme in works by the above authors is that insects capable of inducing galls are thought to have overcome nutritional problems faced by external feeders. The feeding stages of gall-inducers not only become enclosed within plant tissue preventing them from falling off the host, becoming desiccated, and potentially providing escape from parasitoids, but the plant cells they consume are vastly different nutritionally from any of the cell types found within the host organ on which the gall was induced (Bronner 1992). These cells, referred to as nutritive cells, are the sole source of food for developing larvae. The cytology and composition of these cells has been studied in some detail and it is known that they contain high levels of lipids, simple carbohydrates and enzymes relative to outer gall tissues (Bronner 1992). The gut of many species of gall-inducing larvae is blind, limiting the volume of ingested food, which Weis and Berenbaum (1989) consider as further evidence that gall tissue must be extremely nutritious.

Price et al. (1987) proposed that galls are feeding adaptations which provide a superior food resource to gall-inducing insects by acting as "sinks" which concentrate higher levels of limiting nutrients than ungalled plant tissues. This was termed the "nutrition hypothesis of gall evolution." The highest levels of nutritious substances within

galls are likely to be in the nutritive cells and the gall inducer larva (Bronner 1992); however, total gall concentrations are expected to be higher than ungalled tissues. Studies using radioactive carbon tracers have shown that galls can be sinks for carbon (Larson and Whitham 1991). For example, galls of the cynipid wasp *Phanacis taraxaci* (Ashmead) accumulate up to 70% of the total carbon assimilated by their host plant, *Taraxacum officinale* Weber (Bagatto et al. 1996). However, plants have the potential to overproduce carbohydrates and rarely produce their maximum under normal conditions (Harper 1977), meaning that the abundance of assimilated carbon in galls, most likely to be in the form of structural molecules such as cellulose, does not necessarily represent a loss of carbon to the host plant or a nutritional advantage to the larvae. Furthermore, it is unlikely that galls are adaptations for obtaining more sugars and starches since herbivores are typically nutrient (particularly nitrogen) limited rather than carbon or energy limited (Slansky and Feeny 1977). Thus, carbon may not be an ideal element for study in gall systems if the allocation patterns of critical nutrients in gall systems is of interest. In contrast, Thompson and Stewart (1981) proposed that minerals (e.g. nitrogen, phosphorus and potassium) are limiting to plants and thus may provide better insight into resource allocation. Furthermore, Abrahamson and Caswell (1982) recommended considering many nutrients, rather than any one, in physiological studies of galls due to the variability among galls of different populations and different species.

Gall-inducing insects are often fed upon by parasitoids of the superfamilies Chalcidoidea and Ichneumonoidea which procure all of their nourishment from a single inducer larva (Askew 1984). Thus, the highest levels of any particular nutrient to be found in a parasitoid should be equal to or less than the host it consumed. However, Bagatto (1992) and Bagatto and Shorthouse (1997) found that parasitoids of the blueberry-galling pteromalid *Hemadas nubilipennis* Ashmead had higher concentrations of minerals (copper, zinc, magnesium and manganese) than their host inducers. They hypothesized that parasitoids stimulate their host inducers to feed more/longer than normal, thus drawing

more nutrients into the gall, the inducer, and finally into the parasitoid. Therefore, it would be expected that parasitism of gall inducers will lead to an increase in the concentrations of nutrients within gall tissues.

It follows that a study of the nutritional physiology of a gall and gall inducer parasitism requires two key conditions to be successful. Firstly, a well studied, accessible and abundant gall system where galls are sufficiently large for tissue analysis is required. Two species of cynipids, *Diplolepis spinosa* (Ashmead) and *Diplolepis triforma* Shorthouse and Ritchie which gall wild roses in northern Ontario fulfill these requirements.

Secondly, facilities for measuring the levels of many nutrients in plant and animal tissues are required. The opportunity to include measures of organic N in the galls of the above two species and ungalled wild roses was provided with access to a Kjeldahl N autoanalyzer at Agriculture Canada in Ottawa, Ontario. Also, use of an ICP spectrophotometer, which measures the concentrations of a wide variety of elements including most plant mineral nutrients, was made available by the Ontario Geological Survey on the campus of Laurentian University. Thus, the presence of large populations of *D. spinosa* and *D. triforma* near Sudbury, Ontario, and the accessibility of required analytical equipment provided an ideal opportunity to pursue the topic of gall nutrient physiology.

There are approximately 15 species of *Diplolepis* Geoffroy in Canada. Information on most of these species including the identity of their hosts (Shorthouse and Ritchie 1984, Shorthouse 1988, Shorthouse 1994) , their preferred habitats and distribution cross Canada (Shorthouse 1993), characteristics of their galls (Shorthouse 1993), and the occurrence of various parasitoids and inquiline wasps and their relationships in forming component communities (Shorthouse 1973, Brooks and Shorthouse 1997) is available. Furthermore, gall anatomy and developmental morphology (Brooks and Shorthouse 1998), identification of morphogens involved in gall tissue growth and differentiation (Schönrogge et al. 1998)

and physiology of galls (Bagatto et al. 1991, Bagatto and Shorthouse 1994a) has also been studied for this genus of gall-inducing cynipids.

Diplolepis spinosa induces spherical, multi-chambered stem galls on *Rosa blanda* Ait. and *D. triforma* induces fusiform, multi-chambered stem galls on *Rosa acicularis* Lindl. Multi-chambered stem galls are ideal for physiological study because they have adequate gall, inducer and parasitoid biomass for mineral analyses, have variability in size proportional to the number of inducers (Shorthouse 1993, Bagatto and Shorthouse 1994a), have variability in inducer parasitism (Bagatto and Shorthouse 1994a), do not share host tissues with adjacent galls, and ungalled shoots are excellent controls for comparison. Furthermore, both species of host roses often occur in the same habitat and field making it possible to compare gall physiological differences which may result from morphological or host differences rather than site differences (e.g. soil quality and environmental factors.) Galls of both species are inhabited by numerous species of parasitoids, the interactions of which form distinct component communities (Zuchlinski 1979, Shorthouse and Ritchie 1984, Kelleher 1988, Wiebes-Rijks and Shorthouse 1992, Shorthouse 1993, Bagatto and Shorthouse 1994a) and may result in physiological changes in galls.

Thus, the purpose of this study is to search for patterns in the distribution of organic N and mineral nutrients (Ca, Fe, K, Mg, Na, P, and S) in galls of *D. spinosa* and *D. triforma* which would indicate whether or not the role of galls is to accumulate nutrients to the advantage of the inducers. Furthermore, the relationship between inducers and parasitoids on these patterns will be explored. In addition, the results will be used to refute or support the hypotheses developed by Price et al. (1987) to explain the adaptive nature of insect galls. Attributes of plant nutrition and physiology pertinent to gall biology and the biology of *D. spinosa* and *D. triforma* and their galls will be reviewed prior to explaining procedures and results.

II. Attributes of Plants Pertinent to Gall Biology

A. Plant Nutrition

Plant nutrition begins with the uptake of inorganic minerals from the growing medium and subsequent distribution and use of these minerals by the plant (Marschner 1983). Mineral nutrients of plants can be either essential mineral nutrients (elements) or beneficial mineral nutrients (elements). A mineral is considered essential if: 1) there is a positive requirement of the element for normal growth and reproduction, 2) it cannot be replaced in function by other elements and 3) it has a direct or indirect effect on plant metabolism (Marschner 1983). Thus, an element which stimulates growth but does not satisfy all of the criteria is a beneficial element.

Essential mineral elements are constituents of almost every type of organic compound in plants including enzymes, co-enzymes, membrane constituents, secondary compounds and pigments (Marschner 1983). There are presently 14 known essential mineral elements, six are macronutrients (N, K, Ca, Mg, P and S) and eight are micronutrients (Cl, Fe, B, Mn, Zn, Cu, Ni and Mo) (Mehra and Farago 1994, Welch 1995). Macronutrients are 10 - 5000 times more concentrated in plant tissues than micronutrients. Macronutrients are usually measured in percent (%) of dry plant mass and micronutrients are measured in parts per million (ppm = μg/g) of dry mass (Jones et al. 1991). The following is a brief description of the role played by each of the mineral elements pertinent to the present study.

Nitrogen (N) is found in both inorganic and organic forms in plants. It combines with carbon, hydrogen, oxygen and sometimes sulphur to form amino acids, amino enzymes, nucleic acids, chlorophyll, alkaloids and purine bases. The predominant form of nitrogen in plants is organic (high molecular weight proteins) although inorganic nitrogen can accumulate in plants in the form of nitrate (NO_3^-) (Jones et al. 1991). Typically, plant tissues are 1.5 - 6.0 % N by dry mass. Concentrations of nitrogen can vary widely

depending on plant species, stage of growth, and plant organ. Highest concentrations are found in new leaves and total plant nitrogen decreases as the plant ages. Inorganic nitrogen will accumulate at high concentrations in the stems and petioles during the vegetative period of growth. Root uptake of nitrate stimulates the uptake of phosphorus and potassium. However, if nitrogen is being taken up in the form of ammonium (NH_4^+), calcium and potassium levels in the plant may be suppressed. Plants deficient in N are slow growing, weak and stunted with light green or yellow foliage. Nitrogen is highly mobile in plants causing deficiency symptoms in older leaves as N is transported to actively growing regions of the plant. Plants with an excess of nitrogen often appear dark green and are susceptible to disease and insect outbreaks (Jones et al. 1991).

Calcium (Ca) is important in maintaining cell integrity and membrane permeability (Jones et al. 1991). It also plays a role in controlling leaf senescence and high concentrations retard and even prevent abscission (Marschner 1983). Leaf tissues range from 0.2 - 1.0 % Ca by dry mass (Jones et al. 1991). Most of this is bound in cell walls as pectates or sequestered in different organelles. Calcium enhances pollen germination and growth and activates a number of enzymes for cell mitosis, division and elongation. Calcium is required for protein synthesis and carbohydrate transfer, and may even play a role in detoxifying heavy metals in plant tissues by forming molecules with them. An increase in Ca concentration decreases the rate of respiration in that tissue. Deficiency symptoms of Ca include: growing tips (meristems) turn brown and die, leaves curl and margins turn brown, and newly expanding leaves stick together at margins and become shredded when fully expanded. An excess of Ca will result in inhibition of magnesium or potassium uptake and transfer thus causing the deficiency symptoms associated with these elements.

Iron (Fe), the only essential micronutrient considered in this study, is an important constituent of plant enzymes in the electron transport chains in photosynthesis and respiration (e.g. ferrodoxin) (Welch 1995). It is an important enzyme catalyst; it is required

for the synthesis of chlorophyll; in legumes it acts as the O_2 carrier in the process of N_2 fixation. Leaf content can range from 10 - 1000 ppm, although 50 - 75 ppm is typical (Jones et al. 1991). Deficiency of this element leads to chlorosis beginning in younger leaves, then spreading to older leaves because of iron's important role in the synthesis of chlorophyll. Rarely do Fe levels become excessive but when this happens leaves become "bronzed" with tiny brown spots.

Potassium (K) is required in all metabolic processes of plant cells (Mehra and Farago 1994). There are three major roles for K in plant nutrition: 1) an enzyme activator for a number of processes including respiration, protein synthesis and photosynthesis, 2) the most important ion involved in osmotic processes including turgor driven cell growth and plant responses, such as stomatal opening and closing and leaf movements, and 3) charge neutralization, important for membrane transport and the synthesis and translocation of organic anions (e.g. newly formed carbohydrates). Potassium usually makes up about 1 - 5% of leaf dry mass (Jones et al. 1991). Deficiency symptoms include reduced fruit yield and quality, and regressive die back or scorch of older leaves. The most important deficiency symptom is susceptibility to fungi, bacteria and insects (Jones et al. 1991) as a result of synthesis of large polymer compounds such as starch and proteins being inhibited causing an increase in more easily available low molecular weight compounds like sugars and amino acids (Marschner 1983). Excess K will cause symptoms of magnesium and calcium deficiency because of interactions with these elements.

Magnesium (Mg) is the central element of the chlorophyll molecule (Jones et al. 1991). Magnesium is also the activator of more enzymes than any other element (Mehra and Farago 1994). Most enzymes that activate the phosphorylation processes (the release of energy from the ATP molecule) are Mg based. Furthermore, Mg is essential to maintain ribosome structure for protein synthesis. Approximately 0.15 - 1% of leaf dry mass is Mg (Jones et al. 1991). Deficiency symptoms include yellowing of leaves or interval chlorosis beginning in older leaves. Magnesium is known to interact with Ca and K. The interactions

stem from all three elements being strong cations. Magnesium is the weakest of the three and as such, excess of this element rarely causes deficiency of the other two.

Sodium (Na) is usually considered a beneficial mineral element to certain plants and only essential to those which utilize the C4 and CAM photosynthetic pathways (Welch 1995). Since it is not essential to all higher plants, Na is not considered an essential mineral element, although some authors may treat it as such. Mehra and Farago (1994) suggest that Na is a competitor with K in plant nutrition. Sodium augments K when levels are low and can inhibit K deficiency symptoms. However, it can become toxic at higher levels and cause K deficiency.

Phosphorus (P) is a constituent of every living cell (Mehra and Farago 1994). Its main role is in the energy cycle as P is a component of the energy molecule, ATP. Phosphorus is also a major component of enzymes, proteins, RNA, DNA and phytin (Jones et al. 1991). It comprises 0.15 - 1.0 % of leaf dry mass. Highest concentrations are found in new leaves. Deficiency symptoms include: slow growth, dark green colour with older leaves appearing purple and accumulation of sugars and starch in tissues. Excess P leads to deficiency in iron and zinc.

Sulphur (S) is involved in protein synthesis and is part of the amino acids cystine and thiamine (Jones et al. 1991). Sulphur is active in structure and metabolism of plants and often a component of secondary metabolites. Sulphur may play a role in reducing the incidence of disease in plants (Jones et al. 1991). Dry mass of leaves range from 0.15 - 0.5 % S. Deficiency symptoms include: yellowing of leaves and accumulation of inorganic nitrogen in plant tissues (Mehra and Farago 1994). An excess of S is thought to interfere with iron uptake and may cause premature senescence of leaves.

The concentration of each mineral changes over the season (Mattson and Scriber 1987). Nitrogen, P and K show an invariant exponential decay over the entire growing season. Iron and Na have a concave concentration curve, rapid decay over the first part of the season, followed by and increase in the latter part of the season. Calcium and Mg, on

the other hand often show a steady linear increase over the growing season. However, there is much variation in these patterns depending on plant species and soil conditions.

Mineral nutrients are taken up by the roots as inorganic ions and chelates from the soil solution (Marschner 1983). Being separated from regions of photosynthesis, transport of minerals to aerial portions of the plant is necessary. Therefore the uptake and distribution of these minerals is a major part of plant nutrition. Xylem usually transports minerals, in their inorganic form, to the leaves. Phloem usually transports assimilates; however, minerals in inorganic form are plentiful in the phloem as well (Mehra and Farago 1994).

Transport or fluid movement in the xylem is a response to transpiration. However, when transpiration is too low, ions are still able to flow upwards into the stems from areas of high concentration to low concentration. Uptake by roots and rate of transport in xylem of minerals such as P and K are controlled by shoots rather than roots (Marschner 1983). Selective removal of some ions (e.g. Na) while in xylem often occurs through special transfer cells which remove ions from xylem sap and load them into the sieve elements of the phloem. Micronutrients are 4 - 17 times higher in concentration in phloem than xylem indicating that phloem may be more important in transporting these elements.

Not all minerals move through the phloem at the same rate (Marschner 1983). For example, K is considered a phloem-mobile element. It moves easily in the sieve tubes and is found in relatively high concentrations. Calcium, in contrast, is considered immobile and relatively rare in the phloem (Peel 1972). The heavy metal micronutrients (e.g. Fe) fall between K and Ca having moderate phloem mobility. Xylem is critical for the distribution of Ca because of its low phloem mobility.

B. Physiological Sinks

A 'sink' is an organ of a plant which requires more assimilates for growth than it can produce and the converse definition applies to sources (Clifford 1992). Sinks include flowers, fruits and meristems, while the best example of a source is a mature

photosynthesizing leaf. However, every plant organ is a sink at some stage of its development. Vascular bundles of phloem and xylem integrate sinks with sources and although there is considerable information on the operation of individual cells and bundles, regulation of photosynthate in an integrated plant system is poorly understood (Wardlaw 1990). The distance between sources and sinks, the strength of the sinks themselves, the supply of assimilates produced by the sources, the demand for photoassimilates by the sinks and the storage capacity of leaves are all factors that can potentially influence the regulation of photosynthate partitioning (Wardlaw 1990). Furthermore, sinks can cause photosynthesis to increase or decrease, alter the rate of root absorption, regulate which sources deliver to which sinks and regulate the entire plant to produce and deliver to them the assimilates they require (Clifford 1992). Thus, the flux of assimilates is a whole-plant property and can only be predicted from measurements made simultaneously in source leaves, sink organs and the transport system in-between (Farrar 1996).

Traditionally, the terms source and sink were used in reference to photoassimilate partitioning; however, authors have recently broadened the use of these terms to describe the partitioning of mineral nutrients and heavy metals in plants (Abrahamson and Weis 1987, Bagatto et al. 1991, Paquette et al. 1993, Bagatto and Shorthouse 1994a). With this broadening of the definition of a sink has come its use as a comparative term implying greater levels of the substance being measured in the sink tissue than some comparable organ or tissue. The more traditional definition of the term sink does not include this qualifier; sinks do not necessarily attract greater amounts of nutrients than any other plant organ.

III. Biology of *Diplolepis spinosa* and *Diplolepis triforma* and their Galls

A. Biology of the Genus *Diplolepis*

The genus *Diplolepis* belongs to the cynipid subfamily Cynipinae, all of which induce galls (Askew 1984). Adult *Diplolepis* are small, inconspicuous wasps ranging from 3 - 6 mm in length with colouration varying from entirely orange-red to reddish-brown and black to entirely black (Shorthouse 1993). The main character used to distinguish *Diplolepis* from other cynipids is the plowshare-shaped hypopigium (modified eighth metasomal tergite) (Kinsey 1920). The genus is restricted to the Holarctic region but no single species is circumpolar in distribution (Shorthouse 1993). Approximately 10 species occur in the Palearctic region (Dalla Torre and Kieffer 1910; Eady and Quinlan 1963) and 28 species in the Nearctic region (Burks 1979). Two species from the Palearctic region (*D. rosae* (L.) and *D. mayri* (Schlect.) have been introduced to the Nearctic region (Burks 1979).

Diplolepis species are restricted to inducing galls on various organs of the genus *Rosa* and as a result they are categorized as being either leaf-, stem-, or root-gall inducers. The galls of some species are single-chambered or monothalmous, whereas others are multi-chambered or polythalmous (Shorthouse 1993). Most species of *Diplolepis* can induce galls on more than one species of *Rosa*. For example, *D. eglanteriae* (Hartig) is known to induce galls on 7 species of *Rosa* in England (Niblett 1943). The Nearctic species of *Diplolepis* are restricted to 12 endemic species of *Rosa* (Shorthouse 1993); however, several species including *D. spinosa*, *D. radicum* (Osten Sacken) and *D. polita* (Ashmead) also induce galls on the domestic shrub rose *Rosa rugosa* Thunb. (Shorthouse 1988, 1994).

Like all other gall-inducing insects, those in the genus *Diplolepis* cause the formation of an atypical plant structure which provides the immatures with shelter and high

quality food. The gall inducers solicit a wounding response by tissues of the host organ resulting in affected cells becoming the centre of a morphogenic field that overrides normal developmental events (Rohfritsch and Shorthouse 1982). Although adult females may deposit substances at oviposition and their eggs cause some cell lysis and division among adjoining cells, the stimulus for gall formation comes exclusively from the feeding larvae (Rohfritsch 1992). It is also known that cynipid larvae release fluids as they feed; however, it is thought that variation in the physical contact with cells by the larvae is responsible for the structurally distinct galls induced by each species (Magnus 1914, Meyer and Maresquelle 1983).

Galls of all cynipids are classified as prosoplasmic which means that they have characteristic external forms and tissues differentiated into well-defined zones, in contrast to the more primitive kataplasmas which are characterized by little tissue differentiation and lack of constant shape (Dreger-Jauffret and Shorthouse 1992). It has also been suggested that the wide variety of gall structures may be an integral part of the defensive strategy that benefits gall inducers by preventing generalist enemies from developing stereotyped search-and-attack behaviours (Cornell 1983).

The life cycles of all species in the genus *Diplolepis* are similar (Shorthouse 1993). *Diplolepis* are univoltine and overwinter inside their galls in the prepupal stage. The pupal stage lasts approximately 15 days and occurs once diapause has been broken. The adults emerge inside their galls, chew a tunnel to the outside, and immediately search for host plant tissues suitable for oviposition (Shorthouse 1982). Adult females have viable eggs at the time of emergence (Schröder 1967) and mating is not necessary as females can produce both male and female offspring parthenogenetically (Shorthouse 1993). Kinsey (1920) concluded that only primitive cynipids reproduce sexually.

Emergence is synchronized with the stage of host plant development optimum for gall initiation. *Diplolepis* adults live for only 3 - 5 days outside their galls (Kinsey 1920); however, most populations have an extended period of emergence enabling them to overlap

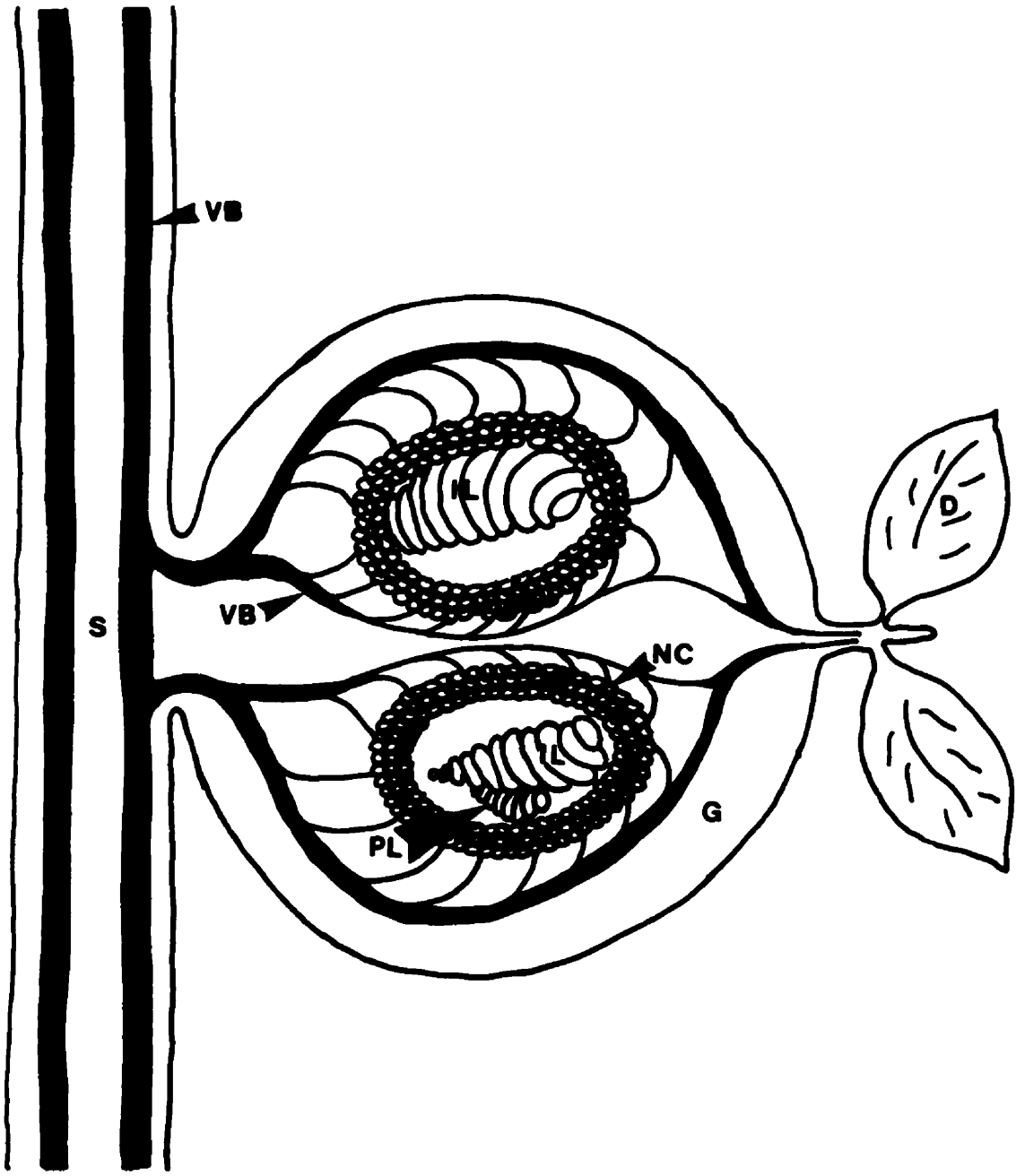
the period of tissue susceptibility (Shorthouse 1992). Eggs are laid in the immature tissues of the host plant organ. The egg stage lasts from 7 - 15 days and the galls are considered to be initiated once the larvae begin to feed (Shorthouse 1982). Cells nearest the larvae become structurally and physiologically modified forming the cytoplasmically dense cells referred to as nutritive cells (Bronner 1992). These cells contain no starch but are rich in small lipid droplets, enzymes and soluble compounds such as glucose, sucrose, and amino acids (Bronner 1992). Nutritive cells line the inside surface of all chambers throughout gall growth and are not only the sole source of food for the larvae, but also serve as the source of all other cells that form the gall. Each larva is encapsulated in its own chamber as they feed on nutritive cells. Like all cynipids, *Diplolepis* have five larval stages (Shorthouse 1993).

Besides initiation, there are two phases in the development of galls of *Diplolepis* referred to as growth and maturation. Gall growth is the phase when biomass of the gall is vastly increased by division and enlargement of both the gall cells and the layer of nutritive cells. Vascular bundles appear within gall tissues soon after initiation (Bronner 1992) and are joined to those of their host organ (Fig. 1). All assimilates and nutrients pass from the host plant into the gall via these bundles and their movement is under the control of the larvae. Larvae feed only minimally during growth phase, but it is important to recognize that both the growth of the gall and physiological movement of assimilates and nutrients occurs only as long as the larvae are alive and active (Rohfritsch 1971). That is, within hours of an inducer being killed, growth and differentiation of gall tissues ceases and assimilates and nutrients are no longer directed into the gall.

Maturation phase occurs while the larvae are in the last instar. This is the main trophic phase of cynipids as they actively feed on the masses of nutritive tissues that have accumulated around them (Rohfritsch 1992). Cynipid larvae do not consume entire nutritive cells, but rather their mouthparts tear into the cells and they imbibe liquids by

Figure 1. Generalization of a *Diplolepis* gall in cross section showing vascular bundles, nutritive cells, an inducer larvae and a parasitized inducer larvae.

D, tissue distal to the gall; G, gall; IL, inducer larva; NC, nutritive cells; PL, parasitoid larva; S, stem.


VB
VB
S
L
D
NC
L
G
PL

sucking movements. The intestine of cynipid larvae is blind and wastes accumulate within the gut (Gauld and Bolton 1988). As nutritive cells are consumed, adjoining parenchyma cells, most of which contain starch granules, quickly develop cytological features of nutritive cells and in turn are fed upon by the larvae (Rohfritsch 1992). This process continues until the chambers of mature larvae are lined with collapsed cells. A sheath of sclerenchyma cells often develops around the layers of nutritive cells and is infiltrated by a network of vascular tissues which connect to the vascular system of the host plant (Rohfritsch and Shorthouse 1982). The sclerenchyma sheath is synthesized only if the larvae are actively feeding. If the larvae are killed just before gall maturation, the sclerenchyma does not differentiate (Rohfritsch 1992). Defecation occurs prior to pupation after feeding is complete and as a result, the larvae avoid fouling their chambers throughout gall development (Shorthouse 1993). The gall and the larvae are mature in approximately 2-3 months and the larvae enter the prepupal stage in the fall (Shorthouse 1980).

The growing tissues of galls of cynipids function in similar ways to those of normal plant organs. That is, masses of cells proliferate and enlarge and are fed nutrients from the host plant through a network of vascular bundles. A major difference is that the development of galls is under the control of the insects within and not the host plant itself. However, the main functions of galls are to shelter the larvae from abiotic factors, provide a certain amount of protection from natural enemies, and provide a continuous supply of high quality food. Indeed, cynipid larvae exert a mobilizing effect on their host plants, drawing food materials to the gall. According to McCrea et al. (1985), galls affect carbon flow through the host plant by either blocking or actively redirecting the normal flow of resources, thus, accumulating sugars, lipids, and other nutrients. Price et al. (1987) proposed that the function of galls is to provide a superior food resource for gall inducing insects compared to the ungalled tissues and called this the "nutrition hypothesis of gall evolution."

Galls of cynipids are susceptible to attack by various species of minute wasps that feed either on the larva of the inducer or on tissues of the gall and it is common for them to kill over 90% of an inducer population (Askew 1984). Those that feed on the inducers are referred to as parasitoids and those that feed on gall tissues are referred to as inquilines. Cynipid galls are apparent, predictable resources for natural enemies and the assemblage of these inhabitants result in component communities (Claridge 1987). Most parasitoids attacking cynipids deposit eggs early in gall development and interactions between the resulting larvae occur throughout the summer until the final composition of the community is established by autumn. It is this assemblage that re-establishes the community the following year. There is also considerable fluctuation from year to year, and from site to site and it is clear that parasitoids and inquilines play an important role in the regulations of the gall-inducer population sizes and densities (Cornell 1983). Most parasitoids and inquilines overwinter in their host galls and exit by chewing their way free the following spring. *Diplolepis* larvae are most commonly attacked by chalcids in the genera *Eurytoma* Illiger and *Torymus* Dalman (Shorthouse 1993) and inquilines of the genus *Periclistus* Förster (Brooks and Shorthouse 1997). Parasitoids of *Diplolepis* larvae are referred to as koinobionts. Koinobionts, in contrast to idiobionts, which permanently paralyze or kill their hosts before the egg hatches, do not kill or paralyze the attacked larva at oviposition (Askew and Shaw 1986). Instead, koinobionts keep the inducer alive until it matures at which time it is consumed (Bagatto and Shorthouse 1994a).

B. Biology of *Diplolepis spinosa* and its Gall

Diplolepis spinosa is a small (4 mm in length) reddish brown and black wasp (Shorthouse 1993). It induces galls on the stems of the wild rose *R. blanda* and the domestic rose *R. rugosa* in central Canada. Galls of *D. spinosa* are also found on *R. woodsii* Lindl. in western Canada (Shorthouse 1993). *D. spinosa* was named by Ashmead (1887) after observing the gall; *Rhodites multispinosus* Gillette (1890) is a junior synonym

(Shorthouse 1988). More recent descriptions of *D. spinosa* and its gall are in Shorthouse (1993) and Bagatto and Shorthouse (1994a).

Galls of *D. spinosa* are spherical (Fig. 2), polythalamous, average 23 mm in diameter when mature and contain an average of 17 inducers per gall (Bagatto and Shorthouse 1994a). Mature galls are hard woody structures, covered in stout spines, and often remain attached to the plant after the inducers have exited. The inhabitants are distributed in a sphere midway to the centre of the gall (Fig. 3).

Adults of *D. spinosa* exit from early May to late June. Matings have been observed both in the field and in the lab and the sex ratio (F/F+M) averages 0.65 (Shorthouse 1993). Females oviposit in immature lateral or terminal leaf buds. Buds at this stage consist of tightly rolled, partially developed leaves enclosing the apical meristem (Shorthouse 1993). Sometimes more than one female will oviposit into a single bud resulting in galls with the offspring of each in the same gall. Each egg is attached to an epidermal cell at a leaf internode (Shorthouse 1993). Cytological differences between galled and ungalled leaf buds appear within 14 days post oviposition. Galled buds are stunted, wider and spinier when compared to ungalled buds (Shorthouse 1993).

Larvae of *D. spinosa* are attacked by several species of parasitoids (Bagatto and Shorthouse 1994a). The most common species are *Eurytoma spongiosa* (Bugbee), *Orthopelma occidental* Ashmead, and one of *Torymus solitarius* (Osten Sacken), *T. bedeguaris* (L.), *T. flavacoxa* Osten Sacken, or *T. chrysochlorus* (Osten Sacken); less common inhabitants are at least one species of *Aprostocetus* Westwood and *Pteromalus* Swederus. In all cases, the parasitoids emerge from previous years galls within 2 - 4 weeks of the gallers and oviposit within immature galls. Parasitoids hatch and feed only after the gall inducers have attained approximately one quarter full size (Bagatto and Shorthouse 1994a).

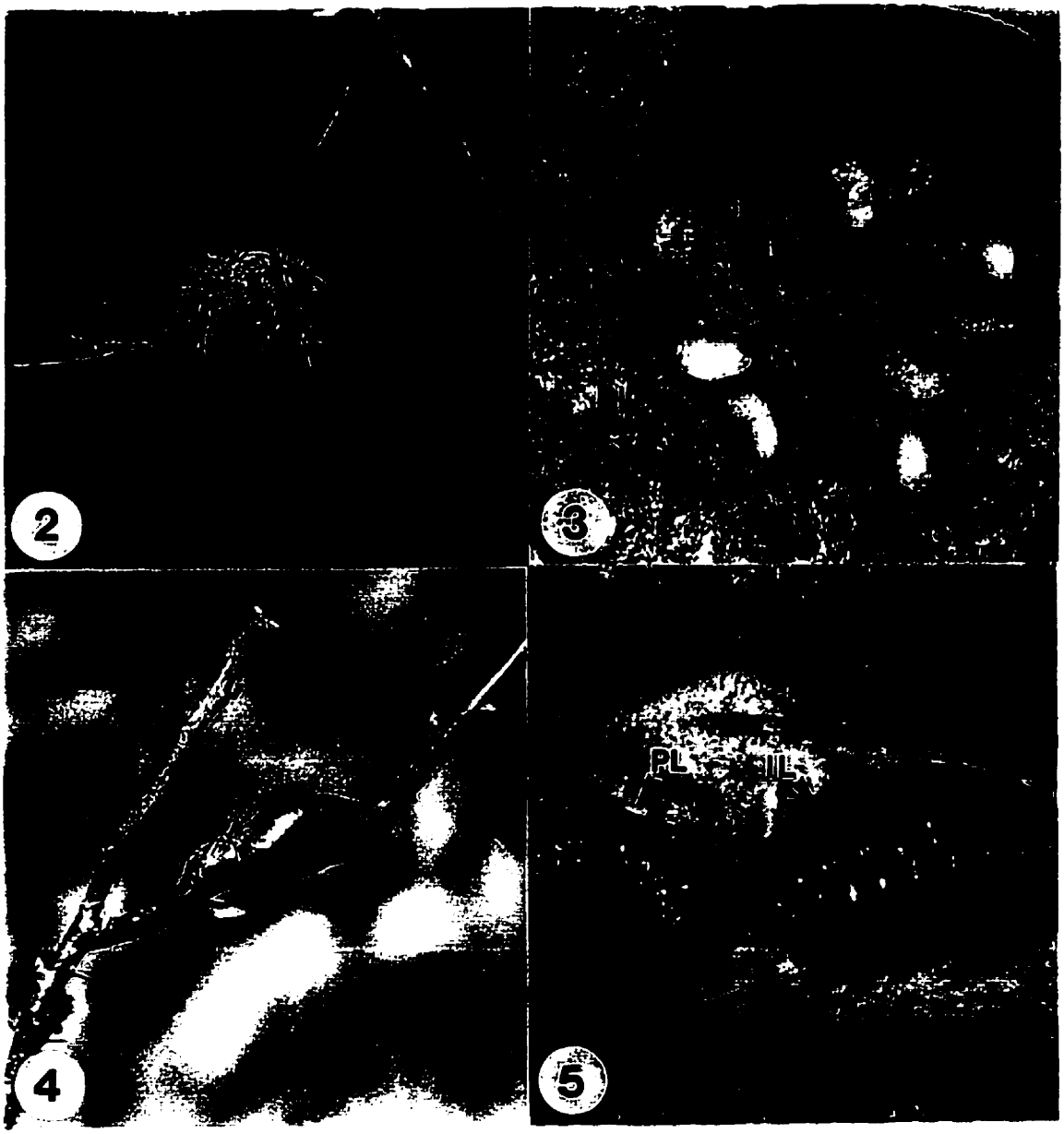
Figure 2. Habitus of a gall of *Diplolepis spinosa*.

Figure 3. Dissection of a mature gall of *Diplolepis spinosa*.

Figure 4. Habitus of a gall of *Diplolepis triforma*.

Figure 5. Dissection of a mature gall of *Diplolepis triforma*.

IL, inducer larva; PL, parasitoid larva.


2
3
4
5

C. Biology of *Diplolepis triforma* and its Gall

Diplolepis triforma and its gall were described by Shorthouse and Ritchie (1984). Adult female *D. triforma* are slightly smaller than *D. spinosa*, averaging 3.4 mm in length (Shorthouse and Ritchie 1984). They are usually completely black except for the clypeusand metasoma which are red. *D. triforma* only induce galls on the stems of *R. acicularis* (Shorthouse and Ritchie 1984). *D. triforma* was so named because it induces three structurally different galls. All are woody and generally ovoid in shape; however, one form has no distal growth, another has some twig growth and a third appears to be a swollen otherwise normal stem (Fig. 4). The latter two forms are covered in stout spines. The difference in gall forms is a result of the maturity of the leaf bud at oviposition (Shorthouse and Ritchie 1984). The first form results from eggs deposited in buds with unfolded leaves. The second form results when oviposition is in buds with most of their leaves unfolded. The third form occurs when females oviposit directly into developing stem tissue. Galls are polythalmous (Fig. 5), averaging approximately 10 inducers per gall (Shorthouse and Ritchie 1984).

The life cycle of *D. triforma* is similar to other *Diplolepis* (Shorthouse and Ritchie 1984). The wasps pupate for 5 - 10 days in the spring then exit from early May to early June. Although the sex ratio for *D. triforma* is 0.78, matings have not been observed (Shorthouse and Ritchie 1984). Eggs are laid in a similar manner to *D. spinosa* and signs of gall development appear 10 days post oviposition (Shorthouse and Ritchie 1984). Larvae of *D. triforma* are attacked by many of the same parasitoids that attack *D. spinosa*. Most common are *E. spongiosa* and an unidentified species of *Torymus* (Shorthouse and Ritchie 1984).

IV. Study Site

All data for this study were obtained from a field of approximately 7 hectares located 45 km northwest of Sudbury, Ontario (46°35'N, 81°12'W). This agricultural field which was abandoned approximately 20 years ago has since become dominated by a shrub layer of *R. blanda* and *R. acicularis.* Other common plants include Devil's Paintbrush (*Hieracium aurantiacum* L.) and patches of shrub willows (*Salix* spp.). A forest community composed mainly of poplars (*Populus* spp.) and pines (*Pinus* spp.) surrounds the field. In addition to *D. spinosa* and *D. triforma, D. fusiformans* (Ashmead), *D. nodulosa* Beutenmüller, *D. polita, D. rosaefolii* (Cockerell), *D. bicolor* (Harris), *D. radicum* and *D. nebulosa* (Basset) were all found at this site.

V. Occurrence of Nitrogen Within Tissues of Galls of *Diplolepis spinosa* and *Diplolepis triforma* and Ungalled *Rosa blanda* and *Rosa acicularis*

A. Introduction

Nitrogen (N) is a major component of all organisms and the role which N plays is largely the same regardless of taxonomic boundaries (Lewis 1986). Molecules ranging from DNA to individual amino acids in proteins and enzymes are all N-based. Furthermore, the energy required for all functions of life is dependent on N-based molecules such as ATP and NAD. The importance of N to all organisms is undeniably enormous.

Next to carbon, N is the most concentrated element in organisms (White 1993). It is also a common element in the earth's crust and the atmosphere. However, despite its abundance, N is a limiting nutrient to most organisms (White 1993). Atmospheric N, which comprises roughly 7% of the total N on earth, is in the form of N_2 gas, an inert molecule unavailable to plants and animals (Lewis 1986). Approximately 93% of the remaining N on earth is bound in the earth's crust and equally unavailable to organisms. The amount of N remaining in the biosphere is approximately 0.0025%, of which less than 4% is in a form available to plants.

Biologically available forms of N (nitrate, nitrite and ammonium) are produced by bacteria in the soil and taken up by plant roots to be converted into organic molecules such as proteins. Compared to animals, which are roughly 7 - 14 % N by dry mass, N requirements of plants are low as most of their tissues are composed of carbohydrates (Mattson 1980). Dry mass of plants can be 1.5 - 6 % (15 - 60 mg/g) N (Jones et al. 1991);

however, it is the primary nutrient in limited supply that affects the growth and productivity of plants (Fernandes and Rossiello 1995).

Animals have much greater N requirements than plants since the primary building blocks of animals are proteins (Mattson 1980, Mattson and Scriber 1987). Animals are also less efficient in metabolizing N than are plants. Furthermore, animals excrete N daily as waste and so must continuously replenish their supplies. Thus, animals take in, contain, and lose considerably more N than plants.

Herbivores must obtain all their N from plants either directly or indirectly through symbionts. Since plants have less than half the concentration of N in animal tissue, and animals are only about 50% efficient at ingesting N, herbivores must consume more than four times as much food matter to support their own biomass (Mattson 1980). However, this is a simplified generalization which does not take into account that N in plants is in various forms, some of which are unusable and some are even toxic (Mattson and Scriber 1987). The toxic forms of N, such as alkaloids, affect the digestibility of food and increase the need for usable N. Often as the amount of total N in a plant increases, alkaloids increase respectively (Bently and Johnson 1991).

Phytophagous insects are particularly disadvantaged with regard to obtaining adequate N. Insects grow relatively rapidly and so require a high N-to-energy ratio in their food compared to mammals (Schoonhoven et al. 1998). For example, larvae of the cabbage butterfly require a 1:1 ratio of N to glucose in their food for optimal development while the same ratio for a domestic cow is 1:7 (Bernays 1982). Therefore, the nutritional value of a plant to an insect is primarily determined by N content. The procuring of sufficient N to survive and reproduce is a constraint on most phytophagus insects that has been demonstrated many times. Excellent reviews of these studies are provided by White (1984, 1993), McNeill and Southwood (1978), and Mattson (1980).

To date, White (1993) wrote the most comprehensive review of the ecological importance of N. He provided convincing evidence from many forms of life: plants,

insects, crustaceans, mollusks, mammals, birds, reptiles and fish, that N is the most limiting nutrient in the environment. He took this idea further to argue that all ecological interactions exist for one reason: to procure enough N to survive and reproduce, and that N, or the lack thereof, is the primary driving force of evolution. Furthermore, White (1993) considers the position of herbivores in food chains pivotal since they must convert food with low N concentrations into their own tissue.

McNeill and Southwood (1978) discussed three general ecological patterns illustrating that phytophagus insects are limited by inadequate sources of N in their environments: 1) growth and reproduction of phytophagus insects is influenced by quality and quantity of proteins and amino acids in their diets; 2) they are vulnerable to low levels of usable N in their hosts; and 3) plants use non-available N as defenses against herbivory in an evolutionary "arms race". Mattson (1980) illustrated that herbivores are affected by variation in the concentration and quality of N among species of plants, organs of plants, seasons and life cycles. He also discussed the numerous adaptations herbivores have evolved to deal with these variations including protracted feeding periods and life cycles, modification of the digestive system (microbial symbionts), dependence on external microorganisms, occasional carnivory, switching food sources, evolution of a larger body size, and regulating plant physiology.

Insects capable of inducing galls are thought to have overcome nutritional problems faced by external feeders by influencing host plant morphology and physiology (Abrahamson and Weis 1987, Price et al. 1987). Furthermore, it has been suggested that concentration of N could be used as an indicator of the phytopathological importance of galls since the most physiologically active plant organs require the most N (Palct and Hassler 1967). The concentration of N in a few galls has been measured in past studies of the nutritive value of gall cells (Molliard 1913, Skuhravy et al. 1980, Andersen and Mizell 1987, Brewer et al. 1987, Hawkins and Unruh 1988, Hartley 1990, Hartley and Lawton 1992) in hopes of finding patterns which would indicate that galls were adaptations for N

acquisition or how detrimental galls are to host plants (Palct and Hassler 1967, Abrahamson and McCrea 1986). Results varied widely, even among closely related taxa (Brewer et al. 1987); however, many gall systems were found to have lower N concentrations than ungalled host tissues (Hartley 1990).

Hartley (1990) and Hartley and Lawton (1992) found that among galls of 16 species of inducers, including two *Diplolepis*, 10 had significantly lower concentrations of N than ungalled tissues and the other six showed no significant differences. Therefore, galls do not seem to regularly accumulate N relative to ungalled host tissues. Furthermore, Hartley and Lawton (1992) demonstrated that the cynipids *Neuroterus quercus-baccarum* L. and *Andricus lignicola* Hartig do not benefit from increased concentrations of N in their galls. Similarly, galls of these species did not accumulate higher levels of N when the host plant was fertilized, even though ungalled leaves showed a significant increase in N concentrations. Rather than refute the nutrition hypothesis, Hartley and Lawton (1992) suggested that galls allow inducers to manipulate plant nutrients for their own benefit, which is essentially the nutrition hypothesis without the assumption that more nutrients implies a better situation for the inducer. This new definition of gall function is similar to the definition of a plant physiological sink (Marschner 1983, Clifford 1992).

The purpose of this chapter is to examine the levels of N within galls of *D. spinosa* and *D. triforma* and to compare these levels with those of ungalled tissues of *R. blanda* and *R. acicularis*, respectively. Results will be used to refute or support the nutrition hypothesis of gall evolution developed by Price et al. (1987) and compared to those of Hartley and Lawton (1992) who experimentally tested the hypothesis. In addition, the influence of inducers and parasitoids on the levels of N within galls of both species will be determined.

B. Materials and Methods

Current year galls of *D. spinosa* and *D. triforma* in the growth phase and ungalled tissues of *R. blanda* and *R. acicularis* were collected from the study site in mid-July and again when galls were in maturation phase in early-September of 1995. Galls were selected by walking through patches of roses and harvesting all galls observed. As each gall was harvested, an analogous ungalled sample was collected meeting the following criteria: current year leaf and stem tissue from a neighboring rose plant (*R. blanda* for *D. spinosa* and *R. acicularis* for *D. triforma*), little or no leaf damage, same location on the plant and plant of equal age and size. All collected tissues were placed in Whirl-pak® bags and stored on ice until they could be frozen at -20 °C upon returning to the laboratory. Tissues remained frozen until they could be processed.

Galls in the growth phase were measured for their maximum diameter (largest gall diameter perpendicular to the shoot) with a digital micrometer (minimum measurement 0.01 mm). Tissue growing distally from the gall (gall distal tissue) was removed and chopped using a razor blade and placed in a separate petri dish. Ungalled tissues of the host plant were chopped with a razor blade and placed in individual petri dishes. All tissues were dried in an oven at 65 °C for 24 hours which was adequate to obtain constant mass. Dried tissues were massed to three decimal places using an electronic balance. Tissues were subsequently ground by mortar and pestle and stored in individual scintillation vials until organic N could be determined by Kjeldahl analysis.

Galls in the maturation phase were dissected and the number of emergence holes and chambers were counted. Gall inhabitants were identified as either parasitoid or inducer and enumerated. Larvae of *Aprostocetus* sp., which exhibit polyembryony, were counted as one larvae if they all inhabited a single larval chamber. Each dissected gall (gall and inhabitants) was placed in a glass petri dish. All tissues were treated similarily as for those of growth phase.

Organic N content of each sample was determined by Kjeldahl analysis at the Grain Quality Laboratory of Agriculture Canada in Ottawa, Ontario. The minimum sample size for analysis was 0.2 g. Growth phase tissues were run in duplicate and so the minimum sample mass required was 0.4 g. If sample masses for any of the tissues were below 0.4 g or 0.2 g (for growth phase samples and maturation phase samples respectively) samples were combined. This was accomplished by adding the smallest sample to the next smallest sample until the desired mass was attained and then using their average mass in subsequent statistical analyses.

At the Ottawa laboratory, samples were weighed onto a sheet of Kjel-Foss Automatic Weigh Paper (A/S N. Foss Electric, Denmark. Part number 221465) using a Sartoris Basic electronic scale (Model #B3105-OKR) with a minimum measurement of 0.001 g. This mass was inputted directly to a spreadsheet (Labtronics, Datatalk) on an IBM PC. Sample and weigh paper were then placed individually into Tecator Kjeldahl digestion tubes with two Kjeltabs (99.9% potassium sulfate, 0.1% selenium, Fisher Scientific Catalog #13-15913) and 12.5 ml of ACS sulfuric acid (BDH Chemical Supply). Digestion tubes were placed on a Tecator Digestion System 1015 Digester in racks of 20 for 45 minutes at 425 °C. The rack was then removed and left to cool for 15 minutes at which time 50 ml of dH_2O was added to each tube. Tubes were then inserted into a Tecator Kjeltec Auto Analyzer 1030 (serial #2809) and ammonia hydroxide was determined with the analyzer set with a conversion factor of 0.876 (standard for organic matter). Output was delivered to the spreadsheet and percent organic N was calculated.

Moisture content of each sample was determined by combining left over samples to make up 2.000 g of tissue. These were weighed before and after drying at 130 °C for 1.5 hours in a Lab Line oven (Model 3511). Percent moisture was calculated as mass before drying less mass after drying, all divided by 2.000 and multiplied by 100. A total of eight measurements (four non-galled tissues and four gall tissues) for growth phase samples and

twelve measurements (six non-galled tissues and six gall tissues) for mature phase samples were made.

Data were statistically analyzed using SPSS 6.1 and Statview 1.03 for Macintosh. All proportion data were arcsine transformed to meet with the assumption of homogeneity of variance. Correlation analyses were performed to determine if fall-exiting parasitoids affected concentration or amount of nitrogen in galls. Since no relationship could be found and few galls had early emergents (evidenced by emergence holes) no further analysis was conducted with these data. An analysis of variance (ANOVA) was performed to compare the concentration of N in the tissues measured. Since homogeneity of variance was not accepted in all cases, a Kruskal-Wallis one-way ANOVA was performed to confirm results. Mean values for the amount of N (mg), concentration of N (mgN/g dry mass), dry mass (g), maximum diameter (mm), number of inhabitants, and percent parasitism (percentage of gall inhabitants which were parasitoids) for galls of both *D. spinosa* and *D. triforma* were compared using a student's t-test. A significance level of $\alpha = 0.05$ was chosen for all ANOVA's and t-tests. Pearson correlation matrices were calculated for the measured parameters of galls collected in September. Correlations greater than or equal to |0.7| were considered significant.

C. Results

The concentration of N in galls of *Diplolepis spinosa* in the growth phase was 10.896 ± 0.899 mgN/g dry mass (mean ± S.E.) (Fig. 6). In contrast, the concentrations of N in tissue distal to the gall and comparable ungalled tissue of *R. blanda* was 15.106 ± 0.462 and 15.264 ± 0.313 mgN/g dry (mean ± S.E.), respectively. The concentration of N in galls of *D. spinosa* in the maturation phase was 7.532 ± 0.484 mgN/g dry mass (mean ± S.E.). In contrast, the concentrations of N in tissue distal to the gall and comparable ungalled tissue of *R. blanda* was 12.648 ± 0.355 and 12.320 ± 0.288 mgN/g dry (mean ± S.E.), respectively. Galls were consistently lower in N concentrations when compared to distal and ungalled tissues and there was a significant decrease in levels of N in all tissues between growth and maturation phase (ANOVA, $F = 35.7$, d.f. = 103, $p < 0.05$).

The concentration of N in galls of *D. triforma* in the growth phase was 13.469 ± 0.854 mgN/g dry mass (mean ± S.E.) (Fig. 7). In contrast, the concentrations of N in tissue distal to the gall and comparable ungalled tissue of *R. acicularis* was 16.390 ± 0.374 and 15.942 ± 0.215 mgN/g dry (mean ± S.E.), respectively. The concentration of N in galls of *D. triforma* in the maturation phase was 12.061 ± 0.549 mgN/g dry mass (mean ± S.E.). In contrast, the concentrations of N in tissue distal to the gall and comparable ungalled tissues of *R. acicularis* was 13.205 ± 0.246 and 11.54 ± 0.509 mgN/g dry (mean ± S.E.), respectively. Galls of *D. triforma* in growth phase were lower in N concentrations when compared to distal and ungalled; however, galls in maturation phase did not have lower N concentrations than ungalled host tissues. There was a significant drop in N levels of all tissues, except for *D. triforma* galls, between growth and maturation phases (ANOVA, $F = 68.1$, d.f. = 96, $p < 0.05$).

The total amount of N in galls of *D. spinosa* in maturation phase was 7.605 ± 1.099 mgN (mean ± S.E.) (Table 1). In contrast, galls of *D. triforma* in maturation phase contained significantly less N, 2.864 ± 0.328 mgN (mean ± S.E.)

Figure 6. Mean concentration of N (mgN/g dry mass ± S.E.) in ungalled *Rosa blanda*, galls of *Diplolepis spinosa* and tissues distal to the galls collected in gall growth and maturation phases.

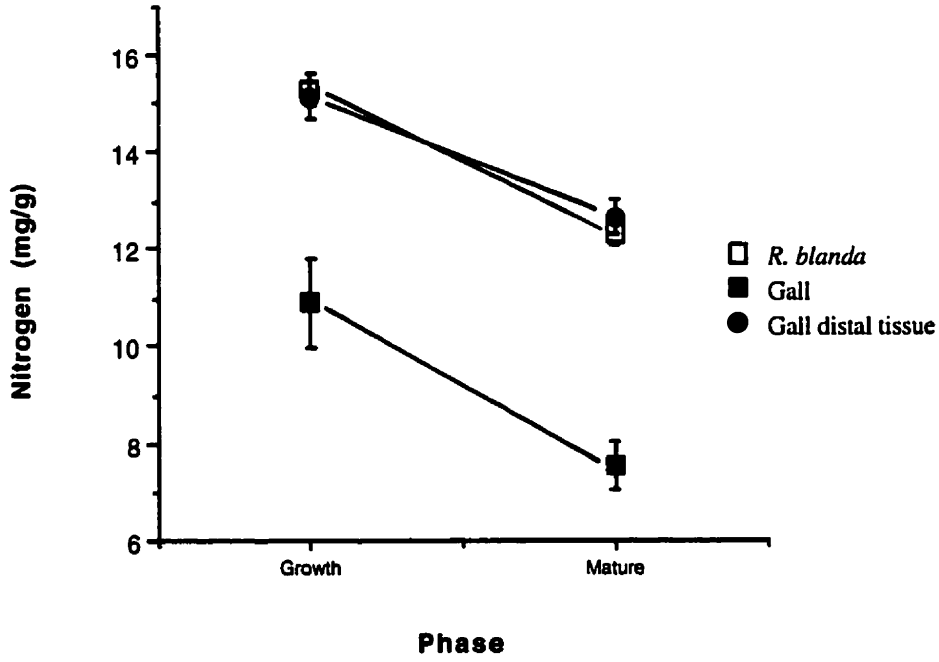

16
14
12
10
8
6
Nitrogen (mg/g)
Growth
Mature
Phase
R. blanda
Gall
Gall distal tissue

Figure 7. Mean concentration of N (mgN/g dry mass ± S.E.) in ungalled *Rosa acicularis*, galls of *Diplolepis triforma* and tissues distal to the galls collected in gall growth and maturation phases.

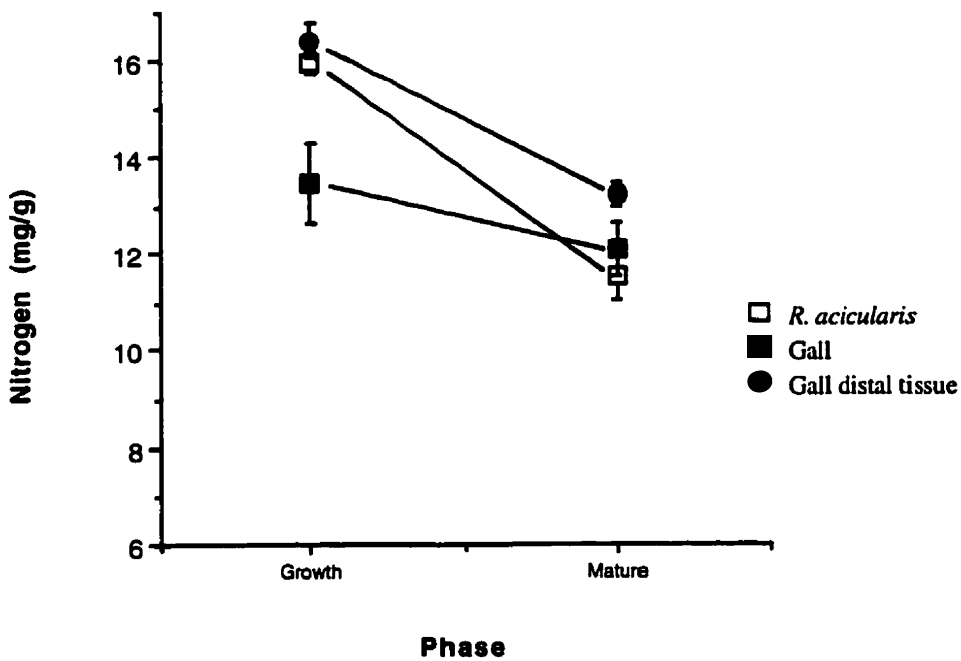

16
14
12
10
8
6
Nitrogen (mg/g)
Growth
Mature
Phase
R. acicularis
Gall
Gall distal tissue

Table 1. Amount (mg) and concentrations of N, dry mass, maximum diameter, number of inhabitants and percentage parasitism of inducers for galls of *D. spinosa* and *D. triforma* in maturation phase. Significantly higher values (2-tailed t-test, d.f. = 54, $\alpha = 0.05$) are indicated by asterisks.

Measurement	Galls of *D. spinosa*	Galls of *D. triforma*
	Mean ± S.E.	Mean ± S.E.
N (mg)	7.605 ± 1.099 *	2.864 ± 0.328
N (mg/g)	7.532 ± 0.484	12.058 ± 0.549 *
Dry mass (g)	1.024 ± 0.139 *	0.230 ± 0.021
Max. Diameter (mm)	18.42 ± 0.83 *	8.76 ± 0.33
Number of Inhabitants	13.204 ± 1.325 *	4.523 ± 0.544
Parasitism (%)	59.371 ± 4.856	69.914 ± 4.405
Observations (n)	27	29

($t = 4.26$, d.f. = 54, $p < 0.05$). However, the concentration of N (mgN/g dry mass) was significantly higher in galls of *D. triforma* ($t = -6.15$, d.f. = 54, $p < 0.05$). Galls of *D. spinosa* had a dry mass of 1.024 ± 0.139 g (mean ± S.E.) which was significantly greater than the mass of *D. triforma* galls which was 0.230 ± 0.021 g (mean ± S.E.) ($t = 5.86$, d.f. = 54, $p < 0.05$). There was a significant difference between the maximum diameter of galls of *D. spinosa* and *D. triforma* ($t = 11.58$, d.f. = 54, $p < 0.05$) which measured 18.426 ± 0.826 and 8.763 ± 0.332 mm (mean ± S.E.), respectively.

The number of inhabitants (inducers + parasitoids) in galls of *D. spinosa* in maturation phase was 13.204 ± 1.325 (mean ± S.E.) (Table 1). The number of inhabitants in galls of *D. triforma* was 4.523 ± 0.544 (mean ± S.E.) and was significantly less than galls of *D. spinosa* ($t = 6.21$, d.f. = 54, $p < 0.05$). The rate of parasitism of larvae of *D. spinosa* in each gall was 59.371 ± 4.856 % while *D. triforma* were parasitized at a rate of 69.914 ± 4.405 %. However, there was no significant difference in the rate of parasitism between the two gall systems ($t = -1.61$, d.f. = 54, $p = 0.113$).

Physical parameters of galls of *D. spinosa* and *D. triforma* in maturation phase, including dry mass (g), maximum diameter (mm), number of gall inhabitants, and parasitism (%) were not strongly correlated (Pearson Correlation Coefficient, $r < |0.7|$) with gall N concentration (mgN/g dry mass) (Tables 2 and 3). However, strong correlations (Pearson Correlation Coefficient $r > |0.7|$) existed between the amount of N (mg) and gall mass (g), maximum diameter (mm), and number of gall inhabitants. The rate of parasitism (%) was not correlated with the amount or concentration of N in galls of either species. Moisture content of tissues analyzed for N averaged 6.3% and 7.3% for growth phase and mature galls of both species respectively in accordance with the standards for Kjeldahl analysis.

Table 2. Pearson correlation matrix for physical attributes of galls of *Diplolepis spinosa* in maturation phase. Significant correlations are indicated by boldface type.

N (mg)	0.352	**0.891**	**0.798**	**0.702**	-0.194
N (mg/g)		-0.060	-0.085	0.014	0.101
Dry mass (g)			**0.929**	**0.758**	-0.290
Max. Diameter (mm)				**0.800**	-0.264
Number of Inhabitants					-0.026
Parasitism (%)					

Table 3. Pearson correlation matrix for physical attributes of galls of *Diplolepis triforma* in maturation phase. Significant correlations are indicated by boldface type.

N (mg)	0.676	**0.885**	**0.735**	**0.718**	-0.240
N (mg/g)		0.276	0.611	0.453	-0.136
Dry mass (g)			0.592	0.663	0.066
Max. Diameter (mm)				0.630	0.027
Number of Inhabitants					-0.065
Parasitism (%)					

D. Discussion

The concentration of N was significantly lower in of galls of *D. spinosa* and *D. triforma* than in ungalled tissues in three of four comparisons while no significant difference was found in the fourth (Figs. 6 and 7). However, a significant seasonal decrease in N levels in *R. acicularis* may have obscured this relationship in Fig. 7. One possible explanation for the substantial decrease in N is that *R. acicularis* has an accelerated phenology compared to *R. blanda* (pers. obs.). Leaves of *R. acicularis* may have been near abscission when collected in September resulting in low N concentrations. The significant decrease in concentrations of N in all tissues, except galls of *D. triforma,* was not surprising since levels of leaf-N in plants are known to decrease over the season, being at their lowest when mature and as senescence begins (Jones et al. 1991). As well, gall tissues had become woody by September. Woody tissues are known to have the lowest concentration of N of all plant tissues (Lewis 1986).

Levels of N reported here for *R. blanda* (13.0 - 15.5 mgN/g dry mass) and *R. acicularis* (11.5 - 16.0 mgN/g dry mass) were lower (Figs. 6 and 7) than those reported in the literature for other species of domesticated roses. The normal levels of N in *Rosa odorata* are between 30 - 50 mgN/g dry mass (Jones et al. 1991) and 30 mgN/g dry mass for 'Royalty' roses (Cabrera et al. 1995). However, these values are for leaves of cultivated roses grown under optimal conditions. In the present study, whole shoots were sampled, rather than leaves, and the roses grew wild without the aid of fertilizer or hydroponics, likely accounting for lower levels of N. When Cabrera et al. (1995) sampled whole, new shoots (analogous with ungalled tissues collected in July), N was present at 19 mgN/g dry mass, much closer to the results reported here.

Although *D. spinosa* and *D. triforma* both induce polythalmous stem-galls on wild roses, their galls are different in many respects (Table 1). Galls of *D. spinosa* are larger (greater dry mass (g) and diameter (mm)) and contain more inhabitants than galls of *D.*

triforma which is a likely explanation for their greater amounts of N (mg). However, galls of *D. triforma* have less mass per gall inhabitant, which explains why concentration of N (mgN/g dry mass) was higher in these galls than those of *D. spinosa*. Furthermore, it is generally believed that evolution of larger gall sizes is a response to parasitoid pressure on gall inducers (Askew 1984); however, the data do not support this theory since there was no significant difference in the percentage of parasitism between these two galls.

It is not unusual for galls of closely related species to have markedly different concentrations of N. For example, Brewer et al. (1987) could find no pattern in the concentration of N (and other nutrients) in five species of cecidomyiid galls, three of which were of the same genus. Furthermore, not only was interspecies variability high, the authors reported high variability from gall to gall. However, mature leaf-galls induced by *D. rosae* and *D. eglanteriae* on *R. canina* were 8.2 and 10.1 mgN/g dry mass respectively (Hartley 1990), which were similar to the results reported for *D. spinosa* and *D. triforma* in this study (Table 1).

It may be that larvae of *D. spinosa* and *D. triforma* were regulating host plants for lower gall-N concentrations (Figs. 6 and 7). An alternative explanation for these results is that host plants cut off the supply of N to the galls; however, results suggest that N must have been transported through the galls to distal tissues at levels equal to ungalled tissues (Figs. 6 and 7). Therefore, lower levels of N in galls of *D. spinosa* and *D. triforma*, relative to their host-plants, were not due to a lack of available host-plant N, nor an ability of the host to stop N from entering the vasculature of the gall. If N is a limiting nutrient for most herbivores (Mattson 1980), the nutrition hypothesis of Price et al. (1987) predicts that N levels in galls should be elevated relative to ungalled host tissues. Thus these results do not support the predictions of Price et al. (1987) at first examination.

In general, results here indicate that galls of *D. spinosa* and *D. triforma* are not adaptations for accumulating high N concentrations (Figs. 6 and 7). These results are in agreement with the findings of Hartley and Lawton (1992) who found that two species of

cynipids regulate galls for lower N concentrations for the provision of increased survivorship. Galls of *D. spinosa* and *D. triforma* seem to regulate the amount of N they receive to the benefit of the inducers as well, evidenced by the highly positive relationship between number of gall inhabitants and amount of N (Tables 2 and 3). This could be a means of avoiding undue stress on the host plant, evidenced by the lower concentrations of N in galls relative to ungalled host tissues (Figs. 6 and 7). These two conclusions suggest that galls are similar to true plant physiological sinks (Section II. B.) and that galls may not be as detrimental to host plants as previously believed (Palct and Hassler 1967). Furthermore, obtaining adequate N is considered the driving force of evolution by some authors (McNeill and Southwood 1978, Mattson 1980, White 1984, White 1993) and it has been suggested that gall inducers, in general, are not N-limited (Abrahamson and McCrea 1986, Hartley and Lawton 1992). Thus, galls of *D. spinosa* and *D. triforma* may have significantly less N than ungalled tissues because the galls provide an environment for inducers where N is not limiting. Therefore, the nutrition hypothesis of gall evolution proposed by Price et al. (1987) has some merit.

It was expected that parasitism of gall inducers would increase the levels of N in galls of *D. spinosa* and *D. triforma* based on work by Abrahamson and McCrea (1986) and Bagatto and Shorthouse (1997). However, the presence of parasitoids in galls had no significant effect on the concentration or total amount of N in galls of either *D. spinosa* or *D. triforma* (Tables 2 and 3). It may be that the amount of N in parasitoids of *D. spinosa* and *D. triforma* is similar to their hosts or the increased amount they require is negligible when compared to the entire gall. Measurements of N concentrations in the larvae themselves and controlled experiments where parasitism of gall inducers is regulated are needed to better determine the effect of parasitism on gall physiology.

In summary, these results support the nutrition hypothesis as stated by Hartley and Lawton (1992); galls regulate the amount of N brought to the gall as a sink organ and that galls are nutritional adaptations. However, results presented here must be interpreted with

caution since key nutrients are likely restricted to the nutritive layer of the galls (Bronner 1992) and so measures of total gall N may be misleading when discussing the requirements of the gall inducers. Similarly, more refined studies may find specific nutrients such as amino acids elevated in galls which would not be detected by the measure of total organic N. Furthermore, the nutritional requirements of gall-inducing insects are unknown, making predictions of nutritional advantages based on gall measurements equivocal at best (Hartley and Lawton 1992).

VI. Occurrence of Ca, Fe, K, Mg, Na, S, and P Within Tissues of Galls of *Diplolepis spinosa* and *Diplolepis triforma* and Ungalled *Rosa blanda* and *Rosa acicularis*

A. Introduction

Phytophagous and entomophagous insects have had to evolve strikingly different behavioural and physiological mechanisms due to the nature of their food. Herbivory may be advantageous compared to entomophagy since there is at least ten times more available plant biomass than predatory insects have in prey (Strong et al. 1984). However, entomophagy has clear advantages in terms of food quality. There are few differences in the composition of predator and prey and thus predacious insects eat a nutritionally well balanced diet. Conversely, the diets of phytophagous insects are typically of different composition than their own bodies. Plants tend to have adequate levels of K, Ca, Mg and Mn but significantly lower concentrations of Na, S, P, N, Fe, Zn, and Cu when compared to insect tissues (Schoonhoven et al. 1998), yet phytophagous insects have the same nutritional requirements as entomophagous insects (House 1965, Hagen 1987).

The nutritional quality of the food of insects is determined primarily by the proportions of essential nutrients rather than absolute amounts of nutrients, provided all essential nutrients are present (House 1969). Thus phytophagus insects must eliminate surplus minerals in their diets until they are restored to optimal balance with more deficient minerals; meaning that excess of essential minerals is wasteful and may even retard insect development. Furthermore, minerals in plants are diluted in a matrix of indigestible cellulose and lignin and a variety of allelochemicals which lower digestibility of plant matter or deter feeding (Schoonhoven et al. 1998). Thus phytophagous insects had to evolve behavioural and/or physiological mechanisms to correct for the discrepancy between the nutrients plants provide and the nutrients insects require.

External feeding, leaf-chewing insects consume large amounts of food since they have little or no control over the physiology of their hosts. Grasshoppers, for example,

consume twice their body weight in food matter daily while developing (Bernays and Barbehenn 1987). This means that external feeders consume and defecate large quantities of nutrients abundant in leaves (e.g. sugars and starches) to obtain dilute nutrients (e.g. nitrogen, Section V). This is a wasteful method of feeding in which the herbivore eats many times its own mass in leaf tissue through its lifetime.

Gall induction is thought to be an adaptation by inducers for obtaining adequate nutrition from an otherwise unbalanced food source (Abrahamson and Wies 1987). However, the control inducers have over the accumulation of mineral nutrients required for larval development is poorly understood. It is assumed that mineral nutrients are attracted to galls and make their way to the nutritive tissue via a rich network of vascular bundles.

Since it is relatively easy to measure the concentrations of minerals within plant tissues, it is not surprising that some students have done so with gall tissues. Bagatto et al. (1991) measured levels of Cu, Zn, Fe, Mn, Ni, Ca, Mg in galls of *D. spinosa* on domestic shrub rose and found that all, except Ca and Mg, were elevated when compared to ungalled tissues. Similarly, Paquette et al. (1993) found galls of *Phanacis taraxcaci* on *Taraxacum officinale* accumulated Mn, Mg, Ca, Cu, Zn, and Fe relative to normal leaves.

Bagatto and Shorthouse (1994a) compared the level of Cu, Zn, Fe, Ca, Mn and Mg in galls of *D. spinosa* collected from two sites, 260 km apart, and from two host plants, *R. blanda* and *R. rugosa*. They found that both site and host had significant influences on the levels of these mineral in the galls. They also found that while parasitism of inducer larvae did not affect gall size, the concentration of many minerals increase in proportion to the number of parasitoids in galls. In another study, it was suggested that the concentrations of minerals in galls will increase with the number of parasitoids since parasitoids cause inducers to feed more than normal as was found for parasitoids of the chalcid galler *Hemadas nubilipennis* (Bagatto and Shorthouse 1997).

Bagatto and Shorthouse (1994a) did not have the opportunity to consider more than one species of *Diplolepis* and they did not compare gall tissues to ungalled tissues. Thus,

the purpose of this chapter is to gain a more detailed understanding of the patterns of mineral movement and accumulation within insect galls and the control gallers and their parasitoids have over host plants. It was hypothesized that the concentrations of the minerals Ca, Fe, K, Mg, Na, P and S would be greater in tissues of galls of *D. spinosa* and *D. triforma* compared to respective ungalled host plant tissues. As well, concentrations and amounts of mineral nutrients within plant tissues protruding from the ends of galls (tissue distal to the galls) of both inducers are measured to determine the level at which minerals are translocated through galls. The concentrations of minerals in inducer larvae will be compared to their parasitoids to determine if parasitoids are accumulating minerals by influencing the feeding behaviour of their hosts. It was hypothesized that levels of minerals in galls will increase with the number of inducers and parasitoids.

B. Materials and Methods

Galls of *D. spinosa* and *D. triforma* and ungalled tissues of *R. blanda* and *R. acicularis* were collected and stored as described in Section V, B. All labware used in preparation of samples was acid-washed in 20% nitric acid and rinsed twice with distilled, deionized water (ddH_2O). Galls and ungalled tissues were prepared for oven drying as described in Section V, B.; however, all galls were dissected prior to drying. Larvae in each growth phase gall were counted and placed in separate petri dishes whereas larvae of inducers and parasitoids from maturation phase galls were separated into individual petri dishes.

Dried tissues were massed to four decimal places using a Mettler 240 Analytical balance and transferred into crucibles and ashed in a muffle furnace at 500 °C. After ashing, samples were washed from their crucibles with two 5 ml aliquots of 100% nitric acid and approximately 5 ml of ddH_2O into 50 ml Pyrex beakers. Beakers were placed on hotplates at a low setting (3/10) and allowed to boil until the nitric acid solution had completely evaporated. The beakers were then removed from heat and an additional 1.5 ml of 100% nitric acid was added if the original sample dry weight was less than 0.5 g, and 3.7 ml of nitric acid was added if the original sample dry weight was greater than or equal to 0.5 g. The sample solution was then filtered through Watman No. 541 filter paper with ddH_2O and made up to either 10 ml (if sample mass was less than 0.5 g) or 25 ml (if sample mass was greater than or equal to 0.5 g) in volumetric flasks resulting in an approximately 15% nitric acid solution. Samples were then transferred into sample tubes for ICP analysis.

All determinations were performed by personnel of the Ontario Geological Survey (O.G.S.) in Sudbury, Ontario using an ICP spectrophotometer. Levels of Ca, Fe, K, Mg, Na, P and S in all samples were measured as parts per million (ppm). Procedural blanks which did not contain biological material were also analyzed with each experimental run. To evaluate the reliability of the analytical methods used in this study, citrus leaf standard

reference material 1572 (National Bureau of Standards) was analyzed and values were compared with the certified values. Furthermore, staff of the O.G.S. analyzed extra reference material including pine needles and calibrated the spectrophotometer regularly. Values of total gall mineral concentrations were calculated to include the combination of both gall and insect tissues.

Data were analyzed statistically using SPSS 6.1 and Statview 1.03 for Macintosh. All proportion data were arcsine transformed to meet with the assumption of homogeneity of variance. Outliers were determined using SPSS 6.1 procedure Examine and removed from further analysis. Ungalled tissues, total gall and tissue distal to the gall, and inducers and parasitoids were compared in terms of the concentrations of minerals in these tissues using multiple analyses of covariance (MANCOVA) with dry mass as the covariate and Tukey's HSD test. Individual one-way ANOVAs were performed to find significant differences among the tissues for each mineral individually. Pearson correlation matrices were calculated for the measured parameters of galls collected in July and September . A correlation was considered significant if greater than or equal to |0.7|.

C. Results

Concentrations of Ca, Fe, Mg, P, and S were significantly higher in ungalled tissues of *R. blanda* compared to growth phase galls of *D. spinosa* (Table 4, Figure 8). There was no significant difference in Na concentration levels in these same tissues while, ungalled *R. blanda* had significantly lower levels of K than galls of *D. spinosa*. Concentrations of Ca, Fe, Mg, Na, and S were all significantly lower in galls of *D. spinosa* relative to tissues growing distal to the galls. There was no significant difference in the levels P in galls or tissues growing distal to the galls. However, levels of K were significantly higher in galls of *D. spinosa* than in both ungalled *R. blanda* and tissues growing distal to the galls. Ungalled tissues of *R. blanda*, growth phase galls of *D. spinosa* and tissue distal to the galls collected in July were all significantly different in mineral composition (MANCOVA Pillais = 0.932, F= 5.86, d.f. = 94, $p < 0.05$).

Concentrations of Ca, Fe, Mg, Na, and S were all significantly higher in ungalled tissues of *R. blanda* compared to maturation phase galls of *D. spinosa*, while concentrations of K were significantly lower and there was no significant difference in the concentration of P (Table 5, Figure 9). Concentrations of Ca, Fe, Mg, Na, and S were all significantly lower in maturation phase galls of *D. spinosa* compared to tissues growing distal to the galls, while there was no significant difference in the levels of K and P between these same tissues. Ungalled tissues of *R. blanda*, maturation phase galls of *D. spinosa*, and tissue distal to the galls collected in September were all significantly different in mineral composition (MANCOVA Pillais = 1.547, F= 22.44, d.f. = 92, $p < 0.05$).

Concentrations of K and S were significantly higher in ungalled tissues of *R. acicularis* compared to growth phase galls of *D. triforma*, while Na was significantly lower and no difference was found in Ca, Fe, Mg, and P concentrations (Table 6, Fig. 10). There were no significant differences in the concentrations of any of the minerals measured between ungalled tissues of *R. acicularis* and tissues growing distal to galls of *D. triforma* collected in July (Table 6, Figure 10). Growth phase galls of *D. triforma* had significantly

Table 4. Mean concentrations of Ca, Fe, K, Mg, Na, P and S and dry mass of ungalled tissues of *R. blanda*, growth phase galls of *D. spinosa* and tissue distal to the galls collected in July.

Measurement	*R. blanda*	Galls of *D. spinosa*	Gall Distal Tissue
	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
Ca (%)	1.197 ± 0.115 [a]	0.310 ± 0.037 [c]	0.637 ± 0.078 [b]
Fe (ppm)	57 ± 4 [a]	29 ± 2 [b]	63 ± 7 [a]
K (%)	0.486 ± 0.024 [b]	0.617 ± 0.029 [a]	0.496 ± 0.033 [b]
Mg (%)	0.385 ± 0.026 [a]	0.135 ± 0.012 [c]	0.227 ± 0.021 [b]
Na (ppm)	228 ± 30 [ab]	183 ± 28 [b]	273 ± 36 [a]
P (%)	0.203 ± 0.010 [a]	0.173 ± 0.006 [b]	0.162 ± 0.009 [b]
S (%)	0.101 ± 0.004 [a]	0.070 ± 0.003 [c]	0.085 ± 0.003 [b]
Dry mass (g)	0.342 ± 0.070 [a]	0.409 ± 0.080 [a]	0.192 ± 0.043 [b]
Observations (n)	24	24	18

Means in rows sharing the same letter designation are not significantly different ($\alpha = 0.05$) as determined using one way ANOVA and Tukey's HSD test.

Figure 8. Relative concentrations of Ca, Fe, K, Mg, Na, P and S in galls of *D. spinosa*, tissue distal to the gall and ungalled *R. blanda* collected in July (circle = gall, square = tissue distal to the gall, and diamond = ungalled tissue). Minerals are shown in order of decreasing concentration within each tissue. Larger font sizes indicate significantly higher concentrations.

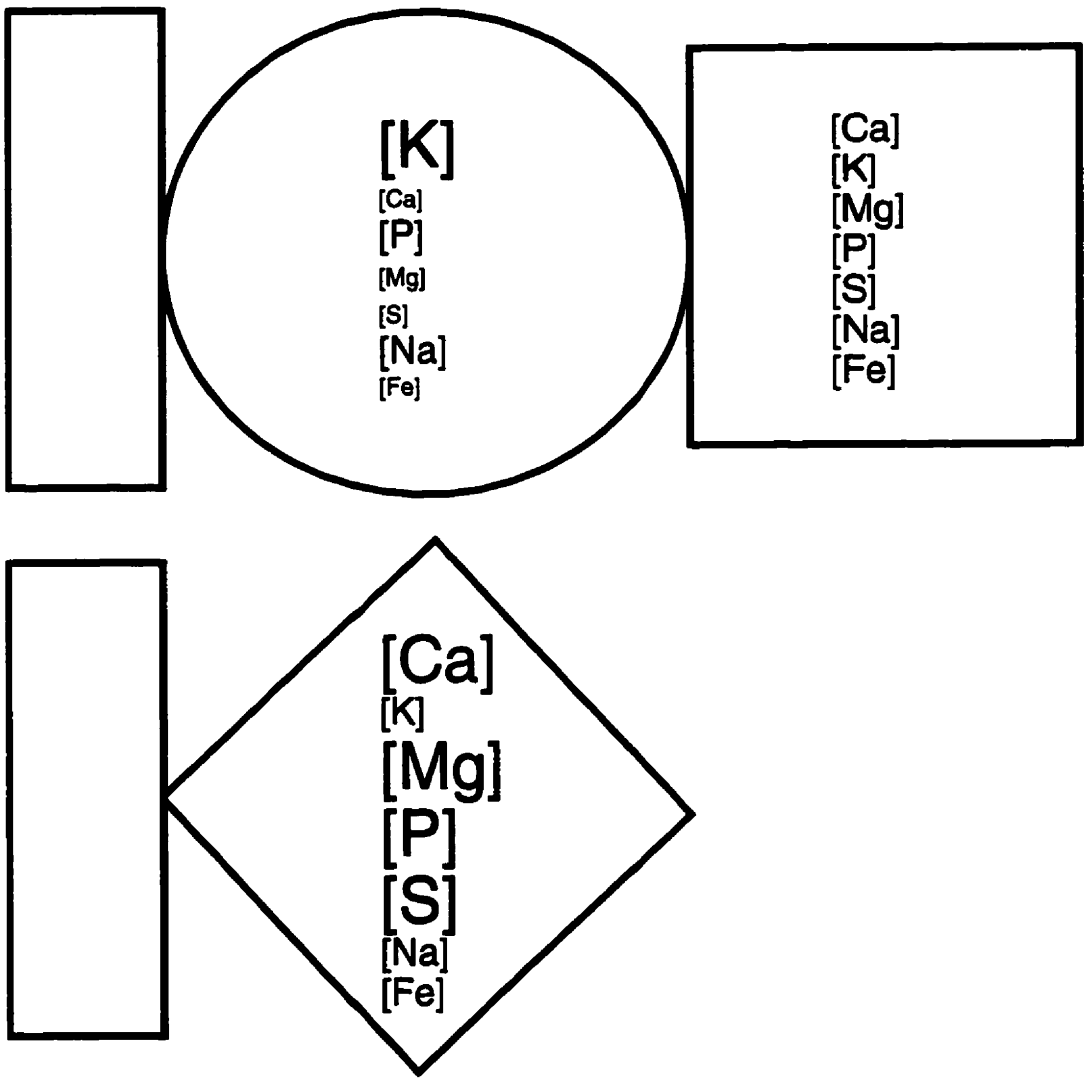

[K]
[Ca]
[P]
[Mg]
[S]
[Na]
[Fe]
[Ca]
[K]
[Mg]
[P]
[S]
[Na]
[Fe]
[Ca]
[K]
[Mg]
[P]
[S]
[Na]
[Fe]

Table 5. Mean concentrations of Ca, Fe, K, Mg, Na, P and S and dry mass of ungalled tissues of *R. blanda*, maturation phase galls of *D. spinosa* and tissue distal to the galls collected in September.

Measurement	*R. blanda*	Galls of *D. spinosa*	Gall Distal Tissue
	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
Ca (%)	0.966 ± 0.045 [a]	0.241 ± 0.025 [c]	0.569 ± 0.053 [b]
Fe (ppm)	52 ± 3 [a]	19 ± 1 [b]	58 ± 6 [a]
K (%)	0.375 ± 0.021 [b]	0.646 ± 0.040 [a]	0.645 ± 0.056 [a]
Mg (%)	0.315 ± 0.019 [a]	0.098 ± 0.006 [c]	0.171 ± .013 [b]
Na (ppm)	130 ± 6 [b]	58 ± 5 [c]	239 ± 33 [a]
P (%)	0.139 ± 0.005	0.123 ± 0.009	0.151 ± .014
S (%)	0.067 ± 0.003 [b]	0.047 ± 0.003 [c]	0.087 ± 0.005 [a]
Dry mass (g)	0.703 ± 0.128 [b]	1.082 ± 0.174 [a]	0.368 ± 0.086 [c]
Observations (n)	27	23	13

Means in rows sharing the same letter designation are not significantly different ($\alpha = 0.05$) as determined using one way ANOVA and Tukey's HSD test.

Figure 9. Relative concentrations of Ca, Fe, K, Mg, Na, P and S in galls of *D. spinosa*, tissue distal to the gall and ungalled *R. blanda* collected in September (circle = gall, square = tissue distal to the gall, and diamond = ungalled tissue). Minerals are shown in order of decreasing concentration within each tissue. Larger font sizes indicate significantly higher concentrations.

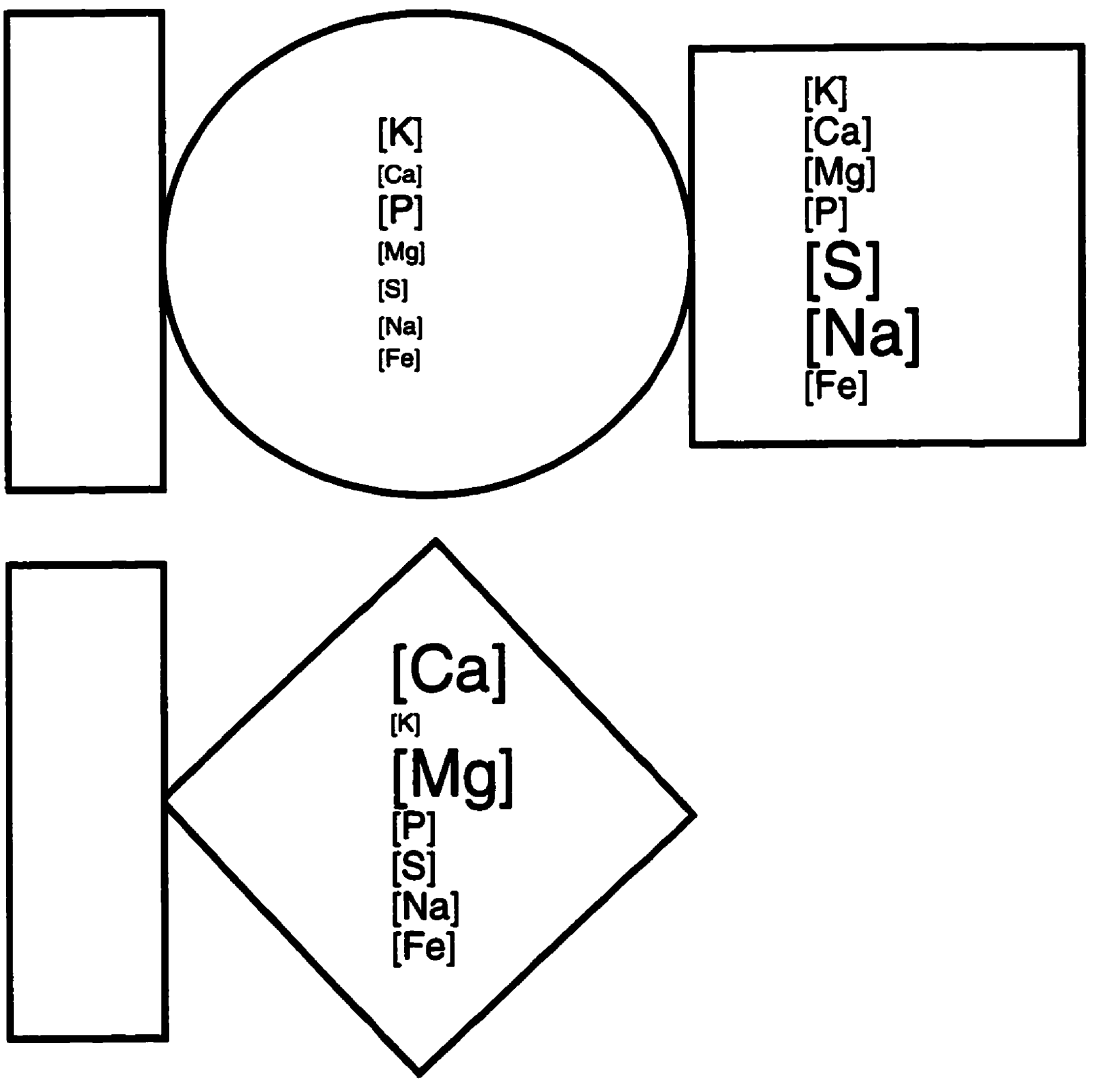
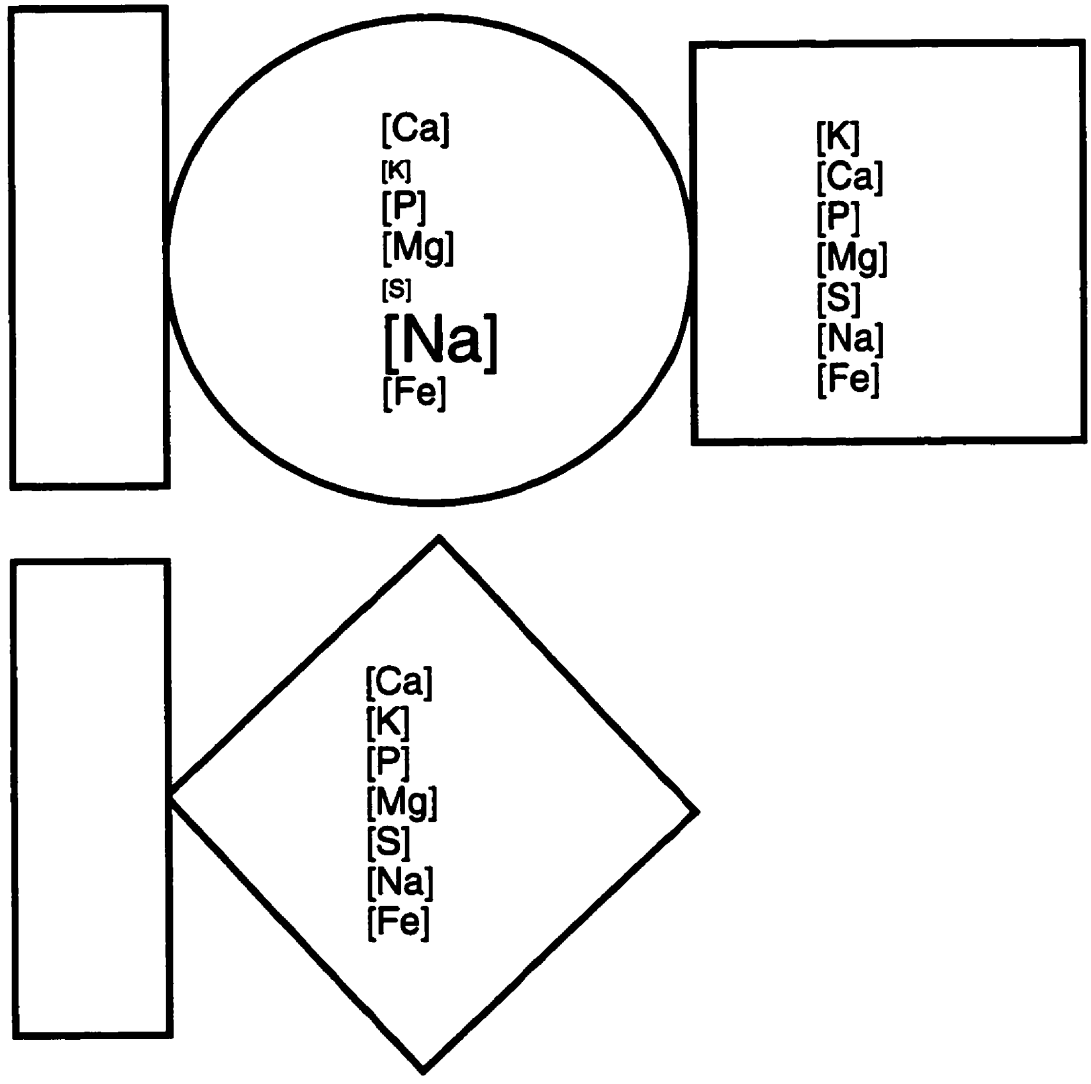

[K]
[Ca]
[P]
[Mg]
[S]
[Na]
[Fe]
[K]
[Ca]
[Mg]
[P]
[S]
[Na]
[Fe]
[Ca]
[K]
[Mg]
[P]
[S]
[Na]
[Fe]

Table 6. Mean concentrations of Ca, Fe, K, Mg, Na, P and S and dry mass of ungalled tissues of *R. acicularis*, growth phase galls of *D. triforma* and tissue distal to the galls collected in July.

Measurement	*R. acicularis*	Galls of *D. triforma*	Gall Distal Tissue
	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
Ca (%)	0.753 ± 0.067	0.712 ± 0.055	0.646 ± 0.054
Fe (ppm)	48 ± 4	52 ± 4	51 ± 4
K (%)	0.622 ± 0.034 [a]	0.508 ± 0.035 [b]	0.712 ± 0.040 [a]
Mg (%)	0.237 ± 0.014	0.224 ± 0.011	0.255 ± 0.012
Na (ppm)	89 ± 11 [b]	285 ± 25 [a]	102 ± 15 [b]
P (%)	0.303 ± 0.022	0.271 ± 0.017	0.296 ± 0.017
S (%)	0.153 ± 0.012 [a]	0.101 ± 0.006 [b]	0.146 ± 0.008 [a]
Dry mass (g)	0.765 ± 0.114 [a]	0.131 ± 0.031 [c]	0.490 ± 0.095 [b]
Observations (n)	26	25	20

Means in rows sharing the same letter designation are not significantly different ($\alpha = 0.05$) as determined using one way ANOVA and Tukey's HSD test.

Figure 10. Relative concentrations of Ca, Fe, K, Mg, Na, P and S in galls of *D. triforma*, tissue distal to the gall and ungalled *R. acicularis* collected in July (circle = gall, square = tissue distal to the gall, and diamond = ungalled tissue). Minerals are shown in order of decreasing concentration within each tissue. Larger font sizes indicate significantly higher concentrations.


[Ca]
[K]
[P]
[Mg]
[S]
[Na]
[Fe]
[K]
[Ca]
[P]
[Mg]
[S]
[Na]
[Fe]
[Ca]
[K]
[P]
[Mg]
[S]
[Na]
[Fe]

lower concentrations of K and S, significantly higher concentrations of Na, and similar concentrations of Ca, Fe, Mg, and P compared to tissue growing distal to the gall. Ungalled tissues of *R. acicularis* and tissue distal to galls were significantly different in mineral composition than growth phase galls of *D. triforma* (MANCOVA Pillais = 0.768, F= 4.63, d.f. = 104, $p < 0.05$).

Concentrations of K, Mg, and S were all significantly higher in ungalled tissues of *R. acicularis* compared to maturation phase galls of *D. triforma,* while Na was significantly lower and there were no significant differences in Ca, Fe, and P concentrations (Table 7, Figure 11). There was no significant difference in the concentrations of Ca and Fe between gall and tissues distal to the galls, while galls had significantly lower concentrations of K, P, Mg and S. Ungalled tissues of *R. acicularis*, maturation phase galls of *D. triforma* and tissue distal to the galls were all significantly different in mineral composition (MANCOVA Pillais = 0.845, F= 4.91, d.f. = 94, $p < 0.05$).

Multiple analysis of variance of the transformed mineral concentrations by tissue type (ungalled *R. blanda* and *R. acicularis*, galls of *D. spinosa* and *D. triforma* and tissues distal to the galls) and phase of gall development (growth and maturation) indicated that significant interactions exist between tissue type and phase of gall development for Ca, K, Na and S (MANCOVA, Pillais = 0.557, F = 3.81, d.f. = 35, $p < 0.05$). Phase of gall development had a significant effect on the concentrations of Ca, K, Mg, Na and P in tissues analyzed (MANCOVA, Pillais = 0.347, F = 15.90, d.f. = 7, $p < 0.05$).

Larvae of *D. spinosa* and their parasitoids were not significantly different in mineral composition (Ca, Fe, K, Mg, Na, P, and S) (MANCOVA Pillais = 0.350, F = 1.233, d.f. = 7, p = 0.342) (Table 8). Likewise, larvae of *D. triforma* and their parasitoids were not significantly different in mineral composition (MANCOVA Pillais = 0.221, F = 0.568, d.f. = 5, p = 0.742). Pooled gall inhabitants (inducers + parasitoids) from galls of *D. spinosa* were significantly different in mineral composition than inhabitants of galls of *D. triforma* (MANCOVA Pillais = 0.496, F = 4.267, d.f. = 6, $p < 0.05$).

Table 7. Mean concentrations of Ca, Fe, K, Mg, Na, P and S and dry mass of ungalled tissues of *R. acicularis*, maturation phase galls of *D. triforma* and tissue distal to the galls collected in September.

Measurement	*R. acicularis*	Galls of *D. triforma*	Gall Distal Tissue
	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
Ca (%)	0.889 ± 0.071	0.830 ± 0.046	1.009 ± 0.065
Fe (ppm)	47 ± 5	44 ± 4	54 ± 4
K (%)	0.374 ± 0.022 [a]	0.230 ± 0.023 [b]	0.444 ± 0.025 [a]
Mg (%)	0.215 ± 0.014 [a]	0.166 ± 0.010 [b]	0.245 ± 0.014 [a]
Na (ppm)	58 ± 4 [b]	191 ± 16 [a]	74 ± 8 [b]
P (%)	0.208 ± 0.013 [b]	0.178 ± 0.013 [b]	0.249 ± 0.017 [a]
S (%)	0.136 ± 0.011 [b]	0.072 ± 0.005 [c]	0.193 ± 0.014 [a]
Dry mass (g)	1.542 ± 0.230 [a]	0.162 ± 0.026 [c]	0.444 ± 0.080 [b]
Observations (n)	26	25	19

Means in rows sharing the same letter designation are not significantly different ($\alpha = 0.05$) as determined using one way ANOVA and Tukey's HSD test.

Figure 11. Relative concentrations of Ca, Fe, K, Mg, Na, P and S in galls of *D. triforma*, tissue distal to the gall and ungalled *R. acicularis* collected in September (circle = gall, square = tissue distal to the gall, and diamond = ungalled tissue). Minerals are shown in order of decreasing concentration within each tissue. Larger font sizes indicate significantly higher concentrations.

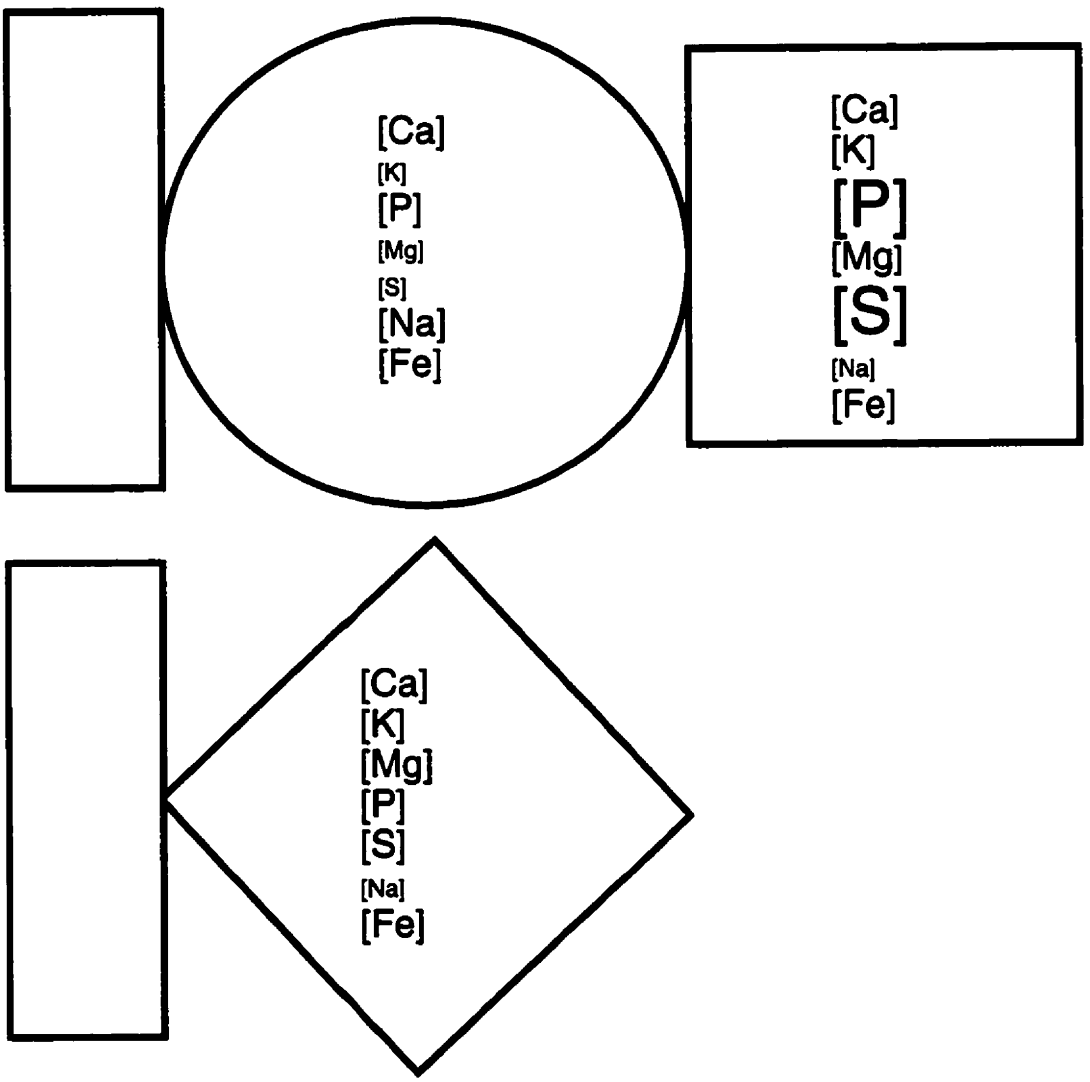

[Ca]
[K]
[P]
[Mg]
[S]
[Na]
[Fe]
[Ca]
[K]
[P]
[Mg]
[S]
[Na]
[Fe]
[Ca]
[K]
[Mg]
[P]
[S]
[Na]
[Fe]

Table 8. Concentrations of Ca, Fe, K, Mg, Na, P, and S; and dry mass of larvae of *D. spinosa*, *D. triforma* and their parasitoids from maturation phase galls.

Measurement	Galls of *D. spinosa*		Galls of *D. triforma*	
	D. spinosa	Parasitoids	*D. triforma*	Parasitoids
	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
Ca (%)	0.341 ± 0.065 ^{b}	0.246 ± 0.030 ^{b}	0.795 ± 0.110 ^{a}	0.677 ± 0.084 ^{a}
Fe (ppm)	341 ± 43 ^{bc}	231 ± 28 ^{c}	547 ± 62 ^{ab}	654 ± 108 ^{a}
K (%)	0.242 ± 0.015	0.241 ± 0.012	0.263 ± 0.021	0.272 ± 0.033
Mg (%)	0.061 ± 0.005 ^{b}	0.064 ± 0.003 ^{b}	0.129 ± 0.020 ^{a}	0.102 ± 0.008 ^{a}
Na (ppm)	2557 ± 428 ^{b}	1095 ± 149 ^{c}	3812 ± 607 ^{ab}	4445 ± 799 ^{a}
P (%)	0.328 ± 0.009 ^{b}	0.323 ± 0.016 ^{b}	0.444 ± 0.033 ^{a}	0.392 ± 0.028 ^{ab}
S (%)	0.248 ± 0.038 ^{a}	0.107 ± 0.013 ^{b}	0.429 ± 0.091 ^{a}	0.364 ± 0.061 ^{a}
Dry Mass (g)	0.0140 ± 0.0040	0.0090 ± 0.0010	0.0020 ± 0.0004	0.0030 ± 0.0010
Observations (n)	18	21	13	17

Means in rows sharing the same letter designation are not significantly different ($\alpha = 0.05$) as determined using one way ANOVA and Tukey's HSD test.

The concentrations of Ca, Fe, K, Mg, P, and S were not highly correlated (Pearson Correlation Coefficient < |0.7|) with the number of larvae (inducers + parasitoids) in either growth or maturation phase galls of *D. spinosa* or *D. triforma* (Table 9). The concentration of Na was highly negatively correlated with the number of larvae in maturation phase galls of *D. spinosa* but not in growth phase galls or galls of *D. triforma* in either phase. The amounts (μg) of Fe, K, P, and S were highly correlated with the number of larvae in growth phase galls of *D. spinosa*. The amounts (μg) of Ca, Fe, K, Mg, P, and S were highly correlated with the number of larvae in maturation phase galls of *D. spinosa*. Mineral concentrations did not correlate highly with the number of larvae in growth or maturation phase galls of *D. triforma* (Table 9). The amounts (μg) of Ca, Fe, K, Mg, P, and S were highly correlated with the number of larvae in growth phase galls of *D. triforma*. The amounts (μg) of Ca, Fe, Mg, and Na correlated highly with the number of larvae in maturation phase galls of *D. triforma*. The rate of parasitism of inducers in maturation phase galls of *D. spinosa* and *D. triforma* (percent of gall inhabitants which were parasitoids) did not correlate highly with the concentration or amount of any mineral measured (Table 9).

Table 9. Pearson correlation coefficients between the concentrations (%, ppm) and amounts (μg) of Ca, Fe, K, Mg, Na, P, and S, and the number of gall inhabitants (larvae) in growth and maturation phase galls. Correlations of percent parasitism of inducers of maturation phase galls of *D. spinosa* and *D. triforma* and mineral concentrations and amounts.

	Galls of *D. spinosa*			Galls of *D. triforma*		
	July	September		July	September	
	Number of Larvae	Number of Larvae	Parasitism (%)	Number of Larvae	Number of Larvae	Parasitism (%)
Ca (%)	-0.535	0.047	-0.019	-0.227	0.176	0.003
Fe (ppm)	-0.471	-0.327	-0.157	-0.483	0.523	-0.009
K (%)	0.117	-0.06	0.078	0.004	-0.563	-0.265
Mg (%)	-0.351	< -0.001	-0.096	-0.085	0.645	0.422
Na (ppm)	-0.5	**-0.787**	-0.03	-0.625	0.260	-0.246
P (%)	-0.496	< -0.001	-0.198	0.135	-0.570	-0.009
S (%)	-0.171	-0.202	-0.059	-0.199	-0.693	-0.058
Ca (μg)	0.141	**0.709**	-0.178	**0.711**	**0.825**	0.232
Fe (μg)	**0.762**	**0.776**	-0.141	**0.768**	**0.798**	0.155
K (μg)	**0.875**	**0.809**	-0.129	**0.825**	-0.109	-0.101
Mg (μg)	0.503	**0.792**	-0.192	**0.768**	**0.822**	0.223
Na (μg)	0.407	0.287	0.043	0.437	**0.835**	0.078
P (μg)	**0.771**	**0.837**	-0.153	**0.862**	-0.043	0.151
S (μg)	**0.804**	**0.824**	-0.096	**0.821**	-0.077	0.115

Strong correlation's (≥ |0.71|) are shown in bold.

D. Discussion

The concentrations of minerals in ungalled tissues of *R. blanda* collected in July were similar to those reported by Jones et al. (1991) for *R. odorata* (0.80 - 2.0 % Ca, 50 - 200 ppm Fe, 1.1- 3.0 % K, 0.21 - 0.5 % Mg, 0.21 - 0.5 % P and 0.20 - 0.7 % S) although concentrations of K and S were slightly lower in *R. blanda* (Table 4). No values for Na were reported by Jones et al. (1991) since it is not considered an essential mineral element to plant growth and development. Although concentrations of minerals in ungalled tissues of *R. blanda* changed slightly by September, levels of Ca, Fe, and Mg remained within the above ranges while K, P and S were slightly lower than those above (Table 5). Similar results were found for *R. acicularis*. All minerals measured were within the ranges reported by Jones et al. (1991) except for K and S both in July (Table 6) and September (Table 7).

Differences between the results reported in the present study and those of Jones et al. (1991) are attributable to three factors: values obtained by Jones et al. (1991) were based on leaves only; leaves were collected from cultivated roses grown in optimal conditions; and a different species of rose was used. The rose tissues in the present study were shoots (leaves and stems) collected from uncultivated and unfertilized wild roses. Considering these differences, the mineral compositions of *R. blanda* and *R. acicularis* were similar to those reported by Jones et al. (1991).

However, the mineral compositions of *R. blanda* and *R. acicularis* were not similar to the known composition of other herbivorous insects. According to Schoonhoven et al. (1998) insects are generally 0.3 % Ca, 125 ppm Fe, 0.7 % K, 0.2 % Mg, 3000 ppm Na, 1.0 % P, and 0.2% S. The concentration of Mg in ungalled tissues of *R. blanda* and *R. acicularis* was at adequate levels for insect consumption (Tables 4 to 7). Concentrations of Ca were substantially higher, K and S were generally half, and Na, P and Fe were substantially lower in ungalled rose tissues compared to typical insect tissue. Thus, if galls of *D. spinosa* and *D. triforma* are adaptations for inducer larvae to ingest highly nutritious

food, it was expected that levels of Fe, K, Na, P, and S would be elevated in galls relative to ungalled rose tissues.

Bagatto et al. (1991) found galls of *D. spinosa* on *R. rugosa* collected in August, 1989 (early maturation phase) to be 0.110 % Ca, 0.100 % Mg, and 128 ppm Fe by dry mass. Their reported levels of Ca are considerably lower than those reported in this study for both galls (Tables 4 to 7); however, they also found folliar levels of Ca to be much lower than those reported here for *R. blanda* and *R. acicularis*. Mg levels in galls reported by Bagatto et al. (1991) were similar to low compared to those reported here; however, they found Fe to be at higher concentrations. A later study by Bagatto and Shorthouse (1994a) found the variability in concentration of Ca in galls of *D. spinosa* on *R. blanda* ranged from 0.1 - 0.3 % and 0.5 - 0.7 % and concentrations of Fe ranged from 40 - 80 ppm and 90 - 150 ppm depending on where the host plants were growing.

Only K in growth and maturation phase galls of *D. spinosa* and Na in growth and maturation phase galls of *D. triforma* were at significantly higher concentrations compared to ungalled tissues (Figs. 8 to 11). Both of these minerals were at levels in *R. blanda* and *R. acicularis* considered sufficient for optimal growth of cultivated roses (Jones et al. 1991). Thus the elevated levels in galls may be the result of what Mattson and Scriber (1987) call "luxury of uptake"; plants were growing in soil particularly rich in K and Na, and took up more than normally required for growth to use in tissues which may benefit from it. It is unlikely that galls are accumulating high concentrations of nutrients when 24 of 28 mineral concentrations were equal to or significantly less than those in ungalled tissues (Tables 4 to 7 and Figs. 8 to 11). Thus, concentrations of gall minerals appear to be sub-optimal for insect consumption. However, had measurements of individual tissue regions in galls been possible, it is likely that nutritive tissues of galls, the only cells consumed by inducer larvae, would have contained levels of minerals similar to typical insect tissues. Bagatto and Shorthouse (1994b) measured the concentrations of Ca, Fe, K, Mg, P, and S along a transect of cells from nutritive cells to epidermal cells of galls of

Hemadas nubillipenis by scanning electron microscopy and found that these minerals were present in the nutritive cells at concentrations similar to those within an average insect (Schoonhoven et al. 1998) but decreased to undetectable levels for much of the cellular region between nutritive and epidermal cells. The present study considered only total gall concentrations, meaning that elevated levels of minerals in nutritive cells would have been masked by the lower concentrations in gall parenchyma.

The lower concentrations of certain minerals in galls compared to ungalled tissues may be a result of the host plant's ability to recognize galls as foreign and respond by decreasing the flow of xylem and phloem sap into galls. However, tissues growing distal to galls of *D. spinosa* and *D. triforma* often had concentrations significantly greater than the galls (Ca, Fe, Mg, Na, and S in tissues distal to galls of *D. spinosa* and K, Mg, P, and S in tissues distal to galls of *D. triforma*) indicating that limiting translocation by the plant was not occurring (Tables 4 to 7 and Figures 8 to 11). Bagatto et al. (1991) found similar results with galls of *D. spinosa* on *R. rugosa*. Tissues growing distally to these galls had significantly higher concentrations of Cu, Fe, Mn, Ni, and Zn than did the galls (Bagatto et al. 1991).

Phase of gall development (July vs. September) had a significant effect on the concentrations of Ca, K, Mg, Na, P and S in ungalled, gall and gall distal tissues (Tables 4 - 7). Concentrations of minerals in plant tissues typically vary over the season, usually resulting in lower mineral concentrations at the end of the season compared to the peak growth period (Section II A.). However, Fe concentrations follow a concave curve over the season (high early in the year, low in the middle and high again late in the year) which may explain why phase of gall development had no effect on the concentrations of this element in any of the tissues measured. There was a significant interaction between tissue type (ungalled *R. blanda* and *R. acicularis*, galls of *D. spinosa* and *D. triforma* and tissues distal to the galls) and phase of gall development for the concentrations of Ca, K, Na and S. It is possible that galls disrupt the 'normal' phenology of their host plants which would

explain this statistical interaction. For example, the concentration of S in *R. blanda* and galls of *D. spinosa* decreased over the season but remained constant in the tissue distal to galls of *D. spinosa* (Tables 4 and 5) perhaps due to influences of galls to alter the phenology of the tissue.

Insect tissues are dominated by the minerals P, K, and Mg; however, there is a large degree of inter-species mineral variation particularly in Ca and Na levels (Mattson and Scriber 1987). Concentrations of Ca and Na in larvae of *D. spinosa*, *D. triforma* and their parasitoids showed a high degree of variation (Table 8). Ca concentrations were twice as high in larvae of *D. triforma* and their parasitoids than they were in larvae of *D. spinosa* and their parasitoids, while Na ranged from approximately 1100 ppm in parasitoids of *D. spinosa* to 4400 ppm in parasitoids of *D. triforma*. Levels of most minerals in the larvae of *D. spinosa*, *D. triforma* and their parasitoids were lower than the averages for insects reported by Schoonhoven et al. (1998) but within the range of values reported by Mattson and Scriber (1987) for other insect species.

Bagatto and Shorthouse (1997) found that parasitoids of *Hemadas nubilipennis* had elevated levels of Cu, Zn, Mn, and Mg, but not Ni, Fe, and Ca, relative to their hosts and concluded that the parasitoids stimulate their hosts to consume more plant tissues than normal and increase the strength of galls as physiological sinks resulting in elevated levels of certain minerals in the parasitoids and the galls. However, a multivariate analysis of larval mineral concentrations indicated that while *D. spinosa* differed significantly from *D. triforma*, parasitoids were not significantly different than their hosts (Table 8). The difference between the findings of Bagatto and Shorthouse (1997) and those reported here may be another example of the inter-species variability of insect mineral composition reported by Mattson and Scriber (1987). Furthermore, the present study did not consider the same group of minerals as Bagatto and Shorthouse (1997). The only difference that can be found between the two studies is in the higher concentrations of Mg in parasitoids of *H. nubilipennis* compared to its host while parasitoids of *D. spinosa* and *D. triforma* did not

have higher concentrations of Mg relative to their hosts. Since the mineral concentrations of parasitoids of *D. spinosa* and *D. triforma* did not differ from their hosts, it is unlikely that they influenced their hosts to increase the sink activity of their galls. This would explain why rate of parasitism did not correlate highly with the concentration or amount of any mineral in galls (Table 9).

Number of gall inhabitants (inducers + parasitoids) was not strongly correlated with the concentrations of minerals in galls of *D. spinosa* and *D. triforma* in most cases; however, strong correlations between total amounts (μg) of minerals and the number of larvae in galls were typical (Table 9). Thus, galls were regulating the amount of minerals they receive and minerals were sufficient for the number of inducers rather than accumulating high concentrations of minerals overall. This finding is consistent with the literature since it is known that each larvae is surrounded by a nutritive layer of cells which are sinks within the gall for nutritious substances such as lipids and sugars (Rohfritsch 1992). Therefore, higher amounts of nutrients were a result of more sink tissue within the galls which were a result of more larvae. However, concentrations would not increase with the number of larvae since gall volume increases with the number of larvae (Bagatto and Shorthouse 1994a) resulting in a dilution effect.

Superficially, the concentration data do not support the nutrition hypothesis since nutrient levels were not elevated in galls; however, the assumption that high concentrations of nutrients in galls is an advantage to the inducer may not be well founded. The highly positive correlation between number of larvae per gall and the amount of nutrients (μg) in galls (Table 9) indicates that the insects are receiving adequate nutrition regulated by the gall while keeping the overall concentration of nutrients in the entire gall low. Not only does this support the nutrition hypothesis but it indicates that *D. spinosa* and *D. triforma* may have a minimal impact on their host plants. Furthermore, parasitoids of *D. spinosa* and *D. triforma* do not accumulate increased concentrations (or amounts) of minerals compared to their hosts, nor do they cause mineral levels in galls to increase.

VII. General Discussion

Galls have been used as model systems for study in ecology, pathology and physiology (Mani 1992), yet our knowledge of gall biology is still far from complete. Much has been written about the galls of various insects including their anatomy (Dreger-Jauffret and Shorthouse 1992), how they develop (Rohfritsch 1992), how they function (Bronner 1992), and the relationships between the many insects which inhabit galls (Weibes-Rijks and Shorthouse 1992); however we still know relatively little about the nutritional physiology of galls. Even so, the physiological attributes of some galls have been studied in an attempt to determine host damage or the specific function of galls as adaptations for the inducer. Generally, students of galls have come to the conclusion, or have made the assumption, that galls are physiological sinks established by the gall inducing insect, drawing assimilates and nutrients to the galls as food for the gallers and causing reduced host-plant fitness (Price et al. 1987).

The presence of large populations of *D. spinosa* and *D. triforma*, together at one study site and the availability of a Kjeldahl N autoanalyzer and an ICP spectrophotometer provided an ideal opportunity to study the nutritional physiology of their galls and the advantages of galling. This study of organic N and mineral nutrients within these two cynipid galls highlights some of the unique physiological attributes of gall-inducing insects and their parasitoids.

No single element is considered more important to the survival of plants and animals than N (White 1993). Required by plants as nitrate (NO_3) and by animals bound in organic molecules such as amino acids, the relative scarcity of these forms of N in the biosphere makes it the most limiting nutrient to the growth, survival and reproduction of plants and animals (White 1993). Many feeding adaptations of herbivorous insects are for the acquisition of N (Weis and Berenbaum 1989). Thus if galls are feeding adaptations evolved for the procurement of superior food by the gall inducer, they are likely to be rich

sources of N. However, galls should also provide the correct balance of all nutrients and minerals required for the survival of the gall inducer. Abrahamson and Caswell (1982) concluded that multiple nutrients must be used in gall nutrient studies due to the variability between species and populations, and Slansky and Feeny (1977) considered measures of mineral element allocation crucial in studies involving multitrophic levels since herbivores are typically nutrient limited rather than carbon or energy limited. Furthermore, since host-plant growth can be limited by mineral-element availability, allocation patterns may be strong indicators of the impact galls have on their host plants.

The concentrations of organic N and minerals (Ca, Fe, K, Mg, Na, P and S) were not elevated in galls of *D. spinosa* and *D. triforma* compared to ungalled host tissues in 28 of 32 comparisons (Figs. 6 and 7, Tables 4 - 7). Thus the hypothesis that organic N and minerals are concentrated in galls relative to ungalled tissues is incorrect. Furthermore, lower levels of N and minerals in galls of *D. spinosa* and *D. triforma*, relative to their host-plants, were not due to a lack of available host-plant nutrients or an ability of the host to stop nutrients from entering the vasculature of the gall. Rather, galls of both *D. spinosa* and *D. triforma,* via larvae of the inducers, may be regulating their host plants for lower levels of N and minerals. This may seem counter-intuitive if galls are feeding adaptations; however, measures of total gall nutrients may be misleading when discussing the requirements of gall inducers since key nutrients are likely restricted to the nutritive layer of cells (Bronner 1992). Had measurements of individual tissue regions in galls been, possible it is likely that nutritive tissues of galls, the sole source of food for inducer larvae, would have contained levels of nutrients similar to the composition of the gall-inducing larvae. Similarly, a more refined study may find specific nutrients such as amino acids elevated in galls which would not be detected by total organic N measurements. Furthermore, the nutritional requirements of gall-inducing insects are unknown, making predictions of nutritional advantages based upon the concentrations of nutrients in galls equivocal at best (Hartley and Lawton 1992).

The concentrations of minerals in larvae *D. spinosa* and *D. triforma* and their parasitoids (Table 8) were within the range measured in other species of insects (Mattson and Scriber 1987). Typically, the amounts, not concentrations, of N and minerals in galls of *D. spinosa* and *D. triforma* were positively correlated with the number of gall insects in each gall (Tables 2, 3 and 9). These results suggests that *D. spinosa* and *D. triforma* are minimizing their host impact by keeping gall nutrient concentrations low while providing increased amounts for each inducer. They appear to be able to acquire sufficient nutrients for survival without causing undue harm to their hosts. It is possible that *D. spinosa* and *D. triforma* are behaving in much the same way as parasites which cause reduced host fitness but are rarely a mortality factor (Abrahamson and Weis 1997).

Abrahamson (1989) argued that the relationship between gall inducer and host is antagonistic and parasitic. He further argued that over evolutionary time, antagonistic relationships between insects and plants have often become mutualistic, antagonistic relationships being less specialized relative to mutualistic ones and so the lack of specialization creates opportunity for selection leading to mutualism. Traditionally, the term parasite is used to describe microorganisms or small animals which feed on larger animals. Abrahamson (1989) argues that gall inducers fit the definition of parasitic animals. Furthermore, gall-inducing insects may have a considerably greater impact on their hosts than externally feeding insects when measured on a per herbivore basis (Stinner and Abrahamson 1979). Gall inducers not only "rob" the host plant of the consumed tissues while procuring their nutritional requirements, but also cause their host to alter tissues that would have served more productive functions in plant growth and reproduction (Abrahamson and McCrea 1986). However, gall inducers may affect their hosts in positive ways as well, depending on levels of herbivore infestation, host-plant organs attacked, host responses and the species involved (Skuhravá and Skuhravy 1973, Abrahamson and Weis 1987). Usually, galls developing on stems or shoots, such as *D. spinosa* and *D. triforma*,

cause little loss of fitness whereas galls on reproductive organs cause more host damage (Harris and Shorthouse 1996).

The present study suggests that *D. spinosa* and *D. triforma* have evolved a less antagonistic/parasitic relationships with their hosts. If *D. spinosa* and *D. triforma* defoliated their hosts rather than induce galls, it is likely that the imbalance of nutrients in the ungalled leaves would have led to increased consumption and thus increase nutrient losses. Palct and Hassler (1967) suggested that the concentration of nitrogen in galls may be a measure of the level of harm caused by the gall to the host. Thompson and Stewart (1981) considered most minerals as good indicators of resource allocation in plants. Therefore, *D. spinosa* and *D. triforma* may be regulating gall tissues for lower than normal concentrations of limiting nutrients to reduce their impact on their host plants. Loss in biomass is not necessarily a loss in host plant fitness because plants are typically overproducers of carbohydrates and rarely photosynthesize to their full potential under normal conditions (Harper 1977). Furthermore, the plant is able to "isolate the gall insect in space and time by forcing it into extreme specialization" by participating in gall induction (Mani 1992). Therefore, gall induction may be more mutualistic than parasitic along the spectrum of plant/herbivore interactions.

If galls are regulating host plant nutrients to the benefit of the inducer without placing undue stress on the host plant, then galls are similar to 'normal' plant sinks. Furthermore, obtaining adequate N is considered the driving force of evolution by some authors (McNeill and Southwood 1978, Mattson 1980, White 1984, White 1993) and thus galls of *D. spinosa* and *D. triforma* may have significantly less N than ungalled tissues because gall inducers are not N limited (Abrahamson and McCrea 1986, Hartley and Lawton 1992). If galls provide an environment for inducers where N as well as other nutrients are not limited, then the nutrition hypothesis of gall evolution proposed by Price et al. (1987) has merit.

It was expected that parasitoids of the inducers would have elevated levels of at least some minerals relative to their host based on the findings of Abrahamson and McCrea (1986) and Bagatto and Shorthouse (1997). That they did not (Table 8) is attributable to the variability normally found among insect species (Mattson and Scriber 1987). The rate of parasitism of *D. spinosa* and *D. triforma* was not correlated with the concentration or amount of any nutrient measured in galls (Tables 2, 3 and 9). That parasitoids of *D. spinosa* and *D. triforma* did not differ in their mineral compositions relative to their hosts (Table 8) explains why rate of parasitism had no significant effect on the amounts or concentrations of minerals within galls. Therefore, parasitoids of *D. spinosa* and *D. triforma* did not cause increased feeding by their host or increased sink activity of galls and the hypothesis that gall inducer parasitism would cause increased levels of nutrients in galls is not supported by the data.

The results of both the organic N and mineral nutrient studies support each other and the nutrition hypothesis of gall evolution. The assumption that higher concentrations of nutrients occur in galls relative to ungalled tssue is not supported by this study. Gall induction represents a very precise and species-specific manipulation of host plant chemistry, and may involve a reduction or an increase in critical nutrients within the gall to improve insect performance (Hartley and Lawton 1992). The highly positive correlation between number of larvae per gall and the amount of nutrients in galls indicates that the inducers are receiving adequate nutrition while keeping the overall concentration of nutrients in the entire gall low. Not only does this support the nutrition hypothesis, it suggests that cynipid gall-inducers, at least those on wild roses, have minimal impact on their host plants. Furthermore, parasitoids of *D. spinosa* and *D. triforma* do not significantly influence the concentrations (or amounts) of organic N or minerals in galls.

VIII. Literature Cited

Abrahamson, W. G. 1989. Plant-animal interactions: An overview. pp. 1-22. *In* W. G. Abrahamson (Ed.), Plant-Animal Interactions. McGraw-Hill, New York, 480 pages.

Abrahamson, W. G. and H. Caswell. 1982. On the comparative allocation of biomass, energy, and nutrients in plants. Ecology 63: 982-991.

Abrahamson, W. G. and K. D. McCrea. 1986. Nutrient and biomass allocation in *Solidago altissima*: Effects of two stem gallmakers, fertilization, and ramet isolation. Oecologia 68: 174-180.

Abrahamson, W. G. and A. E. Weis. 1987. Nutritional ecology of arthropod gall makers. pp. 235-258. *In* F. Slansky Jr. and J. G. Rodriguez (Eds.), Nutritional Ecology of Insects, Mites, Spiders, and Related Invertebrates. John Wiley and Sons, New York, 1016 pages.

Abrahamson, W. G. and A. E. Weis. 1997. Evolutionary Ecology across Three Trophic Levels: Goldenrods, Gallmakers, and Natural Enemies. Princeton University Press, Princeton, New Jersey, 456 pages.

Abrahamson, W. G., P. O. Armbruster and G. D. Maddox. 1983. Numerical relationships of the *Solidago altissima* stem gall insect-parasitoid guild food chain. Oecologia 58: 351-357.

Abrahamson, W. G., K. D. McCrea and S. S. Anderson. 1989. Host preference and recognition by the goldenrod ball gallmaker *Eurosta solidaginis* (Diptera: Tephritidae). American Midland Naturalist 121: 322-330.

Abrahamson, W. G., J. M. Brown, S. K. Roth, D. V. Summerford, J. D. Horner, M. D. Hess, S. T. How, T. P. Craig, R. A. Packer and J. K. Itami. 1994. Gallmaker speciation: An assessment of the roles of host-plant characters and phenology, gallmaker competition, and natural enemies. pp. 208-222. *In* P. Price, W. Mattson,

and Y. Baranchikov (Eds.) Gall-Forming Insects. USDA Forest Service, North Central Experiment Station. General Technical Report NC-174.

Ananthakrishnan, T. N. 1984. Biology of Insect Galls. Oxford and IBH, New Delhi, India, 362 pages.

Anderson, P. C. and R. F. Mizell. 1987. Physiological effects of galls induced by *Phylloxera notabilis* (Homoptera: Phyloxeridae) on pecan foliage. Environmental Entomology 16: 264-268.

Ashmead, W. H. 1887. On the cynipidous galls of Florida, with descriptions of new species and synopses of the described species of North America. Transactions. American Entomological Society 14: 125-135.

Askew, R. R. 1984. The biology of gall wasps. pp. 223-271. *In* T.N. Ananthakrishnan (Ed.) Biology of Gall Insects. Oxford-IBH, New Delhi, India, 362 pages.

Askew, R. R. and M. R. Shaw. 1986. Parasitoid communities: Their size, structure and development. pp. 225-264. *In* J. Waage and D. Greathead (Eds.), Insect Parasitoids. Academic Press, London, 390 pages.

Bagatto, G. 1992. Concentrations of minerals (Cu, Ni, Zn, Fe, Mn, Mg and Ca) in tissues of lowbush blueberry (*Vaccinium angustifolium*) and an insect gall induced by *Hemada nubilipennis* (Hymenoptera: Pteromalidae) and its inhabitants in metal-contatminated and non-metal contaminated sites. Ph.D. thesis. Queen's University, Kingston, Ontario, Canada. 174 pp.

Bagatto, G. and J. D. Shorthouse. 1994a. Mineral nutrition of galls induced by *Diplolepis spinosa* (Hymenoptera: Cynipidae) on wild and domestic roses in central Canada. pp. 405-428. *In* M. A. J. Williams (Ed.) Plant Galls. Clarendon Press, Oxford, 488 pages.

Bagatto, G. and J. D. Shorthouse. 1994b. Mineral concentrations within cells of galls induced by *Hemadas nubilipennis* (Hymenoptera: Pteromalidae) on lowbush

blueberry: evidence from cryoanalytical scanning electron microscopy. Canadian Journal of Botany 72: 1387-1390.

Bagatto, G. and J. D. Shorthouse. 1997. Accumlation of mineral nutrients by the galler *Hemadas nubilipennis* (Hymenoptera: Pteromalidae) and its parasitoids on lowbush blueberry: Implications for feeding behaviour. pp. 159-168. *In* A. Raman (Ed.) Ecology and Evolution of Plant-Feeding Insects in Natural and Man Made Environments. International Scientific Publications, New Delhi, 245 pages.

Bagatto, G., T. J. Zmijowskyj and J. D. Shorthouse. 1991. Galls induced by *Diplolepis spinosa* influence distribution of mineral nutrients in the shrub rose. HortScience 26: 1283-1284.

Bagatto, G., L. C. Paquette and J. D. Shorthouse. 1996. Influence of galls of *Phanacis taraxici* on carbon partitioning within common dandelion, *Taraxacum officinale.* Entomologia Experimentalis et Applicata 79: 111-117.

Beijernick, M. W. 1882. Beobachtungen über die ersten Entwicklungsphasen einiger Cynipidengallen. Naturk. Vech. Koninkl. Akad. Wetenshap. 22: 1-198.

Bently, B. L. and N. D. Johnson. 1991. Plants as food for herbivores: the roles of nitrogen fixation and carbon dioxide enrichment. pp. 257-272. *In* P. W. Price, T. M. Lewiston, G. W. Fernandes and W. W. Benson (Eds.), Plant-Animal Interactions: Evolutionary Ecology in Tropical and Temperate Regions. John Wiley and Sons, Inc., New York, 639 pages.

Bernays, E. A. 1982. The insect on the plant-a closer look. pp. 3-18. *In* Procedings of the Fifth International Symposium on Insect-Plant Relationships, Wageningen, The Netherlands. Pudoc, Wageningen, The Netherlands.

Bernays, E. A. and R. Berbehenn. 1987. Nutritional ecology of grass foliage-chewing insects. pp. 147-176. *In* F. Slansky Jr. and J. G. Rodriguez (Eds.), Nutritional Ecology of Insects, Mites, Spiders, and Related Invertebrates. John Wiley and Sons, New York, 1016 pages.

Brewer, J. W., J. N. Bishop and V. Skuhravy. 1987. Levels of foliar chemicals in insect-induced galls (Dipt., Cecidomyiidae). Sonderdruck aus 104: 503-510.

Bronner, R. 1992. The role of nutritive cells in the nutrition of cynipids and cecidomyiids. pp. 118-140. *In* J. D. Shorthouse and O. Rohfritsch (eds.) Biology of Insect-Induced Galls. Oxford University Press, New York, 285 pages.

Brooks, S. E. and J. D. Shorthouse. 1997. Biology of the rose stem galler *Diplolepis nodulosa* (Hymenoptera: Cynipidae) and its associated component community in central Ontario. The Canadian Entomologist 129: 1121-1140.

Brooks, S. E. and J. D. Shorthouse. 1998. Developmental morphology of stem galls of *Diplolepis nodulosa* (Hymenoptera: Cynipidae) and those modified by the inquiline *Periclistus pirata* (Hymenoptera : Cynipidae) on *Rosa blanda* (Rosaceae). Canadian Journal of Botany. In Press.

Burks, B. D. 1979. Cynipoidea. pp. 1105-1108. *In* K. V. Krombein, P. D. Hurd, Jr., D. R. Smith and B. D. Burks (Eds.) Catalog of Hymenoptera of North America North of Mexico. Vol. 1. Smithsonian Institution Press, Washington, DC., 1198 pages.

Cabrera, R. I., R. Y. Evans and J. L. Paul. 1995. Nitrogen partitioning in rose plants over a flowering cycle. Scientia Horticulturæ 63: 67-76.

Claridge, M. F. 1987. Insect assemblages - diversity, organization and evolution. pp. 141-162. *In* J. H. R. Gee and P. S. Giller (Eds.), Organization of Communities: Past and Present. Blackwell, Oxford, 576 pages.

Clifford, P .E. 1992. Understanding the source-sink concept of phloem translocation. Journal of Biological Education 26(2): 112-116.

Cornell, H.V. 1983. The secondary chemistry and complex morphology of galls formed by the Cynipinae (Hymenoptera): Why and how? American Midland Naturalist 110: 225-234.

Craig, T. P., P. W. Price and J. K. Itami. 1986. Resource regulation by a stem-galling sawfly on the arroyo willow. Ecology 67(2): 419-425.

Craig, T. P., J. K. Itami, J. D. Horner and W. G. Abrahamson. 1994. Host shifts and speciation in gall-forming insects. pp. 194-207. *In* P. Price, W. Mattson, and Y. Baranchikov (Eds.) Gall-Forming Insects. USDA Forest Service, North Central Experiment Station. General Technical Report NC-174.

Dalla Torre, K. W. and J. J. Kieffer. 1910. Cynipidae. Das Tierreich 24, 891 pages.

Dreger-Jauffret, F. and J. D. Shorthouse. 1992. Diversity of gall-inducing insects and their galls. pp. 8-33. *In* J. D. Shorthouse and O. Rohfritsch (Eds.) Biology of Insect-Induced Galls. Oxford University Press, New York, 285 pages.

Eady, R. D. and J. Quinlan. 1963. Cynipoidea. Handbook for the Indentification of British Insects 8: 1-81.

Farrar, J. F. 1996. Sinks-integral parts of a whole plant. Journal of Experimental Botany 47: 1273-1279.

Fernandes, M. S. and R. O. P. Rossiello. 1995. Mineral nitrogen in plant physiology and plant nutrition. Critical Reviews in Plant Sciences 14(2): 111-148.

Gauld, I. and B. Bolton. 1988. The Hymenoptera. Oxford University Press, Oxford, 332 pages.

Hagen, K. S. 1987. Nutritional ecology of terrestrial insect predators. pp. 533-578 *In* F. Slansky Jr. and J. G. Rodriguez (Eds.), Nutritional Ecology of Insects, Mites, Spiders, and Related Invertebrates. John Wiley and Sons, New York, 1016 pages.

Harper, J. L. 1977. Population Biology of Plants. Academic Press, New York, New York, 892 pages.

Harris, P. and J. D. Shorthouse. 1996. Effectiveness of gall inducers in weed biological control. The Canadian Entomologist 128: 1021-1055.

Hartley S. E. 1990. What are galls for? Tests of the nutrition hypothesis. pp. 265-274 *In* T. Jermy, A. Szentesi, G. Dezso & J. Horvath (Eds.), Proceedings of the Seventh International Symposium on Insect Galls. Hungarian Academy of Sciences, Budapest, 577 pages.

Hartley, S. E. and J. H. Lawton. 1992. Host-plant manipulation by gall-insects: a test of the nutrition hypothesis. Journal of Animal Ecology 61: 113-119.

Hawkins, B. A. and T. R. Unruh. 1988. Protein and water levels in *Asphondylia atriplicis* (Diptera: Cecidomyiidae) galls. The Southwestern Naturalist 33: 114-117.

House, H. L. 1965. Insect nutrition. pp. 796-813. *In* M. Rockstein (Ed.) The Physiology of Insecta Volume II. Academic Press, New York, 905 pages.

House, H. L. 1969. Effects of different proportions of nutrients on insects. Entomologia Experimentalis et Applicata 12: 651-669.

Jones, J. B., B. Wolf and H. A. Mills. 1991. Plant Analysis Handbook. Micro-Macro Publishing, Inc., Georgia, 213 pages.

Kelleher, M. J. 1988. Influence of parasitoids on a population of *Diplolepis spinosa* (Ashmead) (Hymenoptera: Cynipidae) found on *Rosa rugosa* Thunb. (Rosaceae) in Sudbury, Ontario. M. Sc. Thesis, Laurentian University, Sudbury, Ontario, Canada, 145 pages.

Kinsey, A. C. 1920. Phylogeny of cynipid genera and biological characteristics. Bulletin. American Museum of Natural History 42: 357-402.

Larson, K. C. and T. G. Whitham. 1991. Manipulation of food resources by a gall-forming aphid: the physiology of sink-source interactions. Oecologia 88: 15-21.

Lewis, O. A. M. 1986. Plants and Nitrogen: The Institute of Biology's Studies in Biology No. 166. Edward Arnold, Great Britain, 104 pages.

Magnus, W. 1914. Die Entstenhung der Pflanzengallen vurursacht durch Hymenopteren. Jena, G. Fischer, 160 pages.

Mani, M. S. 1992. Introduction to cecidology. pp. 238- 257. *In* J. D. Shorthouse and O. Rohfritsch (Eds.), Biology of Insect Induced galls. Oxford University Press, New York, 285 pages.

Marasquelle, H. J. and J. Meyer. 1965. Physiologie et morphogenèse des galles d'original animale (zoocécides). Handbuch Pflanzenphyisiologie 15(2): 280-329.

Marschner, H. 1983. General introduction to the mineral nutrition of plants. pp. 5-60. *In* A. Lauchli and R. L. Bieleski (Eds.), Inorganic Plant Nutrition. Springer-Verlag, New York, 449 pages.

Mattson, W. J. 1980. Herbivory in relation to plant nitrogen content. Annual Review of Ecology and Systematics 11: 119-161.

Mattson, W. J. and J. M. Scriber. 1987. Nutritional ecology of insect folivores of woody plants: nitrogen, water, fiber, and mineral considerations. pp. 105-146. *In* F. Slansky Jr. and J. G. Rodriguez (Eds.) Nutritional Ecology of Insects, Mites, Spiders, and Related Invertebrates. John Wiley and Sons, New York, 1016 pages.

McCrea, K. D., W.G. Abrahamson and A.E. Weis. 1985. Goldenrod ball gall effects on *Solidago altissima*: ^{14}C translocation and growth. Ecology 66(6): 1902-1907.

McNeill, S. and T. R. E. Southwood. 1978. The role of nitrogen in the development of insect/plant relationships. pp. 77-98. *In* J. B. Harborne (Ed.) Biochemical aspects of plant and animal coevolution. Academic Press, New York, 435 pages.

Mehra, A. and M. E. Farrago. 1994. Metal ions and plant nutrition. pp. 31-66. *In* M. E. Farrago (Ed.), Plants and the Chemical Elements: Biochemistry, Uptake, Tolerance and Toxicity. VCH, New York, 292 pages.

Meyer, J. 1987. Plant Galls and Gall Inducers. Gebrüder Borntraeger, Berlin, 291 pages.

Meyer, J. and H. J. Maresquelle. 1983. Anatomie des Galles. Borntraeger, Berlin, 662 pages.

Molliard, M. 1913. Recherches physiologiques sur les galles. Revue Generale de Botanique 25 225-370.

Niblett, M. 1943. The species of *Rhodites* causing pea-galls on *Rosa*: 1. Entomologist 76: 11-15, 34-39.

Paclt, J., and J. Hassler. 1967. Concentration of nitrogen in some plant galls. Phyton (Australia) 12: 173-176.

Paquette, L. C., G. Bagatto and J. D. Shorthouse. 1993. Distribution of mineral nutrients within the leaves of common dandelion (*Taraxacum officinale*) galled by *Phanacis taraxaci* (Hymenoptera: Cynipidae). Canadian Journal of Botany 71: 1026-1031.

Peel, A. J. 1972. The control of solute movement into sieve elements. Pesticide Science 3: 631-641.

Price, P. W. and K. M. Clancy. 1986. Multiple effects of precipitation on *Salix lasiolepis* and populations of the stem-galling sawfly, *Euura lasiolepis*, in Northern Arizona. Ecological Research 1: 1-14.

Price, P. W. and T. P. Craig . 1984. Life history, phenology, and survivorship of a stem-galling sawfly, *Euura lasiolepis* (Hymenoptera: Tenthredinidae), on the arroyo willow, *Salix lasiolepsis*, in Northern Arizona. Annals of the Entomological Society of America 77: 712-719.

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

Price, P. W., G. W. Fernandes and G. L. Waring. 1987. Hypotheses on the adaptive nature of galls. Environmental Entomology 16: 15-24.

Price, P. W., W. Mattson and Y. Baranchikov (Eds.). 1994. Gall-Forming Insects. USDA Forest Service, North Central Experiment Station. General Technical Report NC-174.

Rohfritsch, O. 1971. Dévelopment cécidien et rôle du parasite dans quelques galles d'athropodes. Marcellia 37: 233-339.

Rohfritsch, O. 1992. Patterns in gall development. pp 60-86. *In* J. D. Shorthouse and O. Rohfritsch (Eds.) Biology of Insect-Induced Galls. Oxford University Press, New York, 285 pages.

Rohfritsch, O. and J. D. Shorthouse. 1982. Insect galls. pp. 131-152. *In* G. Kahl and J. Schell (Eds.) Molecular Biology of Plant Tumors. Academic, New York, 617 pages.

Sacchi, C. F., P. W. Price, T. P Craig and J. K. Itami. 1988. Impact of shoot galler attack on sexual reproduction in the arroyo willow. Ecology 69(6): 2021-2030.

Schoonhoven, L. M., T. Jermy, and J. J. A. van Loon. 1998. Insect-plant Biology: From Physiology to Evolution. Chapman and Hall, London, 409 pages.

Schönrogge, K., L. J. Harper, S. E. Brooks, J. D. Shorthouse and C. Lichenstein. 1998. Reprogramming plant development: two approaches to srudy the molecular mechanism of gall formation. *In* P. W. Price, G. Csóka and W. Mattson (Eds). Forest Service-U. S. Dep. of Agriculture. St. Paul, Minnesota. In press.

Schröder, D. 1967. *Diplolepis* (=*Rhodites*) *rosae* (L.) (Hymenoptera, Cynipidae) and a review of its parasite complex in Europe. Commonwealth Institute of Biological Control. Technical Bulletin 9: 93-131.

Shorthouse, J. D. 1973. The insect community associated with rose galls of *Diplolepis polita* (Cynipidae, Hymenoptera). Quaestiones Entomologicae 9: 55-98.

Shorthouse, J. D. 1980. Modification of galls of *Diplolepis polita* by the inquiline *Periclistus pirata*. Bulletin Societe Botanique de France. Actualites Botaniques 127: 79-84.

Shorthouse, J. D. 1982. Resource exploitation by gall wasps of the genus *Diplolepis*. pp. 193-198. *In* Proceedings of the Fifth International Symposium on Insect-Plant Relationships. Pudoc, Wageningen.

Shorthouse, J. D. 1988. Occurrence of two gall wasps of the genus *Diplolepis* (Hymenoptera: Cynipidae) on the domestic shrub rose, *Rosa rugosa* Thunb. (Rosaceae). The Canadian Entomologist 120: 727-737.

Shorthouse, J. D. 1993. Adaptations of gall wasps of the genus *Diplolepis* (Hymenoptera: Cynipidae) and the role of gall anatomy in cynipid systematics. Memoirs of the Entomological Society of Canada 165: 139-163.

Shorthouse, J. D. 1994. Host shift of the leaf galler *Diplolepis polita* (Hymenoptera: Cynipidae) to the domestic shrub rose *Rosa rugosa*. The Canadian Entomologist 126: 1499-1503.

Shorthouse, J. D. and A. J. Ritchie. 1984. Description and biology of a new species of *Diplolepis* Fourcroy (Hymenoptera: Cynipidae) inducing galls on the stems of *Rosa acicularis*. The Canadian Entomologist 116: 1623-1636.

Shorthouse, J. D. and O. Rohfritsch (Eds.). 1992. Biology of Insect-Induced Galls. Oxford University Press, New York, 285 pages.

Skuhravá, M. and V. Skuhravy. 1973. Die Gallmücken (Cecidomyiidae, Diptera) des Schilfes (*Phragmites communis* Trin.) Studie CSAV. Praha, Nr. 3.

Skuhravy, V., M. Skuhravá and J. W. Brewer. 1980. Evaluation of plant damage by three species of gall midge (Diptera: Cecidomyiidae). Zeitschrift fuer Angewandte Entomologie 90: 184-190.

Slansky, F. and P. Feeny. 1977. Stabilization of the rate of nitrogen accumulation by larvae of the cabbage butterfly on wild and cultivated food plants. Ecological Monographs 47: 209-228.

Stinner, B. R. and W. G. Abrahamson. 1979, Energetics of the *Solidago canadensis* stem gall insect-parasitoid interaction. Ecology 60: 918-926.

Strong, D. R., J. H. Lawton and S. R. Southwood. 1984. Insects on Plants: Community Patterns and Mechanisms. Blackwell Scientific Publications, Oxford, 313 pages.

Thompson, K. and A. J. A. Stewart. 1981. The measurement and meaning of reproductive effort in plants. American Naturalist 177: 205-211.

Wardlaw, I. F. 1990. The control of carbon partitioning in plants. New Phytologist 116: 341-381.

Weis, A. E. and W. G. Abrahamson. 1985. Potential selective pressure by parasitoids on a plant-herbivore interaction. Ecology 66(4): 1261-1269.

Weis, A.E. and W.G. Abrahamson. 1986. Evolution of host-plant manipulation by gall makers: Ecological and genetic factors in the *Solidago-Eurosta* system. American Naturalist 127: 681-695.

Weis, A. E. and M. R. Berenbaum. 1989. Herbivorous insects and green plants. pp. 123-162. *In* W. G. Abrahamson (Ed.), Plant-Animal Interactions. McGraw Hill, New York, 480 pages.

Weis, A. E., W. G. Abrahamson and K. D. McCrea. 1985. Host gall size and oviposition success by the parasitoid *Eurytoma gigantea*. Ecological Entomology 10: 341-348.

Welch, R. M. 1995. Micronutrient nutrition of plants. Critical Reviews in Plant Sciences. 14(1): 49-82.

White, T. C. R. 1984. The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. Oecologia 63: 90-105

White, T. C. R. 1993. The Inadequate Environment: Nitrogen and the Abundance of Animals. Springer-Verlag, Heidelberg, Germany, 425 pages.

Wiebes-Rijks, A. A. and J. D. Shorthouse. 1992. Ecological relationships of insects inhabiting cynipid galls. pp. 238- 257. *In* J. D. Shorthouse and O. Rohfritsch (Eds.) Biology of Insect Induced galls. Oxford University Press, New York, 285 pages.

Williams, M. A. J. (Ed.). 1994. Plant Galls. Clarendon Press, Oxford, 488 pages.

Zuchlinski, J. A. 1979. Interactions among inhabitants of *Diplolepis* (Hymenoptera: Cynipidae) bud galls on *Rose acicularis* (Rosaceae) in northern Ontario. M.Sc. Thesis, Laurentian University, Sudbury, Ontario, Canada, 104 pages.

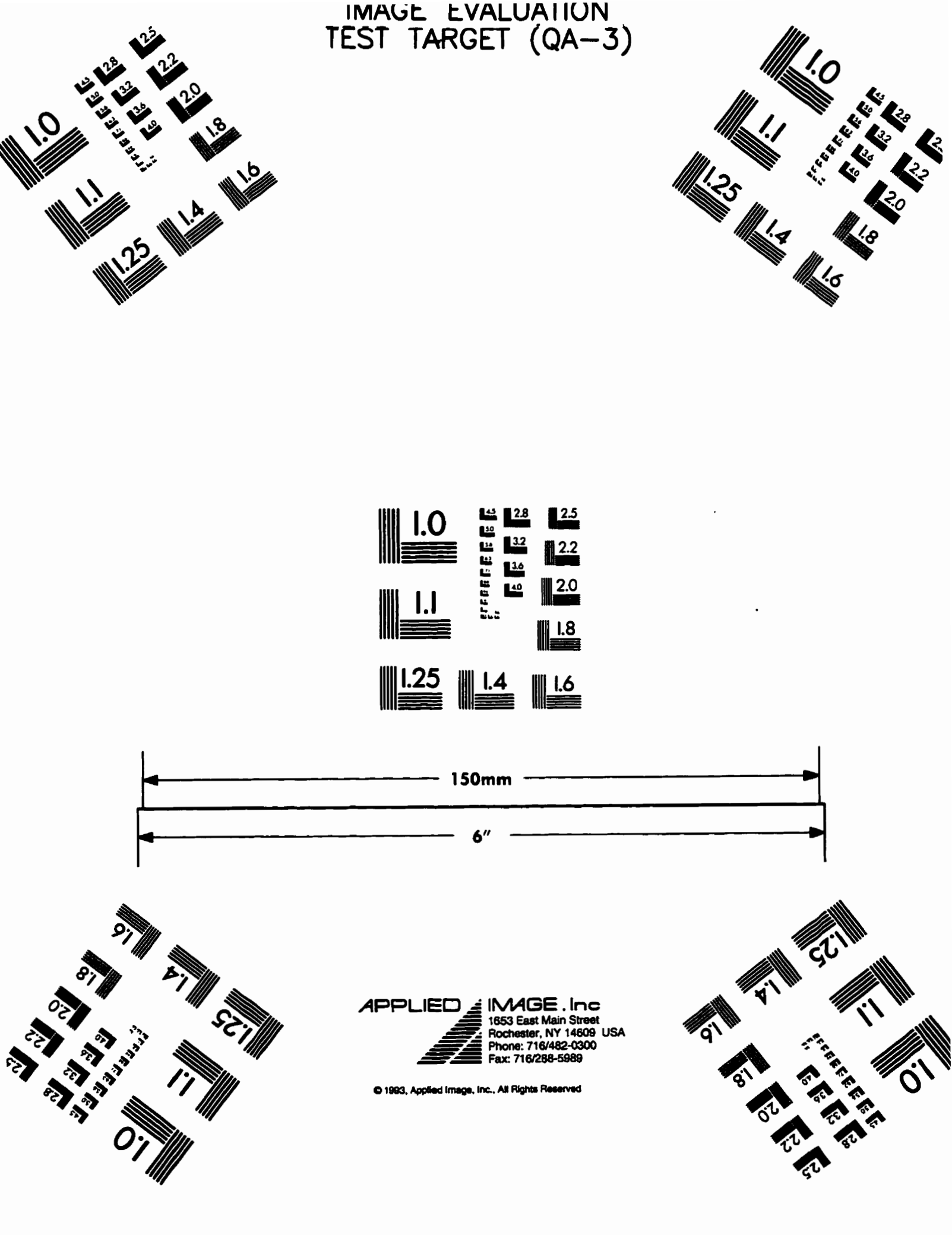


IMAGE EVALUATION
TEST TARGET (QA-3)
1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0
150mm
6"
APPLIED IMAGE, Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989
© 1993, Applied Image, Inc., All Rights Reserved