Stress Physiology and Biological Weed Control: A Case Study with Canada Thistle (Cirsium arvense (L.) Scop.)

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Abstract

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Stress Physiology and Biological Weed Control : A Case Study with Canada Thistle (Cirsium arvense (L.) Scop.).

The success of biological weed control programs has been limited by a lack of understanding of the stress physiology of insect damage and pathogen development. This case study with the perennial weed, Cirsium arvense, (L.) Scop. evaluated the stress of five natural enemies. Attack by a seed head predator, Orellia ruficauda (F.) caused about 21.5% predation and may reduce seed dispersal. The stress of stem gall formation (Urophora cardui (L.)) is greatest when the gall occurs on young plants and on the mainshoot and defoliation simulation (Cassida rubiginosa Muller) is most effective at high levels on young plants. In nature, however, the latter two natural enemies are not synchronized with these susceptible stages, thereby reducing their effectiveness. Although Cleonus piger Scop., a root crown inhabitant, can result in plant death, regeneration of damaged vascular tissue can occur. Plants which emerge systemically infected with Puccinia punctiformis (Str.) Rohl. (rust) rarely survive the season. A matrix model simulating the effects on Canada thistle population dynamics by the natural enemies was applied.

Résumé

Ph.D Sheila Forsyth Phytotechnie

Physiologie du stress et lutte biologique des mauvaises herbes: Une etude sur le cas du chardon des champs (Cirsium arvense (L.) Scop.).

Le succes de la lutte biologique contre les mauvaises herbes est limité par le peu de compréhension du stress physiologique provoqué par les dégâts causés par les insectes et les microbes pathogènes. Ce travail évalue le stress provoqué chez la mauvaise herbe vivace Cirsium arvense (L.) Scop. par cinq de ses enemis naturels. Le predateur des capitules en fruit, Orellia ruficauda (F.) attaque 21.5 pourcent des capitules et est susceptible de diminuer la dispersion de la semence. Le stress provoqué par le développement des galles dues a Urophora cardui (L.) est le plus grave lorsque les galles se développent sur les jeunes plantes et sur la tige maîtresse. La defoliation artificielle simulant les dégâts causes par Cassida rubiginosa Muller est le plus afficace quand elle est severe sur des jeunes plantes. Cependant, en milieu natural, les attaques de ces deux derniers enemis ne sont pas synchroniques aux stades sensibles de la plante ce qui reduit leur morbidité. Quoique Cleonus piger Scop. réside dans le pied de la plante et peut causer sa mort, les tissus vasculaires souvent se régénerent. Les plantes qui au moment de la levée renferment une infection generlisée de la rouille Puccinia punctiformis Str. Rohl. ne survivent que rarement la saison. Un modele matriciel simulant les effets de ces enemis naturels sur la dynamique des populations du chardon des champs est utilise.

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I. INTRODUCTION

"Cursed is the ground... thorns also and thistles shall it bring forth to thee." Genesis 3:17,18.

1. Introduction

Biological control is the deliberate use of natural enemies (phytophagous or pathogenic organisms) to reduce the density of a weed to an economic or a tolerable level (Goeden 1977, Watson 1977). There are four approaches to biological control: (1) classical or conventional which involves the importation and release of parasite-free host-specific natural enemies from the native range of introduced weed species, (2) augmentation, periodic release and/or redistribution of natural enemies often augmented by laboratory reared insects, (3) biological herbicides (mycoherbicides), the application either of an introduced or an endemic plant pathogen, and (4) conservation, a method rarely used which involves determining the effects of the available natural enemies on the weed and aiding the most effective by eliminating competition and/or parasites or predators (Watson 1977, Batra 1979).

The principles and procedures of the biological control of weeds (especially with insects) are well documented and the method has produced

effective and safe controls (Huffaker 1958, Wapshere 1974, Batra 1979).

However, recent evaluation of biological control programs indicate that progress has been limited by an incomplete knowledge and understanding of the type and level of damage caused by natural enemies and the resulting physiological response or stress by the plants (Andres 1980 b Harris 1981).

The focus of biological control has been primarily on the introduction of exotic natural enemies which do not damage non-target plants, without regard for the degree of control. Harris (1973a, 1973b) noted that much attention is given to the selection of host-specific agents for the biological control of weeds but little is given to the selection of effective agents nor to the vulnerability to attack of different plant organs or life stages. Consequently, although no accidents have occurred, the desired degree of control has not been realized in many cases. Andres (1980) and Harris (1982) recognize the importance of examining ways to explain the success or failure of biological control programs. Both acknowledge that the concept of stress may be important for increased effectiveness of natural enemy selection. As Harris (1981) states, "Little work has been done in this regard with weed-feeding insects."

There are a number of factors that control plant numbers including factors within the plant itself (genetics, reproductive rate and mode, age, dormancy characteristics of seeds and propagules, density) (Harper 1977), the environment (edaphic conditions, effects of other plants (allelopathy, competition), climate (rainfall, light quantity and quality, temperature), topography, altitude et cetera), and by any disturbance caused by the environment (abiotic- drought, temperature stress, nutrient deficiency, toxic

chemicals and biotic- competition from other plants, damage by insects and/or pathogens). In this study, the stress caused by biotic agents is examined to attempt to shed some light on how workers in the field of biological weed control may chose more effective agents. In Canada, a cost estimate for the development and implementation of a biological control program per weed species is 18.8 to 23.7 scientist years or $1.2 to $1.5 million in 1979 (Harris 1979). More and better information on how a certain level and type of damage could potentially affect weed population dynamics may result in a more efficent and effective use of time and money.

2. Stress Physiology

Stress caused by insect pests and pathogens has been recognized almost as long as man has tilled the soil ("that which the palmerworm hath left hath the locust eaten; and that which the locust hath left hath the cankerworm eaten; and that which the cankerworm hath left hath the caterpillar eaten" Joel 1:4). The concept of biological weed control is to use nature as a tool (in the form of natural enemies) to return a natural balance to the noxious weeds which have been introduced without their limiting factors.

Stress was first defined for the mammalian system in the early 1950's by Selye as, "the nonspecific response of the body ... to any demand made upon it" (Selye 1974). Subsequent definitions are more generalized and include concepts of the recognition of a normal range of responses, the necessity for quantification of stress and the realization that stress may produce some disadvantage to the organism (Brett 1958, Bayne 1975). More recent definitions extended to the ecosystem include that of Barrett et al. (1976), "a perturbation (stressor) applied to a system (a) which is foreign to that system

or (b) which is natural ... but applied at an excessive level".

These definitions are all limited and tend to each omit some salient point for an accurate understanding of stress. In addition, scientific and colloquial uses of the term have caused a loss of clarity for the term since it has been variably used as both the causer of and the resultant response. The meaning has been so misused and distorted that Harper (1982) says it has become almost redundant and quotes Pickering (1961), who, "sets it aside... because I do not know what it means." The author therefore proposes that the following definition of stress be kept in mind throughout this work:
measurable adaptation or response of an organism to some perturbation in the environment. Physiology is defined as a branch of biology that deals with the functions and activities of life or living matter and of the physical and chemical phenomena involved. Stress physiology in this context would therefore be an examination of the effects of different types of disturbances and perturbations on the normal activities of plant life.

There are three possible sources of perturbation; climatic (temperature, water, light quality and quantity), edaphic (soil texture and structure, pH) and biotic factors (insect damage, disease development, competition, allelopathy). In response, plants may exhibit one of three degrees of stress reaction; (1) positive response (plant growth and development is stimulated) (2) zero or no response (the plant is not affected), and (3) negative response (reduction of plant growth and development). It is the latter which is desired for successful biological control.

Pest attacks can potentially initiate or accelerate a complex series of metabolic changes in the host (stress). Insects and pathogens may exert stress

on a plant through:

(a) loss of photosynthetic area. Defoliation can put a plant at a competitive disadvantage because of possible reduction of light reception, decrease of photosynthetic efficiency and possible alteration of regular assimilate distribution (Hewett 1977).

(b) loss of absorptive areas. Root damage can cause a deficiency in water and nutrient uptake with resulting repercussions such as wilting, typical deficiency symptoms et cetera.

(c) blockage or damage to translocation elements (xylem and phloem). Blockage ,modification or severing of the vascular system resulting from gall formation, pathogen infestation et cetera could cause interruption of photosynthate, nutrient and water flow.

(d) loss of reproductive capacity

(i) seed production. Loss of seed production can occur directly by feeding on the seeds and flowers of the plant or indirectly by the stress from damage to another plant organ which does not allow seed formation.

(ii) vegetative. Root bud, tuber et cetera production could also be directly or indirectly affected as for seed production.

(e) toxins. Several organisms which attack plants are capable of producing toxins that adversely affect plants (most notably plant pathogens).

(f) alteration of physiology. An example of this is growth hormone imbalance.

Other factors which may affect the plant's response include the type of tissue damaged, but also the degree of damage and the importance of the damaged plant organ to the growth and development of the plant. Synchrony with plant phenology (attack at the most susceptible period of plant growth) and also the additional effect of the climate may be an important aspects of control. The effect of the climate has been shown to be crucial for the success of some programs (Tyria jacobaea L. against Senecio jacobaeaeL.; the winter frosts in eastern Canada)(Zwölfer 1973, Harris et al. 1978a, 1978b, Myers 1980) and Chrysolina quadrigemina Suff. against Hypericum perforatum L.;dry summers (Harris 1973a). The lack of information and an appropriate theory as a result of sporadic research has as Andres (1980) states caused, "an inability to predict the impact" of biological control candidates. Therefore, basic research and a theory of how certain types and amounts of damage can potentially affect plants could be invaluable for a more effective biological control program.

In order for stress physiology to be applied properly, the target weed phenology, physiology and morphology should be understood and as Myers (1980) states, "It is striking how rarely this is done." The following sections (3 and 4) describe the target weed, Canada thistle and the biological control program against it.

3. The Case Study Organism-Cirsium arvense

> "find their frailties and by exploiting their frailties we shall come nearer to weed elimination." (Chancellor 1971).

3.1 History and Geographical Distribution

Canada thistle (Cirsium arvense (L.) Scop.), a member of the Carduinae subtribe of the Cardueae tribe of the Asteraceae family, is indigenous to Eurasia and northern Africa. It was introduced into Canada early in the colonial period in the 17th century as a crop seed impurity. Dewey (1901) reports its introduction into east New York state with oats in the late 1700's (Rousseau 1968). Although it has been speculated that the weed spread from French Canada into the United States (Hodgson 1968, Erickson 1982), Hansen (1918) states that it was probably introduced as a contaminant of crop seed into New France and New England independently at about the same time (Moore 1975, Rousseau 1968).

In the 16th century, Canada thistle spread throughout Europe and by the mid 18th century was common throughout Europe (Dewey 1901). It spread to west Asia, north India, Australia, and New Zealand by the early 1900's (Hodgson 1968, Amor and Harris 1975).

In North America, the seriousness and range of the weed continued to increase after introduction. The first control legislations were enacted in Vermont in 1795 and in New York in 1831 (Moore 1975). Holmes reported it as common in Montreal in 1821 and Macoun affirms it was abundant in Ontario in 1884 (cited from Rousseau 1968). In 1957 it was considered a noxious weed in the seed laws of all the states of the United States except Alaska, Arkansas, Hawaii and New Mexico. In Canada, Canada thistle was listed as a noxious weed in the Canadian Seeds Act in 1937 and is considered as a noxious weed in most provinces and is listed in the Revised Statutes of Quebec (1964), Ontario (1970), Manitoba (1970), Saskatchewan (1965), Alberta (1970) and British Columbia (1960) (Moore 1975).

Canada thistle is naturalized in several northern hemisphere countries. It is found in Canada in all provinces as far north as 58-59° N. In Europe, it has been found as far north as 68° N (Scandinavia) but Kolokolinikov (1931) reported it fails to flower north of 58° N in Siberia. It has been naturalized in the United States north of the 37° N but does not survive at lower latitudes. In north Africa and Afghanistan it occurs as far south as approximately 30° N. In the southern hemisphere, it is naturalized in South Africa, New Zealand and southeast Australia (Moore 1975).

Canada thistle is rated by Holm et al. (1979) as a serious weed in Finland, Lebanon, Portugal, Turkey and the United States and as a principal weed in Belgium, Bulgaria, Canada, England, Germany, Greece, India, Iran, Italy, New Zealand, Pakistan, Romania, South Africa, the Soviet Union, Tunisia and Yugoslavia and as a weed in many other parts of the world.

3.2 Botanical Description and Habitat

Canada thistle has been often described (Detmers 1927, Hayden 1934, Hodgson 1964, 1968, Moore 1975, Moore and Frankton 1974, Arnold 1980). It is a perennial weed, with slender green (sometimes brown or reddish-purple) stems of 30 to 150 cm in height which branch freely at the top. The leaves are alternate, base sessile and clasping or shortly decurrent, deep green and spiny. They are generally oblong in outline, with marginal variations from entire to deeply pinnate. Variations of the margin and of other leaf characteristics (texture, vestiture, segmentation and spininess) are the bases of varietal differentiation. The plants are dioecious (Moore 1975) or imperfectly dioecious (Hodgson 1964, Lloyd and Myall 1976), the only thistle of this type in Canada. The flower heads are numerous, small and the male and

female flower heads are easily distinguished after the bud stage. The florets are all tubular, rose-purple or less commonly white (Moore 1975). The pappus is plentiful, beige, feathery, 20 to 30 mm long on the mature achene. The achenes are 2.5 to 4 mm X 1 mm, straight or slightly curved, straw or light brown. The creeping horizontal roots, from which arise ramets, perpetuate the plant. Thistle clones occur in circular patches.

There are four recognized infraspecific varieties; var. horridum, var. vestitum, var. arvense (the type specimen, formerly mite) and var. integrifolium. These varieties are distinguished primarily by leaf characteristics. They interbreed freely and all have a chromosome number of 2n=34 (Moore and Frankton 1974). Detmers (1927) grew seedlings of all four varieties from seeds of a var. vestitum plant. The variety horridum is the most common. In Quebec, all four varieties are present as well as a white-flowered plant, forma albiflorum of the var. horridum. In addition, ecotypes differ in phenology, photoperiodism, vigour, growth habit, stomatal frequency, response to herbicides , seed dormancy and seed germination (Moore 1975).

Canada thistle prefers open mesophytic areas, but is well adapted to a wide range of conditions. A moderate temperature range of 0 to 32 ^{o}C is best, but lows of -27 to -35 ^{o}C are common through much of the range of the weed (Detmers 1927, Moore 1975). Precipitation of 400 to 750 mm/year is favourable but the weed has been found in areas with levels ranging from 300 to 1000 mm (Hodgson 1968, Amor and Harris 1974). Canada thistle grows best in deep productive, well-aerated soil (Hunter and Smith 1972) and is well suited to silt loam (Hodgson 1968) and clay soil (Detmers 1927). However, it grows on a wide variety of soils, according to Rogers (1929) in any soil except peat and

up to 2% salt. Growth is limited in poorly aerated soil or soils with a high water table (wet conditions)(Hodgson 1968). It can survive very dry conditions; Canadian specimens have been collected from clay and sandy loams, sandy clay and even sand dunes (Moore 1975). Good light intensity is required for good growth. In shaded areas, the plants are etiolated, of lower density and produce few flower heads (Bakker 1960). The southerly distribution of the plant is limited by its long day requirement; upwards of 14 hours of light are needed for flowering to occur (Link and Kommendahl 1958, Hunter and Smith 1972).

In Canada, Canada thistle occurs in almost every type of plant community, most frequently along roadsides, on railway embankments, in lawns, in gardens and in abandoned fields. It is also found in many agricultural situations; pastures (Doyon 1968), cereal crops (wheat, oats, barley), other field crops and hayfields. It occurs on stream banks, lakeshores, cleared swamps, grassy clearings in woods, margins of both deciduous and coniferous woods, wet ditches and muskeg edges, but not in extremely wet areas (Moore 1975). The fact that it occurs on both arable land and in grasslands is considered unusual among weeds (Sagar and Rawson 1964).

3.3 Phenology and Physiology

Shoots of field populations of Canada thistle originating from perennial roots emerge when soil and air temperatures have warmed to a mean weekly temperature of 5 ^{o}C (Hodgson 1968). Rosettes develop first followed by stem elongation 3 weeks later to a maximum of 3 cm/day in the last two weeks of June. From July, the growth rate gradually decreases until it is negligible in early August (Hodgson 1968). Flowering occurs in Canada from mid June or early July until September. Canada thistle is a long day plant, which flowers with

greater than 12 hour days (Link and Kommendahl 1958). Hunter and Smith (1972) found variation in the number of light hours required for flowering between ecotypes and also found some to be temperature dependent.

Seedling Development

After germination, a pair of ovoid cotyledons emerge to 1 to 2 cm above the soil, and the primary roots grow vertically down. The first foliage leaves are ovate to round in shape with coarse marginal hairs, which become spinose. Subsequent leaves are of typical shape and spininess (Hayden 1934). Lateral roots can be produced as early as the two leaf stage (Forsberg 1962). The roots thicken and extend laterally to as much as 1.5 cm (Sagar and Rawson 1964) and 1.25 m respectively in the first season (Bakker 1960). McIntyre and Hunter (1980) found that a seedling root can reach 538 m in length and produce 219 visible root buds in the first season. Later, adventitious buds from the main root can grow to the surface from 1 to 90 cm in depth (Freisen 1968).

Root Phenology and Physiology

The root system is perennial and although the shoots are killed by frost, the roots survive the winter. Rogers (1929) reported on root activity in the winter in Iowa. According to Arny (1932), the majority of the roots occur from 8 to 12 inches (20 to 30 cm) below the surface and some to a depth of 16 inches (40 cm), but vertical roots can go 6 to 10 feet (1.8 to 3 m) deep. Freisen (1968) found that most roots occur from 6 to 36 inches (15 to 90 cm) with the main concentration at 6 to 18 inches (15 to 45 cm). Hodgson (1968) reported that 54% of the roots were within 3 to 9 inches of the surface (8 to 23 cm), 30% from 9 to 15 inches (23 to 38 cm) and 16% from 15 to 21 inches (38 to 53 cm), therefore 84% are within 15 inches (38 cm) of the surface. Malzew (1931)

found thistle roots to a depth of 5.5 m in Russia (Moore 1975). In pastures, roots can reach 0.76 m below the surface. The depth depends on soil type, depth of the water table and the nature of the subsoil (Arnold 1980).

Carbohydrate Storage

Root carbohydrate content fluctuates throughout the season. It is lowest from mid June to mid July, when the flower buds begin to appear and increases until it reaches a high in mid August to September when it levels off (Arny 1932, Sagar and Rawson 1964, Hodgson 1968, Bybee et al. 1977, Ozer and Koch 1977). The main storage product is believed to be inulin (Ozer and Koch 1977).

3.4 Reproduction, Dispersal and Weediness

Seed Production

Seed production is variable, being dependent on the distance between male and female clones. Hayden (1934) found abundant seed with distances of less than 33 m and only 2 to 3 seeds/head for distances between 160 and 200 m. Hodgson (1968) found large numbers of seeds with a separating distance of 16.5 m and Amor and Harris (1974) found heads with some seed at distances up to 390 m. Salisbury (1961) cites a maximum distance of 30 m and Bakker (1960) of 50 m for seed production. The flowers are cross-pollinated usually by insects, chiefly by honeybees (Detmers 1927). Wind pollination is not as effective (Hodgson 1968).

Estimates of seed production and the number of heads per plant are variable (Table 1). The maximum number of seed recorded is 30,189 seeds/m^2 (Bakker 1960), but seed production is normally very restricted (Chancellor 1970). Canada thistle was once thought to produce no seeds at all (Boys 1905).

Table 1. Seed and Head Production of Canada Thistle

Seed Production	Reference
Seeds/head	
98 max	Hayden 1934
Seeds/plant	
5300/average 1530	Hay 1937
Seeds/head	
40-50 average	Derscheid and Schultz 1960
83 max	Derscheid and Schultz 1960
Seeds m^{-2}	
100-64,300 viable	Amor and Harris 1974
Heads/Plant	
up to 100	Detmers 1927
13-37 (average 12)	Hayden 1934
32-69	Bakker 1960
24-85	Amor and Harris 1974

Dispersal, Dormancy and Longevity of Seeds

Long distance dispersal by seeds is limited due to the habit of the pappus detaching from the seed. Bakker (1960) found only 9.9% of the pappi at 10 m from the thistle patch had an attached achene and only 0.2% at one kilometer. In Canada, seeds were rarely found beyond 244 m from the thistle patch (Alley and Chamberlain 1962). Long distance dispersal may occur by other means such as by irrigation water (Hope 1927).

The old practice of cutting the heads at the bud stage significantly reduces seed yield since development ceases after mowing (Gill 1938, Derscheid and Schultz 1960). Plants cut in bud and early bloom produce no viable seed. High percentages of viable seeds were found only 11 days after flowering and none at all until 8 to 10 days (Derscheid et al. 1956, Derscheid and Schultz 1960). After 18 days, the number of seeds/head decreased, indicating that shedding of seeds begins at this time (Derscheid et al. 1956).

The literature on dormancy and longevity of seeds is contradictory. Niethammer (1943), Buchli (1936) and Bakker (1960) state that there is a dormancy period, at least for high levels of viability, whereas Hayden (1934) and Kolk (1947) say there is none or little dormancy (Sagar and Rawson 1964).

Longevity (which includes a test of viability) has been rated at between 22 and 54 months when stored under running water (Bruns and Rasmussen 1957) and 30 months under similar conditions by Bakker (1960) and also under 40 cm of soil. In the famous Duvel buried seed experiment, up to 4.5% of the seeds were viable after 21 years at 42 inch (105 cm) (Goss 1924) but not at any of the later sampling dates (Toole and Brown 1946), whereas Chepil (1946) found no

viable seeds after 3 years in cultivated soil and rated their longevity as short to intermediate (1 to 3 years). Chippendale and Milton (1934) also found a low longevity when the seeds were buried under grassland.

Germination is usually low, variable (Table 2) and as expected influenced by germination conditions. With increased seeding depth there is slower growth and lower percent germination (Lund and Rostrup 1901, Kolk 1947).

There is some disagreement as to the importance of seed in establishing stands of Canada thistle. The invasion of the Zuider Zee seems to have been by seed (Bakker 1960), however, there is the general belief that stand establishment by seed is rare.

Seedlings require high light intensity to survive. They are slow to establish and are sensitive to shading and competition (Hodgson 1968). Bakker (1960) states that seedlings die if the light intensity received falls below 28% full sunlight and growth is reduced in 60 to 70% full sunlight. Amor and Harris (1974, 1975) found no seedling establishment in pastures, even after artificial seeding.

Reproduction by seed is less important for population maintenance and growth than vegetative propagation but due to the reported longevity, prevention of seed production is advisable.

Roots

The most impressive and obnoxious attributes that contribute to the aggressiveness of this plant are; (1) the extensive creeping root system and (2) the ability of the roots to regenerate after fragmentation.

Table 2. Germination of Canada Thistle Seeds with Different Storage and Germination Conditions

% Germ.	Storage (if known)	Germination Conditions	Reference
10-27	6 months	87 °F (~31 °C)	Hayden 1934
15-43	2 years	ster. soil	"
up to 95	fresh	unknown	"
38	dead ripe	unknown	Gill 1938
78	fresh	unknown	Derscheid et al. 1956
7-20	fresh	unknown	Bakker 1960
100	3-6 months	unknown	"
1		20 °C-light	Kumar and Irvine 1971
7.6		25 °C-light	"
37.6		30 °C-light	"
40.2		35 °C-light	"
0		20 °C and 25 °C-dark	"
16.6		30 °C-dark	"
25.4		35 °C-dark	"
52-97 ave.-78	6 months	15/30 °C, 12h light	Amor and Harris 1974

Root System

Seedlings with only two true leaves (4 to 5 weeks old) have creeping roots and produce ramets when only 6 to 8 weeks old (Bakker 1960). A plant started as a seedling can extend 1.25 m (radius) and 4.9 m^2 (area) in its first year and 5.0 m (radius) and 78.9 m^2 (area) in its second year. Established plants can extend radially 12.2 m (467.7 m^2 area) in one year (Hodgson 1960). The underground root weight of established plants can reach 2 tonnes/ha (Abromov 1969).

Sagar and Rawson (1964) classified three types of organs in the roots; (1) roots of 1 to 3 mm in diameter (2) non-typical thickened roots from 0.5 to 1.5 cm in diameter which cannot be identified as either roots or shoots and (3) subterranean shoots bearing stem apices at or below ground level and identified by scale leaves and typical stem anatomy. Food reserves in the roots facilitate the initiation of new shoots for up to one and a half years (Hodgson 1968). Roots have dormancy periods during which shoot emergence is restricted, especially in October and November and somewhat in March and April (Henson 1969). Root tissue can withstand a high level of drought. Ramet production is unaffected down to 20% of original moisture content and some shoots are still produced at 5% moisture content. However, oven dried roots produce no shoots (Forsberg 1963).

Regeneration

The ability of small fragments of roots to produce new plants (Table 3) makes Canada thistle tolerant or even dependent on cultivation. Large fragments easily produce shoots, but smaller ones may die, especially under adverse conditions (Chancellor 1970). Root bud production is stimulated by

Table 3. Regeneration Capabilities of Canada Thistle Roots

Length	Diameter	Regenerative Capacity	Reference
Threadlike roots			Prentiss 1889
1/16 inch (1.6 mm)		no plants	"
1/4 inch (6.4 mm)		no plants	"
1 inch (2.5 cm)		some plants	"
Stouter Roots			
1/16"(1.6 mm)	1/8,1/4" (3.2,6.4 mm)	no plants	"
1/4(6.4 mm)	"	14%	"
1/2(1.3 cm)	"	100%	"
5-6 cm	0.5-1.5 cm	90%	Sagar and Rawson 1964
3 cm	4-6 mm	75-85%	Henson 1969

nitrogen fertilization (McIntyre and Hunter 1980).

These characteristics of the roots make Canada thistle an aggressive and difficult to control weed.

3.5 Economic Importance

Detrimental

Canada thistle causes heavier losses in crop yield than any other perennial weed (Hunter and Smith 1972). Canada thistle is found in most crops; wheat and other cereals, corn, peas, beans, sugarbeets, potatoes, et cetera and causes yield and quality losses (Freisen 1965). It is also common in pastures and ranges, where it decreases forage yields and quality (Hodgson 1968). Since it competes for the environmental factors necessary for growth, crop yield is often reduced (Table 4) (Peschken et al. 1980). In grasslands, it is of greatest importance in poorly managed pastures and decreases the amount of grass and grazable area (Chancellor 1970).

Canada thistle harbours economically important insects and pathogens; bean aphid and stalk borer. Grazing animals scratched by the spines may develop infections (Moore 1975). Immature buds can be harvested with canning crops (peas) (Link and Kommendahl 1958).

Canada thistle has allelopathic effects on a number of plants including itself (also oats, other thistles, kochia, marshelder, foxtail barley) (Helgeson and Konsack 1950, Bendall 1975, Hoefer and Haderlie 1980).

Beneficial

The fragrant flowers are attractive to honey bees and Canada thistle may

Table 4. Economic Losses due to Canada Thistle

Thistle* Density	Yield loss #	Reference
	Wheat	
2	15	Hodgson 1963
2.4	18	Molberg 1955
2.4	1.04	Peschken et al. 1980
6	18	Cameron 1936
14	35	Hodgson 1968
24	61.3	Cameron 1936
24	10.4	Peschken et al. 1980
29.75	12.9	"
30	60	Hodgson 1963
	Soybean	
14	62	Elakkad and Behrens 1974
20	24	"
	Corn	
21	36	"
	Spring Cereals&	
32-43	40-70	Timmons 1955
	Barley	
9	22	O'Sullivan et al. 1982!
	Alfalfa	
23	52	Schreibner 1967

2

* per m
\# percentage
& Barley is most adversely affected
! An example from a yield loss equation for barley.

be classed as a honey plant (Detmers 1927). Young shoots are sometimes eaten by grazing animals. Limited human consumption is reported in Russia and by north American Indians (Rogers 1929).

3.6 Herbicide and Other Control Measures

Consistent with most perennial plants with ramifying root systems, cultivation and chemical control of Canada thistle are unreliable.

Cultivation

Cultivation is effective only if repeated regularly (Kirkland 1980). There is evidence that the weed is most vulnerable at or just before the buds become coloured and Hodgson (1968) suggested this as the best time to cultivate.

Mowing

Mowing, as cultivation, is most effective if repeated and can control small infestations in pastures (Gilchrist 1923, Welton et al. 1929, Moore 1975). To prevent viable seed production, mowing should be conducted 8 to 9 days after flowering (Derscheid et al.1956). Arny (1932) found that cutting at full bloom delayed emergence the following year. Grazing affects the weed as does mowing (Moore 1975).

Competitive crops

Alfalfa and some forage grasses can control Canada thistle infestations (Robbins et al. 1942, Thrasher et al. 1963, Schreibner1967, Moore 1975). Canada thistle is not common in older grassland, probably due to reduced light intensity, which limits growth (Fykse 1980). Crop rotation including a competitive crop is also important as a control measure (Bower 1980).

Herbicides

Canada thistle is rated as intermediate in response to 2,4-D ((2,4-dichlorophenoxy)acetic acid),MCPA ([(4-chloro-o-tolyl)oxy]acetic acid), 2,4-DB((2,4-dichlorophenoxy)butyric acid), mecoprop (2-[4-chloro-0-tolyl)oxy]propionic acid), and between susceptible and intermediate for dicamba (3,6-dichloro-0-anisic acid) (Ont. Weed Comm. 1983).

Herbicide control both on arable land and grassland is difficult. Although shoots and parts of the root system are killed, complete kill of the root system is virtually impossible, except with picloram (4-amino-3,5,6-trichloropicolinic acid) (Kreps and Alley 1967, Hodgson 1968, Moore 1975, Vanden Born 1980). This is because the herbicide is generally not translocated into the root system (Burt 1974, Barradarie et al. 1980). Treatments that increase root bud activity (growth hormones) may increase the amount of herbicide translocated to the roots. Research with growth hormones shows some promise (Kossatz et al. 1980, Sterrett and Hodgson 1983).

2,4-D and MCPA use has reduced the prevalence of Canada thistle especially in small cereals (Chancellor 1973). 2,4-D and MCPA are more effective with a competitive crop (wheat, pasture grass or silage corn) and with nitrogen fertilization (Hodgson 1968, Carpenter 1972). However, repeated treatments of 2,4-D and MCPA are needed to control Canada thistle (Moore 1975). Some herbicides are more effective if applied at bud to bloom stage (glyphosate (N-(phosphonomethyl)glycine)(Eady 1980), amitrole (3-amino-s-triazole)(Billett 1980)) and others between vegetative stage and bud stage (dicamba, phenoxys) (Marriage 1980). Soil sterilants can provide control for the season, but root recovery occurs (Moore 1975). Repeated sterilization would be required and is

not recommended.

A problem with Canada thistle control is a differential response to herbicides by ecotypes (Freisen 1968, Hodgson 1970, 1973, Hunter and Smith, 1972, Marriage 1973). This is not due to differences in lipid content (Hodgson 1973), stomatal variations (Hodgson and Moore 1972, Hunter and Smith 1972), leaf weight (Hunter and Smith 1972) or phenotypic variations (leaf and flower) (Hodgson 1970). Burt and Muzik (1970) found differences in alteration of nitrogen metabolism between resistant and susceptible ecotypes.

4 Biological Control of Canada Thistle

Classical biological control

The classical biological control program for Canada thistle was initiated in the late 1950's. Surveys for phytophagous insects associated with Canada thistle began in 1959 in western and west-central Europe, parts of the Mediterranean area, Iran, northern Pakistan and Japan (Zwölfer 1965, 1969). Zwölfer (1965) found 78 insect species, six of which were monophagous; Urophora cardui (L.), Lobesia fuligana Hb, Lema cyanella (L.), Altica cirsii Istrelson, Chomaphis cirsii CB, and Capitophorus braggi (Gill.). Several narrowly oligophagous species were also found; Urophora stylata (F.), Altica carduorum Guer. and Tingis ampliata (H-S). Five of the above species were selected for further detailed study, three have been released in Canada; Urophora cardui, Altica carduorum Guerin-Meneville and Ceutorhynchus litura (F.). Lema cyanella and Tingis ampliata have not been authorized for free release although Lema cyanella is being evaluated in restricted field trials in cage experiments.

Altica carduorum (Coleoptera: Chrysomelidae)

This leaf defoliating and shoot feeding beetle was the first of the candidates to be studied. After examination of the life history and ecology and preliminary screening (Zwölfer 1965), the insect was submitted to intense host specificity tests with a reported feeding range restricted to Cirsium, Silybum, and Carduus of the subtribe Carduineae with a preference for Canada thistle (Harris 1964, Zwölfer 1969). Releases were made from 1963 to 1970 across Canada, in South Dakota and other parts of the United States and in Britain. None of the colonies survived (Baker et al. 1972, Andres and Davis 1971, Peschken et al. 1970, Peschken 1977a, Andres 1980, Peschken et al. 1980a). Analysis of the results indicate that failure was probably due to combined factors of unsuitable climate (Peschken et al. 1970, Baker et al. 1972, Schaber et al. 1975, Peschken 1977a) and predation (Peschken et al. 1970, Peschken 1977a).

Ceutorhynchus litura (Coleoptera:Curculionidae)

C. litura, rated by Zwölfer (1965) as almost monophagous, is a weevil with a variety of attack strategies depending on its stage. The imagines and ovipositing females feed on the leaves of the rosette and older plants and the larvae mine the stems down to the root collar and sometimes into the root. Heavy larval attack seriously reduces plant vigour. Host specificity tests indicate that C. litura is restricted to Cirsium, Silybum, and Carduus genera with Canada thistle as the primary host (Zwölfer and Harris 1966). Releases made between 1965 and 1967 in Ontario were found to thrive (Peschken and Beecher 1973, Peschken 1979). Further releases made between 1973 and 1978 in four other provinces (Saskatchewan, Alberta, British Columbia and New Brunswick) are all increasing (Schroeder 1980). Although exact effects on population density are uncertain, it no doubt stresses the plant and it is

reasonable to continue releases (Andres 1980, Peschken 1980a). Also, this insect does not interfere with the life cycles of previously existing fauna; an important consideration in biological control. C. litura rates at between 17 and 20 on Harris' scale (Harris 1973a) of effectiveness (Peschken and Wilkinson 1979).

Lema cyanella (Coleoptera:Chrysomelidae)

Zwolfer (1969) found in host specificity tests in Europe that L. cyanella feed exclusively on the Cirsium, Carduus and Silybum genera; similar to the feeding patterns of the two other beetles already discussed. Adults and larvae feed on the leaves of Canada thistle and cause considerable damage. It is compatible with other released insects. Further host specificity tests were performed in Canada (Peschken and Johnson 1979) and it was recommended for release, once it was reared clean of a Nosema disease. It survived cage tests in Saskatchewan and was rated by Harris' effectiveness score (Harris 1973a) at 22 of a possible 45. However, since this time, release has been prevented until questions concerning the threat of extinction or severe damage to endangered native Cirsium are addressed (Andres 1980, Peschken et al. 1980b).

Tingis ampliata (H.S.) (Tingidae:Heteroptera)

Zwolfer (1965) rated T. ampliata as oligophagous and in Europe it is confined to Cirsium arvense. This lace bug sucks on the leaves and apparently produces a toxin that causes the leaves to yellow, wrinkle and die (Peschken et al. 1980b). Since T. ampliata was considered host specific by Southwood and Scudder (1956), further tests were performed on insects imported from Britain to Canada. The insect was found capable of production of viable eggs and of

development on the economic plants safflower and globe artichoke (both grown in North America). It was therefore recommended that I. ampliata not be released in Canada (Peschken and Johnson 1979, Peschken et al. 1980b).

Urophora cardui (L.) (Diptera:Tephritidae)

The larvae of this host specific fly cause the formation of large stem galls. This insect has been released in Canada since 1974 and is surviving in several Canadian locations. Since this is one of the case study insects, details will be given later.

Unintentional or accidental introduction

Several endemic or at least accidentally introduced natural enemies already occur on Canada thistle. Some of these insects include Orellia ruficauda F.. The larvae of this small fly feed upon the developing seeds of Canada thistle. Cleonus piger larvae feed in the root crown region of Canada thistle plants. This insect was first found in Canada in the early 1900's. Several stages of the tortoise beetle Cassida rubiginosa defoliate the leaves of Canada thistle. This insect was first reported in Canada in 1902. Zwölfer (1965) rated these insects as oligophagous. In addition, a host specific rust Puccinia punctiformis also occurs in Canada and was included with the insects as part of this study.

The purpose of this study was to examine the stress physiology of a perennial weed under the effects of several natural enemies attacking different plant organs. Both quantitative and qualitative methods were used to determine damage levels, for a better understanding of the effectiveness of the various types of damage and responses of the plant to the damage. The weed chosen for

the case study was Cirsium arvense and the natural enemies chosen were: Orellia ruficauda- a seed head fly; Cassida rubiginosa- a leaf defoliating beetle; Urophora cardui- a stem gall-causing fly; Cleonus piger- a root crown inhabiting weevil and Puccinia punctiformis- a systemic rust.

II. EXPERIMENTAL

A. Field Experiments

A.1 Materials and Methods

Over two summers (1981 and 1982) seasonally permanent quadrats (0.25 m^2) were chosen by a modified stratified technique in four fields on the Macdonald College farm (Fig. 1). The number of quadrats sampled per site were: 75 in 1981 and 60 in 1982 for site 1, 40 in both years for site 2, 20 in 1981 and 30 in 1982 for site 3 and 40 in both years for site 4. The initial plant populations examined were: 463 plants in 1981 and 394 in 1982 for site 1, 322 in 1981 and 188 in 1982 for site 2, 156 in 1981 and 208 in 1982 for site 3 and 177 in 1981 and 92 in 1982 for site 4. Data taken at each quadrat at each sampling date included; number of plants, plant height, diseases and insects attacking each plant, numbers and life cycles of natural enemies, timing of flower bud and flower production and flower number. Soil texture was determined for each site.

For each date, the heights of the "healthy" or non-attacked plants were compared to the heights of attacked plants by the Student's t-test. Each category was compared with its counterpart over the two years (where available) for any height differences by the Student's t-test. For simplicity, the occurrence of Puccinia punctiformis will be referred to as Rusted(systemic or sys) when the infection is systemic and as Rusted(secondary or SR) when the infection is secondary or localized; Cassida rubiginosa as Defoliated(Defol); Urophora cardui as Galled (MS- mainshoot and SS-sideshoot) and Cleonus piger as Root Crown inhabited (RCrown); unidentified stem borer as Bored; Sheep damage as Trampled; Aster Yellows as Asters and unidentified brown aphid as Aphid.

Figure 1:

Map of Macdonald College farm and research areas indicating the locations of field sampling sites

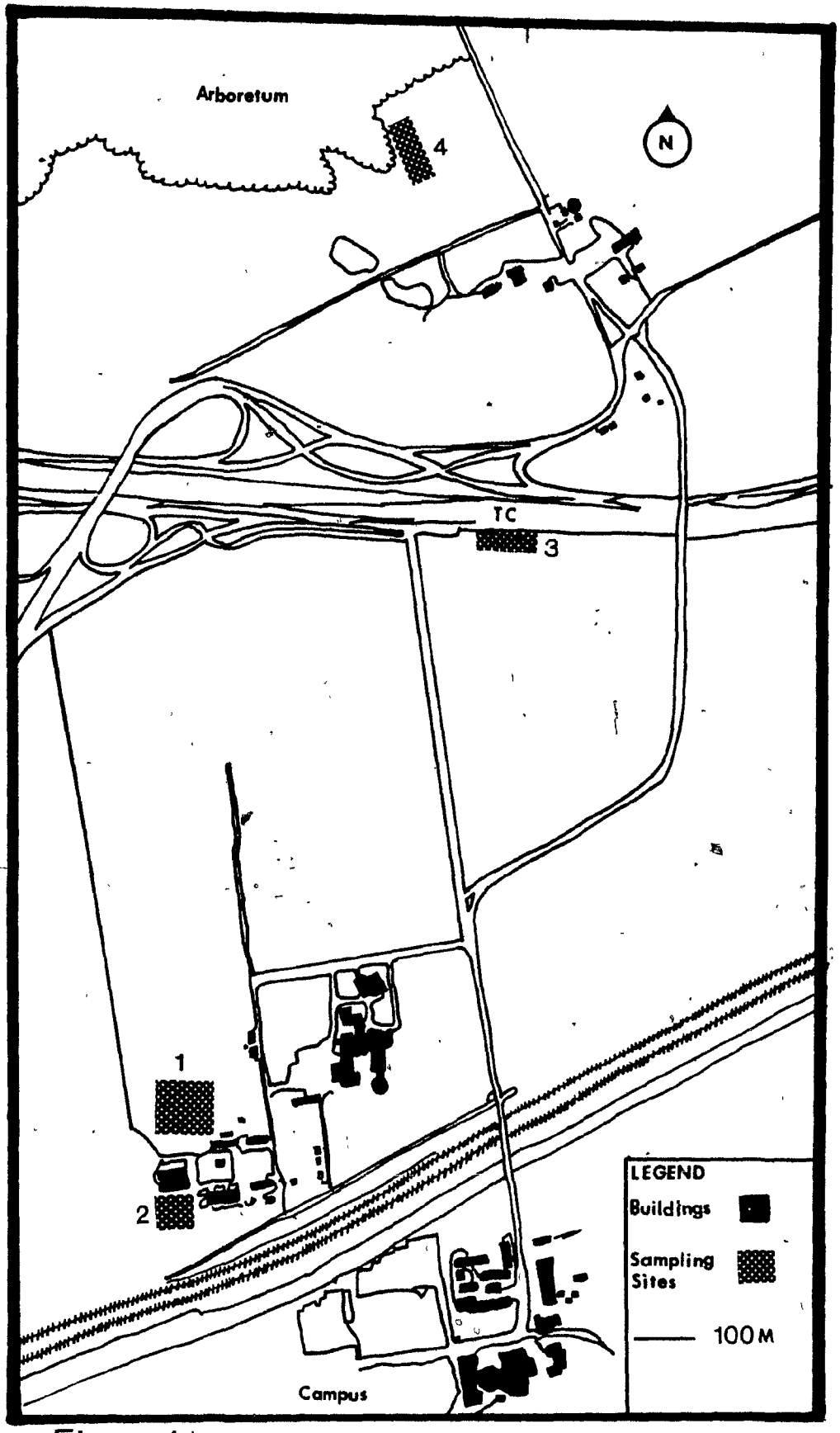

Arboretum
4
N
TC
3
1
2
LEGEND
Buildings
Sampling Sites
100 M
Campus

Figure 1.

A.2 Results

Site 1: Cow Pasture

This is an approximately 5 ha field containing a number of thistle patches and is grazed by dairy cattle. The soil texture was determined to be a clay loam. The area sampled covered a hollow which was fairly well drained and a region of moderate incline. The thistle was of the variety horridum and the main population was a female clone. The associated vegetation was close-cropped grass, clover (Trifolium) spp., dandelion (Taraxacum officinale Weber), plantain (Plantago major L.) and timothy (Phleum pratense L.).

Canada thistle density was fairly high with a maximum of 25-27 plants/m^2. The natural enemies present included rust(systemic) (5-24%), defoliation (low) and gall (increased to 7% in 1982) (Table 5). Rust and defoliation plants were significantly taller and gall(MS) plants were significantly shorter than healthy plants. Rust(systemic) plants had no flower production, defoliation and gall plants seemed to have more plants bearing flowers than healthy plants. The plant heights were slightly lower in 1982, as was flower production (Table 6).

Site 2: Sheep Pasture

This is an approximately one ha field and was used to pasture sheep. The soil texture was determined as a sandy loam, but more fine textured than the other sites. The area sampled was on a slight incline, the soil was well drained and there was a good stand of mixed pasture grasses. The thistle was of the variety horridum and there were two clones; one male and the other female. The sheep caused considerable damage to the thistle population,

Table 5. Population Dynamics of Natural Enemies of Canada Thistle Site 1

Plant Condition	Sampling Dates 20/05//26/05@	04/06//08/06	24/06//22/06	24/07//21/07	19/08//16/08
	----------Density(per m^2)(Frequency(%)){Proportion of total(%)}----------				
Total					
1981	24.8(100){-}	23.2(100){-}	20.0(100){-}	18.4(100){-}	22.8(100){-}
1982	26.4(100){-}	26.4(100){-}	24.4(100){-}	12.0(100){-}	13.6(100){-}
Healthy					
1981	23.6(100){96}	22.4(100){96}	16.8(100){96}	16.4(87){93}	16.8(100){93}
1982	24.8(100){82}	16.8(100){79}	12.0(73){70}	4.0(35){26}	0.3(8)
Rusted (Systemic)					
1981	1.2(11){4}	1.2(11){4}	0.6(8){3}	0.8(10){5}	0.4(1.4){5}
1982	5.2(45){10}	4.4(42){20}	4.4(43){24}	1.2(21){8}	0.1(3)
Rusted (Secondary)					
1981	- - -	- - -	- - -	- - -	- - -
1982	- - -	0.1(3){1}	10.4(8){6}	9.2(88){60}	12.0(97)
Defoliated /Rusted(Secondary)					
1981	- - -	- - -	- - -	- - -	- - -
1982	- - -	- - -	0.8(16){4}	0.4(9){3}	0.2(5){2}
Galled					
1981	- - -	- - -	- - -	0.4(3.4){2}	1.2(4){2}
1982	- - -	- - -	- - -	- - -	- - -
Galled/Rusted(Secondary)					
1981	- - -	- - -	- - -	- - -	- - -
1982	- - -	- - -	0.8(2){1}	0.4(9){2}	0.8(15){5}
Defoliated					
1981	- - -	- - -	0.1(3){1}	0.1(3){1}	0.2(4){2}
1982	- - -	- - -	- - -	- - -	- - -

@ Sampling date for 1981//date for 1982.

Table 6. Heights and Flower Production -Site 1.

Plant	20/05//26/05		04/06//08/06		24/06//21/06		24/07//21/07		19/08//16/08	
Cond.	1981	1982	1981	1982	1981	1982	1981	1982	1981	1982
Height(cm)										
Total			&	&	&	&	* &	&		
	14.0	15.0	28.9	24.0	43.6	34.9	51.3	43.8	48.8	46.6
Healthy			&	&	&	&	&	&		
	14.2	14.8	28.8	25.1	41.3	33.0	49.4	43.6	53.0	44.4
Rusted(sys)		*		*		*				
	15.5	18.4	29.8	33.3	41.3	42.1		42.1		
GalledMS		*		*		*		*		*
		5.0		13.3		17.0		24.5		26.5
GalledSS										*
		15.8		27.6		42.4		56.5		60.3
Defol/Rusted(sys)				*		*		*		
		17.4		31.7		45.7		63.3		
% with flower buds										
Total	0	0	0	9	46	59	25	27	23	
Healthy	0	0	0	13	51	78	29	12	25	-
% with flowers										
Total	0	0	0	0	0	0	39	45		
Healthy	0	0	0	0	0	1	42	-		

#flowers±S.D. at last sampling date

	1981	1982
Total	27.4±24.4	7.8±7.0
Healthy	23.1±17.3	14.0±10.1
Rusted(sys)	0	0
Defoliated	3.0±0	9.0±5.8
GalledMS	-	0
GalledSS	-	10.0±0
Rusted(SR)	34.5±27.3	7.0±6.6

t-tests: within years — no indication-no significant difference from healthy; *-significantly different from healthy-0.05 level

among years — &- significantly different at 0.05 level; no indication- no significant difference

@- sampling dates- 1981//1982

especially in 1982, when a large flock was grazed and a large number of the thistles within the quadrats were trampled and died. Associated vegetation was primarily tall mixed pasture grasses. This field was also the location of the release site for Urophora cardui made in the fall of 1979 (relocated from Compton, Quebec).

Thistle density decreased from 26.4 m^2 in 1981 to 18.8 m^2 in 1982. The natural enemies present included; defoliation (more prevalent here than the other three sites), gall, rust (more in 1982 than 1981), and borer. Gall density and frequency were low and consisted of a small percentage of the total population, but increased in 1982 to an average of about 10% of the population (Table 7). Defoliation plants and gall(SS) plants were at times significantly taller than healthy plants, whereas the MS galled plants were significantly shorter. The percentage of plants with flowers is very high for gall(SS) and borer plants, indicating little inhibition of flowering by these natural enemies. Flower production was similar for all natural enemy combinations, except for the MS galled plants which had not produced flowers by the sampling date. There is no apparent difference in growth and development between the two years (Table 8).

Site 3: Trans-Canada

This is a small area (< 0.4 ha) of ground parallel to the fence beside the Trans-Canada (Highway 40) and near a Macdonald farm field. The soil is gravel. The quadrats ran parallel to the fence. Two clones of thistles, one male and the other female were included in the quadrats, both of the variety horridum. The soil was well drained. Associated vegetation included; mixed grasses, tufted vetch (Vicia cracca L.); mustard spp., Euphorbia spp.

Table 7. Population Dynamics of Natural Enemies of Canada Thistle- Site 2

Plant Condition	Sampling Dates 16/05//16/05@	01/06//02/06	18/06//17/06	17/07//30/07	23/07//29/07
	Density(per m^2){Frequency(%)}{Proportion of total(%)}				
Total					
1981	26.4(100){-}	24.0(100){-}	20.8(100){-}	19.2(100){-}	18.4(100){-}
1982	18.8(100){-}	20.4(100){-}	10.4(100){-}	12.4(100){-}	- - -
Healthy					
1981	23.6(94){90}	21.6(96){90}	18.0(98){86}	14.4(97){74}	9.6(100){54}
1982	10.0(93){62}	10.0(100){62}	5.6(50){46}	4.0(35){3}	- - -
Defoliated					
1981	1.2(18){4}	1.2(22){5}	2.4(33){12}	3.2(39){1}	2.4(31){13}
1982	0.8(8){4}	1.2(31){10}	2.8(41){21}	2.4(47){21}	- - -
Galled					
1981	- - -	- - -	- - -	0.8(19){4}	3.2(45){18}
1982	- - -	- - -	- - -	1.4(24){11}	- - -
Rusted(Systemic)					
1981	1.6(14){6}	1.2(15){5}	0.4(6){23}	0.4(6){23}	0.8(14){5}
1982	5.6(33){34}	4.0(8){23}	0.8(9){5}	- - -	- - -
Defoliated/Galled					
1981	- - -	- - -	- - -	0.4(11){3}	1.6(24){9}
Bored					
1982	- - -	0.3(8){2}	0.2(6){2}	- - -	- - -
Trampled					
1982	- - -	0.3(8){2}	0.3(44){25}	4.8(65){36}	4.0(60){42}
Aphid					
1982	- - -	0.1(3){1}	0.2(6){2}	- - -	- - -

@ Sampling dates 1981//1982

Table 8. Heights and Flower Production - Site 2.

Plant Condition	Sampling date 16/05//16/05 1981	1982	01/06//02/06 1981	1982	18/06//17/06 1981	1982	07/07//30/06 1981	1982	23/07//29/07 1981	1982
Height(cm)										
Total	21.1		39.8	45.0	53.8	55.0	65.7	69.8	69.2	
Healthy	23.4		41.7	46.2	51.6	58.5	64.6	70.5	73.8	83.6
Defol	* 18.6		& 40.7	& 51.0	62.0	61.1	* 81.8	72.4	* 90.8	81.7
Galled/Defol	21.6		44.8	* 42.0	& 67.3	* & 32.0	& 85.3	&* 51.0	85.9	
Galled/MS	* 13.7		* 23.7		* 29.3		* 32.5		* 38.8	
GalledSS	21.9		40.6	* 54.5	55.8	* 71.8	76.2	* 91.3	75.0	
Rusted(Sys)	* 18.7		35.7	* 32.3	* 33.0	* 39.8	* 43.0			
Bored				* 52.0				* 49.0	49.0	
% with flower buds										
Total	0	-	0	4	41	65	16	-	11	10
Healthy	0	-	0	8	64	91	31	79	25	5
Defol	0	-	0	0	93	-	-	-	-	21
GalledSS	0	-	0	0	0	0	17	25	25	0
Rusted(Sys)	0	-	0	0	0	11	-	-	0	0
Bored	-	-	-	0	-	100	-	100		
% with flowers										
Total	0	-	0	0	0	0	31	-	42	60
Healthy	0	-	0	0	0	0	31	-	43	82
Defol	0	-	0	0	0	0	87	-	87	-
GallSS	0	-	0	0	0	0	20	-	100	100

 # flowers ± S.D. at last sampling date

	1981	1982
Total	30.5±32.5	
Healthy	15.0±8.2	31.7±16.5
Defol.	52.8±46.8	40.0±22.1
GalledSS	44.7±45.9	38.0±0
GalledMS	only Flower buds	
Bored		34.5±29.0

t-test Within Years: no indication-not significantly different from healthy; *: significantly different at 0.05 level from healthy

Among years: &: significantly different at 0.05 level; no indication: no significant difference

milkweed (Asclepias syriaca L.), dandelion, burdock (Arctium minus Hill), and some shrubs and small trees (Poplar spp).

Thistle density was high with greater than 30 plants per m^2. The natural enemies present included; defoliation, rust(Systemic and SR), asters and root crown inhabitor. Rust was a common natural enemy and contributed 15-30% to the total population. Defoliation occurrence was variable and was less in 1981 than in 1982 (Table 9). All heights were significantly lower in 1982 than in 1981. Rust(syst) plants were taller early in the season than healthy plants. Defoliation and root crown inhabited plants were often taller than healthy plants. Flowering parameters were similar for all conditions (Table 10).

Site 4: Beef Barn

This is an abandoned field (approximately 0.5 ha) near the Morgan Arboretum. The land is level and the soil texture was determined as a sandy clay loam. The plants were of the variety horridum s.v. albiflorum. Associated vegetation included goldenrod (Solidago spp.), Physalis spp., mixed grasses, vetch and dandelion.

Thistle density was lower at this site than the other sites with less than 20 plants/m^2. The rust(syst) proportion of the total population remained the same at 22% for 1981 and 1982. The natural enemies present were defoliation, rust(syst), root crown inhabitor, borer and asters. Asters disease was most common at this site (maximum of 24%) (Table 11). There was no great height difference found over the two years. Rust(syst) plants were significantly taller at the beginning of the season than healthy plants. There was little difference in flower production for plants attacked by different natural enemy combinations (Table 12).

Table 9. Population Dynamics of Natural Enemies on Canada Thistle- Site 3.

Plant Condition	Sampling Dates 29/05//26/05@	18/06//24/06	22/07//22/07	20/08//19/08
	------Density(per m^2)(Frequency(%)){Proportion of total(%)}---------			
Total				
1981	31.2(100){-}	26.4(100){-}	14.8(100){-}	14.0(100){-}
1982	33.2(100){-}	27.6(100){-}	20.0(100){-}	22.0(100){-}
Healthy				
1981	20.8(100){67}	16.4(83){62}	6.8(60){45}	4.4(55){31}
1982	21.2(96){65}	18.0(100){57}	12.0(86){54}	4.0(55){22}
Defoliated				
1981	0.4(10){2}	1.6(22){6}	- - -	- - -
1982	6.4(60){20}	8.0(83){25}	6.4(68){3}	4.8(50){25}
Rusted(Systemic)				
1981	8.4(55){27}	5.2(50){20}	- - -	- - -
1982	8.0(40){15}	2.0(30){7}	0.6(10){3}	- - -
Rusted(Systemic)/Defol.				
1981	0.4(5){1}	0.4(6){2}	- - -	- - -
1982	0.4(8){1}	2.0(13){2}	- - -	- - -
Defol./Rusted(SR)				
1981	- - -	- - -	1.2(13){9}	2.0(36){15}
1982	- - -	1.6(17){4}	1.6(18){7}	2.8(40){14}
Rusted(SR)				
1981	- - -	- - -	2.0(33){14}	6.8(64){49}
1982	- - -	1.6(17){4}	1.2(23){5}	6.4(75){34}
Root Crown Inhabited				
1982	- - -	0.2(5){1}	0.2(5){1}	0.4(5){2}
Bored				
1982	- - -	0.4(9){1}	0.2(5){1}	0.2(5){1}

@ Sampling dates 1981//1982

Table 10. Heights and Flower Production - Site 3.

	Sampling Dates 29/05//26/05@ 1981	1982	18/06//09/06 1981	1982	03/07//24/06 1981	1982	22/07//22/07 1981	1982	20/08//19/08 1981	1982
Height(cm)										
Total	& 28.5	& 20.9	& 61.3	& 34.0	& 86.5	& 44.2	& 92.8	& 59.6	92.5	60.9
Healthy	& 26.4	& 19.5	& 59.6	& 29.5	& 84.2	& 44.6	& 89.9	& 59.8	84.4	
Rusted(Sys)	*& 30.9	*& 22.7	& 64.4	*& 35.9	& 89.6	& 44.0		57.0		
Rust(sys)/Def	* 37.0	* 35.0	& 63.0	*& 52.0	77.3					
Defol.		* 21.5	& 63.5	& 33.7	*& 95.3	& 47.0	*& 102.3	& 61.1	* 101.8	56.9
Root Crown		* 24.5		* 44.7		* 52.7		* 72.2		76.0
Rusted(SR)					45.3		& 98.8	& 60.6	84.4	65.8
% with flower buds										
Total	0	0	24	21	44	50	17	7	0	-
Healthy	0	0	34	24	44	50	17	7	0	-
Rust(syst)	0	0	0	0	8	8	0	0	0	0
Rust(sy)Def	0	0	0	50	0	-	-	-	0	33
Defoliated	-	0	38	27	75	30	6	-	-	-
Rust(SR)	0	0	0	0	-	75	0	-	-	-
Root Crown	-	0	-	16	-	50	-	0	0	3
% with flowers										
Total	0	0	0	0	7	1	63	58	84	66
Healthy	0	0	0	0	14	1	78	53	59	
Defol.	-	0	0	0	0	27	72	60	80	
Rust(SR)	-	-	-	-	-	-	-	71	-	93

 # flowers ±S.D. at last sampling date

	1981	1982
Total	25.5±25.7	21.7±17.4
Defol.	23.1±17.1	25.1±23.9
Root Crown	43.2±47	22.0±12.7
Rust(SR)	22.3±22.3	21.9±18.2

t-test Within years no indication- not significantly different from healthy
*= significantly different at the 0.05 level from healthy
Among years &= significant difference at 0.05 level
no indication= no significant difference

@ sampling dates- 1981//1982

Table 11. Population Dynamics of Natural Enemies of Canada Thistle- Site 4.

	Sampling Dates 27/05//01/06@	17/06//15/06	02/07//30/06	22/07//01/08	08/08//24/08
	----------Density(per m^2)(Frequency(%))(Proportion of total(%))--------				
Total					
1981	17.6(100){-}	15.2(100){-}	14.0(100){-}	14.0(100){-}	12.4(100){-}
1982	17.6(100){-}	18.4(100){-}	18.0(100){-}	12.8(100){-}	12.4(100){-}
Healthy					
1981	12.4(88){73}	10.0(90){62}	10.0(93){66}	9.2(88){67}	8.4(91){70}
1982	11.6(85){74}	7.6(75){57}	5.2(68){33}	4.8(69){42}	3.6 69 60
Rusted(Systemic)					
1981	3.6(28){22}	3.6(27){21}	1.2(14){8}	0.8(8){5}	0.4(5){3}
1982	2.8(30){18}	2.8(23){21}	2.8(26){17}	0.8(16){10}	- - -
Asters					
1981	- - -	2.0(27){12}	2.4(32){16}	3.2(84){24}	4.0(62){20}
1982	- - -	- - -	2.8(37){22}	0.8(16){5}	0.8(15){10}
Aster/Defoliated					
1981	- - -	- - -	0.8(14){6}	0.2(4){1}	- - -
Defoliated/Rusted(sys)					
1982	0.2(5){1}	0.3(8){2}	0.1(3){1}	- - -	- - -
Bored					
1982	0.3(3){2}	0.8(13){7}	0.8(16){5}	0.4(17){5}	0.3(8){5}
Rusted(SR)					
1982	- - -	- - -	2.0(16){12}	2.0(29){18}	- - -
Defoliated					
1981	0.8(10){6}	0.8(12){5}	0.4(14){4}	0.4(12){3}	0.8(14){6}
1982	0.8(15){5}	1.6(28){12}	1.6(32){10}	1.2(34){13}	0.3(8){5}

@ Sampling dates 1981//1982

Table 12. Heights and Flower Production- Site 4.

	Sampling Dates									
	27/05//01/06@		17/06//15/06		12/07//30/06		22/07//11/08		08/08//24/08	
	1981	1982	1981	1982	1981	1982	1981	1982	1981	1982
Height(cm)										
Total	&	*&	&	*&	&	*&		*		*
	16.9	33.4	41.1	46.1	54.1	60.2	66.3	70.5		73.0
Healthy	&	&								
	15.8	30.1	40.7	40.9	52.6	50.6	65.3	62.5		67.6
Rust(sys)	*&	&	&	*&		*		*		
	20.8	35.5	40.9	47.3	50.2	58.8		53.0		
Rust(sys)/Def.		*								
		50.0		61.7		77.5				
Aster/Rust(SR)						*		*		*
		29.4		44.4		61.8		74.2		76.5
Bored		*		*		*				
		35.0		37.5		41.0		52.0		60.0
Defol.				*						
		34.4		49.5		58.5		76.6		78.9
Rust(SR)						*				
		31.8		43.9		57.9		69.6		71.5
% with flower buds										
Total	0	0	15	22	28	51	-	1	0	0
Healthy	0	0	12	25	38	52	5	2	10	0
Rust(sys)	0	0	0	0	0	10	-	0	-	-
Defol.	-	0	-	40	-	73	-	0	-	0
% with flowers										
Total	0	0	0	0	0	0	33	58	64	52
Healthy	0	0	0	0	0	0	37	56	87	42
Rust(sys)	0	0	0	0	0	0	-	17	-	-
Defol.	-	0	-	0	-	0	-	63	-	63
Asters	0	0	0	0	0	0	-	-	58	67

	# of Flowers ±S.D. at last sampling date	
	1981	1982
Total	21.4±17.1	17.5±14.1
Healthy	18.6±17.3	12.1±9.4
Rust(SR)		17.5±10.6
Asters	17.8±13.4	17.8±18.5
Defol.		21.8±15.5

t-tests Within years ns- not significantly different from healthy
*- significantly different at 0.05 level from healthy
Among years &- significantly different at 0.05 level
no indication- no significant difference

@ sampling dates 1981//1982

Overall View:

Density and frequency of healthy plants slowly decreased over the summer, as a larger proportion of the plants acquired damage by a natural enemy. Rust (syst) was the most common in site 3 (Table 13). The root crown inhabitor was most noticeable at site 3, where the soil was the poorest. Galls were found in two sites ; sites 1 and 2 and had also migrated to site 3 in 1982 at a very low level (none in the samples). Heights were generally lower in 1982 than in 1981. Flowering was reduced by MS gall occurrence and by rust, but not generally affected by the other natural enemies.

Table 13. Comparison of Selected Variables for all Sites at a Date near to end of the Season for Both Years

	Density		Frequency		% flowering		Height(cm)		Percent	
Category	1981	1982	1981	1982	1981	1982	1981	1982	1981	1982
Total										
Site 1	18.4	12.0	100	100	23	26	51.3	43.8	-	-
Site 2	18.4	9.6	100	100	53	69	69.2		-	-
Site 3	14.8	20.8	100	100	56	25	86.5	44.2	-	-
Site 4	14.0	12.8	100	100	43	59	66.3	70.5	-	-
Healthy										
Site 1	16.8	4.0	100	35	67	13	39.4	26.7	73	26
Site 2	9.6	3.6	100	40	68	86	73.8	83.6	54	36
Site 3	20.8	21.6	100	96	59	56	84.2	44.6	67	65
Site 4	19.2	4.8	88	69	40	58	65.3	62.5	67	42
Rusted(Systemic)										
Site 1	0.8	1.2	10	21	0	0	39.4	42.1	5	8
Site 2	0.8	-	14	-	0	0	-	-	5	-
Site 3	8.4	8.0	55	40	8	8	89.6	44.0	27	15
Site 4	0.8	0.8	8	16	0	7	-	53.0	5	10
Defoliated										
Site 1	0.1	-	3	-	50	-	-	64.0	-	1
Site 2	2.4	1.2	31	27	-	43	90.3	81.7	13	14
Site 3	-	6.4	-	68	75	30	95.3	47.0	3	3
Site 4	0.4	1.2	12	34	-	64	-	76.6	3	13
Root crown inhabited										
Site 3	-	0.2	-	5	-	100	-	52.7	-	1
Galled										
Site 1	0.4	0.4	3	9	-	100	26.5/60.3(MS/SS)		-	2
Site 2	3.2	0.8	45	20	-	100	38/75(MS/SS)		-	18 8
Rusted(SR)										
Site 1	-	9.2	-	88	-	-	-	43.6	-	60
Site 3	2.0	1.2	33	23	-	75	-	45.3	14	5
Site 4	-	2.0	-	29	-	-	-	69.6	-	-

A.3 Discussion:

Field results indicated that in general, Cassida rubiginosa was not a common natural enemy (low density and frequency) and that the plants with C. rubiginosa damage are generally taller than plants which are not attacked by any natural enemies (healthy). This may be due to the possibility that the insects were attracted to the taller plants in the population or that the low level of defoliation (only a few plants had greater than 50% defoliation) stimulated plant growth. C. rubiginosa were regularly attracted to systemically rusted plants. The percentage of plants that flowered and the flower production for defoliated plants were similar, although somewhat elevated above those of healthy plants. From this data, it can be said that in the field, damage caused by C. rubiginosa does not adversely affect plant growth or floral production.

Urophora cardui galls were not present at site 1 in 1981, but the following year consisted of 5% of the total population indicating a rapid spread of the fly since its release in the fall of 1979. The gall proportion decreased in site 2 (release site) over the same time period, but this was probably the consequence of a general decrease in plant density due to sheep grazing. Sideshoot galled plants were generally taller than healthy plants, whereas mainshoot galled plants were shorter. Floral production was similar to the healthy for plants with sideshoot galls , but was reduced for plants with mainshoot galls. This indicated either that mainshoot galls are effective in reducing plant growth and flower production or that plants with mainshoot galls were shorter or later emerging than healthy or sideshoot galled plants. In addition, in 1982, galls were also found at site 3, 1.3 kilometers distant from

the release site at site 2, indicating a favourable migration of the fly population.

Plants attacked by the stem borer, as with plants attacked by Cassida rubiginosa are taller than the healthy plants, but floral production was similar. The occurrence of the borer was erratic. Although plants attacked by the borer sometimes had shoot dieback and reduced floral production the insect has a minor effect on Canada thistle population dynamics, since it was relatively rare and erratic.

Cleonus piger attacked plants were generally taller than the healthy plants. This may also be due the selective choice of larger plants by the insect. Plant death due to Cleonus piger was found only at Site 3 which has poor soil. Peschken (unpublished) also found C. piger was capable of causing plant death if the plants were growing in poor soil.

Systemically rusted plants generally were taller than healthy plants early in the season but did not survive the season and rarely produced flowers. Rusted plants attained a maximum of 34% of the total population, but normally had a range between 10 and 20% early in the season. Rust was least common in the cow pasture (Site 1), followed by the beef barn (Site 4) and was most common in sites 2 and 3. Both sites 2 and 3 had good grass growth and are more mesic tham sites 1 and 4. Secondary rust was very common at the end of the season in 1982, especially at site 1.

Heights generally were lower in 1982 than in 1981. This could have been due to climatic influences. The weather in 1982 was much drier than in 1981 and probably affected plant growth.

Aster yellows symptoms were observed at Site 1 and 4. Generally, the flower production of aster affected plants was not reduced, but the flowers were not normal and probably produced little pollen or seed.

In summary, these natural enemies are no doubt affecting the population dynamics of the weed or at least stabilizing the population. The exact nature of the effects of the various types of damage are not well elucidated. In the following chapters, the effects of five of these natural enemies on Canada thistle growth and development are examined in more detail.

B. GALL FORMATION - CASE STUDY ORGANISM UROPHORA CARDUI

B.1 Introduction

B.1.1 Gall Formation

Galls are defined as the pathological development or abnormal growth reaction of plant cells or organs by hypertrophy (overgrowth or abnormal cell enlargement) and hyperplasy (cell proliferation or an abnormal increase in plant cell numbers) as a result of parasitic attack by bacteria, fungi, nematodes, mites or insects (Adler and Straton 1894, Mani 1964, Felt 1965, Darlington 1968). They appear generally as enlargements on otherwise normal tissue. The mite and insect orders which cause galls are: Arachnida (Eriophyidae or Phytoptidae), Hemiptera (Aphididae, Psyllidae and Coccidae), Diptera (Cecidomyiidae and Tephritidae), Coleoptera (Buprestidae), Lepidoptera (Gelechiidae) and Hymenoptera (Cynipidae and Tenthredinidae) (Cook 1904). Gall causers are generally host specific to a plant group and in some cases even to a family, genus or species (Mani 1964, Shorthouse and Watson 1976).

In North America, most galls are formed on the following plant families: Poaceae, Fagaceae, Salicaceae, Rosaceae and Asteraceae (Shorthouse and Watson 1976). Galls can occur on any plant organ with the % frequency of galls at 65% on leaves, 20% on stems, 12% on buds and the remainder on flowers, fruits and roots (Mani 1964). Galls are derived from host plant tissues. The structure and complexity of galls depends primarily on the plant organ attacked, the

plant species and the gall inducing organism (Adler and Straton 1894). Gall forms range from nearly normal to highly abnormal outgrowths. Although the external form of galls is very variable, the types of histological structures are limited. Five types of galls were defined by Adler and Straton (1894).

Gall formation usually is associated with immature insect stages and reproduction of the gall causer (Felt 1918, Darlington 1968). The gall development stimulus, once believed to be a chemical injected by the "gall mother" (adult female for insects), probably originates from secretions (Jost 1907, Hewett 1977) and as a result of feeding actions of the developing parasite. Constant stimulation is required since if the larvae dies, gall growth ceases (Mani 1964). Recent literature indicates that substances similar to plant hormones may be a salivary component of insect larvae (Miles and Lloyd 1967, Miles 1968, Shorthouse and Watson 1976).

Cook (1923) defined three stages in gall formation; (1) cell enlargement and/or division, (2) failure to differentiate into characteristic tissues of the affected organs and (3) the differentiation of specific gall tissues. At the onset of gall formation, neoplastic gall tissue (generally parenchyma) grows around the gall causer. Gall cells are generally larger, have higher water content, enriched cytoplasm, an increase in vacuole number and larger nuclei than normal cells. In most galls, cell differentiation occurs radially around the gall causer and not in reference to normal development (Mani 1964). Galls are either kataplasmas (structure completely undifferentiated parenchyma) or prosoplasmas in which there is differentiation into other tissue types. All galls contain tannin (Cook 1904). As gall development proceeds, the gall acquires its own vascular system (Adler and Straton 1894). Species dependent differentiation determines the amounts and types of tissues forming the gall.

The array of cells and tissues of the gall is abnormal, not in the sense of the forms being altered, but rather in an abnormal abundance in unusual places and/or at unusual times or combinations (Mani 1964). As the tissue ages, tannin concentration decreases and the epidermis hardens (Adler and Straton 1894).

At maturation, there is an abrupt transition. Cell proliferation decreases and stops, protein synthesis ceases, cell walls become thickened and cell contents disappear or change (Mani 1964).

Most galls are composed of various tissues or zones including nutritive, support, vascular and epidermal cells. Directly surrounding the larval cavity, there is usually a zone of "nutritive tissue" composed of cells usually rich in protein, fats or starch. Some nutritive tissue has enriched cytoplasm and some is composed simply of enlarged cells. In some galls, an outer ring of "reserve" material is positioned exterior to the sclerenchyma (Meyer 1951, 1952 a,b, 1954, Mani 1964). Mechanical tissue (thick-walled cells; lignin, sclerenchyma) occurs in prosoplasmic galls and the arrangement can be complex. In galls with central larval cavities, it generally develops radially around the larval cavity. It can occur at any level within the gall (peripheral, near the surface or deeper, sometimes near the larval cavity). These cells are derived from parenchyma and are polygonal or short palisade-like cells (Mani 1964). The vascular system modifications by gall formation can include weakened tracheids and inhibition of normal development because the closed ring can become spread widely. New vascular tissue is differentiated towards the larval cavity, generally ending in phloem elements. These connection are called "faisceux d'irrigation" if the larval cavity is near the vascular bundles or "liber elements" if farther away (Mani 1964). Gall epidermis is generally characterized by larger than normal cells. Other possible modifications

include thickened cell walls or cuticles, fewer stomata and lignification.

B.1.2 Stress from Gall Development

Early biological control documentation considered that the effectiveness of gall insects would be minimal (Harris 1973b). Harris (1973b) states that, "an insect found routinely on a weed regardless of the plant density is presumably not inflicting serious damage and is therefore unlikely to be the most effective" agent... "many gall-forming insects are probably in this category" ...having achieved "a homeostasis with their host that renders them incapable of inflicting serious damage to it." Although Felt (1918) stated that "gall insects live at the expense of their hosts", the degree of damage may be minimal. Darlington (1968) states that, "mortal damage to the colonized plant is the exception rather than the rule." However, there can be no doubt that the plant suffers loss of substance, deviations of growth, differentiation and nutrient transport, and possible premature decay due to the gall (Mani 1964).

The question of whether gall formation is a defense mechanism of the host plant or its response to the activities and chemical secretions of the parasite remains unresolved (Mani 1964). The gall causer derives three benefits from the association with its host plant; shelter, food and a place to breed. Conversely, the plant utilizes material in forming the gall which otherwise would be used in its own metabolism (Darlington 1968). Intuitively, gall development should be detrimental to the host plant.

Gall causers have been and are being used in biological control programs. In Hawaii Procecidochares utilis Stone was introduced to attack pamakani weed (Eupatorium adenophorum Sprengei) and was effective in reducing the weed

population. A gall midge (Agrilus hyperici (Creatzer)) was introduced into New Zealand to control St. John's wort (Hypericum perforatum L.). In Canada, the gall insects Urophora affinis Frld. and U. quadrifasciata (Meigen) have been introduced to attack Centaurea diffusa Lam. and C. maculosa Lam.; Tephritis dilacerata for Sonchus arvensis and Urophora cardui for Cirsium arvense and Rhinocyllus conicus Froelich on Carduus spp. (Shorthouse 1980) and a gall-forming nematode (Paranguina picridis Kirj. & Ivan.) has been released on Russian knapweed (Acroptilon picridis (L.) DC.) (Watson and Harris in press).

Damage to the host plant can be structural and/or physiological. Structural damage either disrupts plant systems such as vascular system or displaces seed if the galls occur in the flower heads (Tephritis dilacerata, Urophora affinis, U. quadrifasciata and Rhinocyllus conicus) (Shorthouse 1980). Physiological damage occurs when assimilates are redirected towards the gall tissues (Shorthouse 1980). Jankiewicz et al. (1969) found that galls can be physiological sinks and this may be more common than previously thought (Shorthouse 1980). Competition for assimilates may reduce the reproductive capacity (floral and vegetative) and may reduce plant vigour (Shorthouse 1980).

There is some evidence that the presence and relative amounts of nutritive cells are proportional to the amount of damage. Therefore, those galls with thicker nutritive zones or more larvae per gall may be more effective for weed control(Shorthouse 1980). Gall causers appear to have a role in biological control and even if the stress is limited, in combination with stresses of other biological control agents and climatic conditions, reduction of weed populations may occur.

B.1.3 Case Study Organism: Urophora cardui L. (Diptera:Tephritidae).

Urophora cardui L. is widely distributed in Europe from Sweden in the north, to the Mediterranean in the south and from France in the east to the Crimea in the west (Peschken and Harris 1975). Canada thistle is the principal host of U. cardui (Hamm 1918, Hendel 1927, Curtis 1928, Mik 1958, Seguy 1932 cited in Phillips 1946). Other plants listed from the older literature include Artemisia spp., Cirsium lanceolatum, C. oleraceum (Hendel 1927, Phillips 1946), however, these reports have not been substantiated recently (Zwölfer 1967).

Life History Adult flies emerge from the previous season's galls primarily from May to June in Europe. The adult insect exits through callus-filled channels formed during gall formation (Lalonde and Shorthouse 1982). After mating, gravid females oviposit one to several eggs among the immature leaves at the growing points of the mainshoot or the sideshoots of Canada thistle plants, mostly in June (Zwölfer 1967). The larvae hatch within 4 to 8 days and enter the stem tissue. Galls are visible within 15 days of oviposition (Peschken and Harris 1975, Lalonde and Shorthouse 1982).

The galls are pleurilocular and each cavity is unilarval. The galls are spherical to fusiform, smooth, prosoplasmic, usually involving several internodes topped with deformed leaves. The gall is soft and yellowish green when immature and becomes woody from the centre and light brown when mature (Darlington 1968, Lalonde and Shorthouse 1982). Within 90 to 100 days of oviposition (August-September), the galls contain mature diapausing larvae, which pupate within the gall in the spring (Zwölfer 1969, Lalonde and Shorthouse 1982). Mature larvae removed from the gall will pupate immediately (Quentin 1954) indicating that permeability to air is required for pupation to occur. The insect is univoltine.

Biological Control Program: U. cardui was recognized as monophagous and a potential biocontrol agent during European surveys in 1965 and was recommended for further study for the biological control program against Canada thistle in Canada (Zwolfer 1965). Host specificity tests indicated that U. cardui was specific to Canada thistle (Peschken and Harris 1975) and releases were made in six provinces in Canada from 1974 to 1977 with evaluation still in progress (Peschken et al. 1982). Initial breeding occurred in all but one release site, but the insect failed to establish in British Columbia, Alberta, Saskatchewan and Western Ontario, whereas it thrived in Eastern Ontario, New Brunswick and Quebec (Peschken 1979, Peschken et al. 1982). A possible reason for failure in the western provinces is insufficient moisture to allow for callus breakdown in the gall and therefore for proper fly emergence (Lalonde and Shorthouse 1982).

In the eastern provinces, the colonies in New Brunswick spread up to four kilometers, with low gall frequency (6%, trace) (Peschken et al. 1982), whereas in Ontario, the gall frequency was higher (approximately 40% in 1978, 30% in 1980) (Laing 1977).

Stress Physiology of U. cardui on Canada thistle (Evaluation): Controlled environment experiments conducted as part of the screening protocol indicated that plants with galls had reduced root weight, reduced combined stem/leaf weights and lower ramet production. Also, the growth rate was reduced and a stimulation of sideshoot elongation resulted from gall formation (Peschken and Harris 1975).

An evaluation protocol was followed for most field releases in which the heights of the plants were recorded for galled and nongalled thistles. Summarized results indicate that plants with one, two or more sideshoots are

not significantly different in height from nongalled plants. However, shoots with mainshoot galls were significantly shorter than nongalled plants and plants with sideshoot galls. A modification of the protocol initiated by the author, in which nongalled plants were distinguished by emergence before and after the oviposition period (Watson et al. 1980) is considered a more "valid" approach (Peschken et al. 1982). In controlled field releases, up to 13 galls per plant have been found, with no significant effect on height, number of seedheads, dry weight or plant spread (Peschken et al. 1982). There is some evidence that the fly prefers shaded areas (Zwolfer 1965, Peschken 1971, Watson and Muirhead 1977, Peschken et al. 1982).

There is a strong positive correlation (0.8 to 0.9) between gall size and the larvae number (Zwolfer et al. 1970, Peschken et al. 1982). It is possible there is also a positive correlation between the amount of nutritive tissue and the stress on the plant with the number of larvae (Peschken et al. 1982).

A relocation of galls from Compton, Quebec to a site on the Macdonald College farm (Site2, Fig. 1) made in the fall of 1979 formed the release site examined in this study.

The purpose of this portion of the study was:

(1) to continue the evaluation of the field release at Macdonald College, to observe the progression of the population in density and also in area covered.

(2) to evaluate the stress caused by gall formation on Canada thistle.

There are two possible types of stress a gall can cause; structural and physiological. In order to evaluate the structural damage, microtechnique was utilized to examine the arrangement and differentiation of tissue types in the gall and the staining characteristics thereof. To evaluate physiological stress; protein and sugar contents were determined for various tissues for both galled and nongalled plants. In addition, radioactive precursor experiments were performed to determine if the gall was a physiological sink. Also, basic yield and growth parameters (biomass, height, flower production et cetera) were examined to evaluate the effect of gall formation. Based on the hypothesis that there may be a positive correlation between the amount of nutritive tissue and the number of larvae and supported by the correlation between gall size and the number of larvae, comparisons were made between plants with galls containing different numbers of larvae to see if a higher number of larvae, resulted in greater stress.

B.2 Materials and Methods

B.2.1 Systematic Field Sampling near Release Site

In 1980 and 1981, a 20X20 m area was staked out around the original field release site and 100 sampling points were randomly chosen (10 points on 10 line transects). At each sampling point a 0.25 m^2 quadrat was placed and the following data were taken for each Canada thistle plant within the quadrat: condition (galled, nongalled), number and position of galls (mainshoot or sideshoot), flower production , height, age (young versus old). Due to a decrease in the thistle population by severe sheep grazing, a smaller sample of 20 quadrats was taken in 1982. The data were analyzed by analysis of variance as a completely randomized design followed by Duncan's multiple range test.

B.2.2 Effect of Gall Formation on Plant Growth

Plants were started from root pieces, between 1-2 cm in length in plastic flats filled with Promix. The flats were watered regularly and the plants were transplanted into 155 mm diameter pots at the three to five leaf stage. Plants were maintained in growth chambers or on a growth bench with conditions of 14 hour daylength at 20 °C (300 microEinsteins m^{-2} sec^{-1}) and 10 hour dark at 15 °C unless otherwise indicated. The plants were fertilized regularly with a water soluble 20:20:20 fertilizer.

Larvae of *U. cardui* were carefully dissected from fresh galls or from galls stored at 5 °C and placed on filter paper in a Petri dish in a cage at ambient temperature. Pupation and emergence occurred in the cage. Water and a sucrose solution were supplied.

The flies were collected with an aspirator and the sex ratio determined. They were released into a cage ($1\ m^3$) containing Canada thistle plants. The plants were changed every 48 hours and at each change newly hatched flies were added to the cage.

Experiment 1: Twenty-two plants (16 exposed to U. cardui and 6 controls) with heights between 20 and 30 cm, grown in the greenhouse under lights and potted in a soil mixture (pasturized soil:sand:peat moss=3:1:1) were examined every 7 to 14 days after exposure to the flies. Height, gall occurrence and flowering time were determined. The experiment was terminated after four months.

Experiment 2: Twenty-nine plants with heights between 10 and 27 cm (25 exposed to the flies and 4 controls) were grown on a growth bench under previously stated conditions. Data taken were height, flowering time, and number and height of ramets and gall size.

Experiment 3 and 4: These experiments involved short plants (5-10 cm at the onset). Twenty-five and 23 plants were exposed to the flies with 5 and 4 controls respectively for experiments 3 and 4. Similar growth conditions and data were taken as for experiment 2. Additional data on the number of sideshoots, the number of mainshoots and the number of root buds as well as fresh and dry weights of the gall, remaining leaves and stem (called plant), roots and ramets were taken. Three galled and nongalled (each) roots were collected and planted to determine shoot emergence.

Data from experiments 1 to 4 were analyzed by analysis of variance as a completely randomized design followed by least-significant-difference (LSD) tests, F-tests and Student's t-tests where appropriate.

B.2.3 Sugar and Protein Content

Samples of leaves, stems (composed of stem1- just below gall level and stem2- approximately 5-10 cm below gall position), roots and galls (for galled plants) were collected (from five galled and four controls for one experiment and four apiece for the other). The fresh weight of the samples was determined prior to extraction. The samples were cut into small pieces, extracted two times with distilled water (amount proportional to the sample weight) by grinding with a Virtis homogenizer and stored in test tubes covered with Parafilm at 4°C until analyzed (1 to 4 weeks). Contaminated samples were discarded. Prior to analysis, the samples were centrifuged at maximum speed (4750 rpm, 2575Xg) for 3 to 5 minutes in an IEC clinical centrifuge to remove particulate matter from the supernatant.

For sugar analysis, the method of Yemm and Willis (1954) for anthrone's reagent was used with some modifications. Instead of 1.0 mL of sample, 0.9 mL of distilled water and 0.1 mL of sample were added to the chilled reagent. The samples were boiled for 12 minutes. Three replicates of each sample were analyzed. Optical densities were read on a spectrophotometer (Spectronic 20) at 620 nm. Samples which were too dense were diluted 1:3 with the same acid/water mixture used to make the reagent. Blanks were prepared with 1 mL of water. The sugar content was determined from a calibration curve (Appendix A).

For protein analysis, the procedure of Lowry et al. (1951) was followed with the following modifications. Only reagents A, B and C were prepared. Reagent E was diluted to 1 N from a commercial preparation. Three replicates of each extract were performed. Samples for analysis were prepared from 0.75 mL water and 0.25 mL of supernatant. Optical density was read at 550 nm and

protein content was determined by calibration curve (Appendix B).

Total nitrogen content was determined by the microKjedahl method according to procedures outlined by Horowitz (1975) and protein content determined by multiplying by the accepted factor of 6.25 (Appendix C for sample calculations).

Analysis of the data was done using analysis of variance for a completely randomized design followed by Duncan's multiple range test.

B.2.4 Radioactive Precursor Experiments

Plants were sampled four (9 galled, 2 control), six (4 galled, 1 control) and eight weeks (8 galled, 4 controls) after exposure to flies. The plants were removed from the growth bench to a radioactive fumehood. Each plant was injected with 15 μCi (20μCi for 8 weeks) of fructose,D-[^{14}C(U)] (specific activity 359.1 mCi/mmol obtained from New England Nuclear) in the stem near the soil level with a 50 μl syringe. Lights (50 microEinsteins m^{-2} sec^{-1}) were affixed over the plants and the plants remained in the radioactive fumehood for 24 hours.

After 24 hours, the plants were separated into leaves, stem, ramets, roots and galls. Fresh weights were determined and the tissue was oven dried for 24-36 hours at 65-70 ^{o}C then finely ground. Three replicates of each sample were prepared for liquid scintillation counting. Twenty milligrams of tissue were placed in 20 mL scintillation vials with foil-lined caps. Tissue solubilization was performed in 0.5 mL of 60% perchloric acid: 30% hydrogen peroxide (1:2 solution) incubated at 70-80 ^{o}C in the tightly capped vials for 8-14 hours until the solution was clear and colourless. After cooling, 15 mL

of scintillation fluid (made from 12 g PPO (2,5 diphenyloxazole), 2 L toluene and 1 L ethylene glycol monoethyl ether) was added to each vial (procedure adapted from Mahin and Lofberg 1966).

Liquid scintillation counting was performed on a Packard Tri-Carb Scintillation Counter (Model 3003) with an internal standard. Counts were done for one minute and counts per minute (cpm) were transformed to disintegrations per minute (dpm) using the B/A channel ratio method (Appendix D).

Analysis of the data was done with analysis of variance for a completely randomized design followed by Duncan's multiple range test.

B.2.5 Microtechnique

Small pieces of fresh young galled and nongalled tissue were fixed in 3.0% glutaraldehyde in 0.025 M phosphate buffer pH 6.8 for 12 to 24 hours at 4 °C. Dehydration was performed in a methyl cellosolve, ethanol, n-propanol and n-butanol alcohol series at 5 °C. Samples were stored at -10 °C in the final change of n-butanol (Feder and O'Brien 1968).

The tissue was then infiltrated for 2 weeks and embedded in gelatin capsule in glycol methacrylate (GMA) monomer mixture. Sections (1-2 μm) were cut on a Reichert Ultramicrotome and mounted in distilled water on glass slides and dried down for 12 to 18 hours at 37 °C. Hand sections of fresh tissue were also cut. Sections were stained with 0.05% toluidine blue O in benzoate buffer (O'Brien and McCully 1981). Plastic sections were also stained by the periodic acid Schiff's (PAS) reaction (modified from O'Brien and McCully 1981) and Sudan III/IV stain (O'Brien and McCully 1981). Sections were examined and photographed on a Zeiss microscope equipped with bright field optics.

B.3 Results

B.3.1 Evaluation of Field Release on Macdonald College

The density and frequency of galled plants at the field release site (marked by X on Fig. 2), initiated in the autumn of 1979, increased over the observation period (1980-1982) (Table 14). In 1980, the flies dispersed throughout the pasture containing the release site (pasture A) and into thistle clones in pasture B (approximately 100 m from X) and along the fence lines of pasture B. In 1981, the galls were dense near the original release site (50 m radius). In pasture B, the number of galls increased and had dispersed farther in the field, but were no longer present on thistle plants along the fence lines. By 1982, the galls were still present near the release site despite severe sheep grazing, had dispersed considerably more into pasture B and had also dispersed as far as 1.3 km northeast to the next nearest thistle clone (site 3 of field data, Fig. 1).

B.3.2 Effect of Gall Formation on Plant Growth

Galls can occur on the mainshoot (Fig. 3) or on the sideshoots (Fig. 4) of Canada thistle plants. When the galls occurred on the sideshoots in the field, there was no significant height reduction from the control (nongalled thistles), but when the galls occurred on the mainshoot, the height was significantly reduced (Table 15).

A cessation of height increase was found during mainshoot gall formation (Fig. 5) with plants with initial height (at fly introduction) between 20 and 30 cm grown in the greenhouse. However, when the expermiment was repeated, little difference in height was found (Fig. 6). Additional parameters were also

Table 14. Gall Population Dynamics at the Macdonald College Field Release *

Parameters	1980		1981		1982	
	Total	Galled #	Total	Galled	Total	Galled
Density (shoots/m^2)	32.0	1.4	18.3	2.0	21.0	3.8
Frequency (%)	93	29	93	28	100	60

* 100 quadrats sampled in 1980 and 1981 and 20 quadrats sampled in 1982.

\# plants with one or more galls.

Table 15. Effect of U. cardui Gall Formation on Height of Canada Thistle in the Field

Year	Nongalled		Galled	
	old	young	side	main
	Mean Height (cm)			
1980	60.78a *	27.17c	47.35b	25.92c
1981	62.98a	21.31c	66.37a	36.94b #
1982	86.38a	17.88b	96.84a	n.d.

* Means with the same letter in a year (row) are not significantly different (α =0.05, Duncan's muliple range test)

\# n.d. no data; no plants with mainshoot galls occurred in the sample

Figure 2:

The field positions of Canada thistle clones and Urophora cardui galls for the years 1980 to 1982 at the release site at Macdonald College. The X on the figure indicates the approximate position of the original release site.

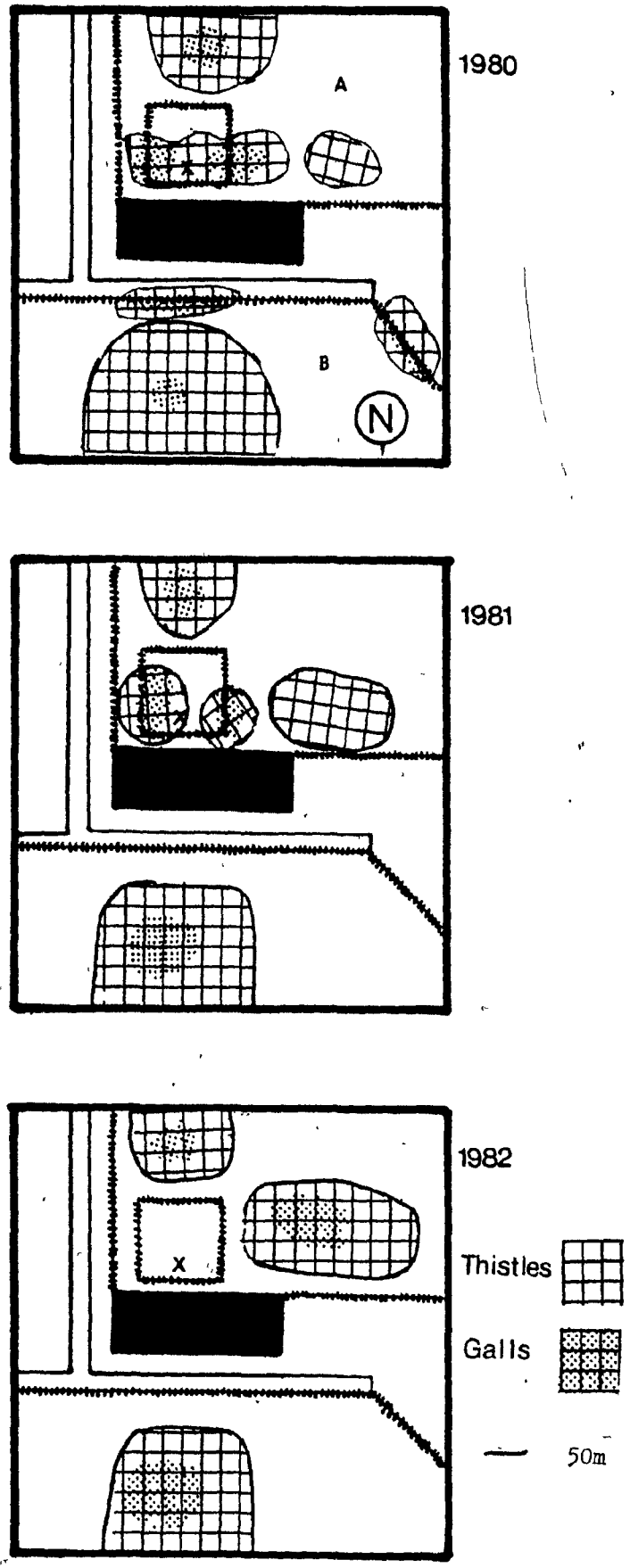


FIGURE 2

Figure 3:

Canada thistle plant with a mainshoot gall.

Figure 4:

Canada thistle plant in the field with a sideshoot gall. (Arrow points at the gall).


3

Figure 5:

The effect of gall formation on height of thistles with initial height at fly introduction between 15 and 30 cm. X=galled and $=nongalled plants.

Figure 6:

Effect of gall formation on thistle height with initial heights of between 13 and 27 cm. X=galled and $=nongalled plants.

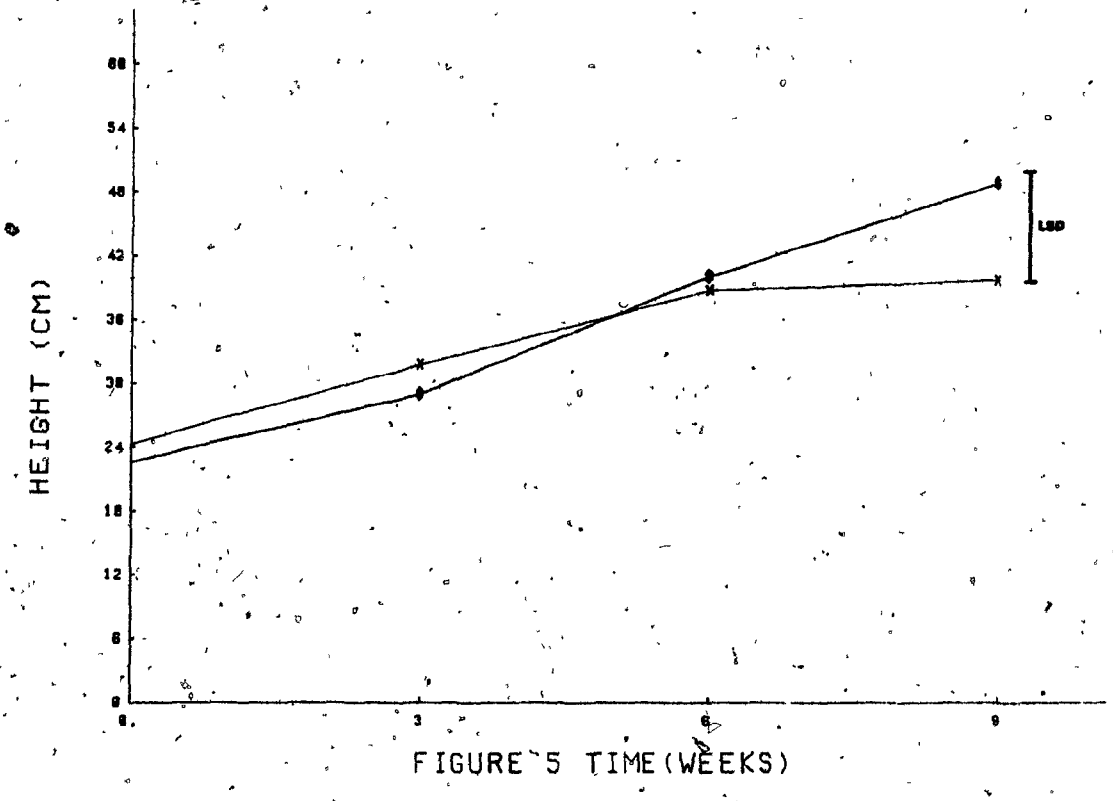


FIGURE 5 TIME(WEEKS)

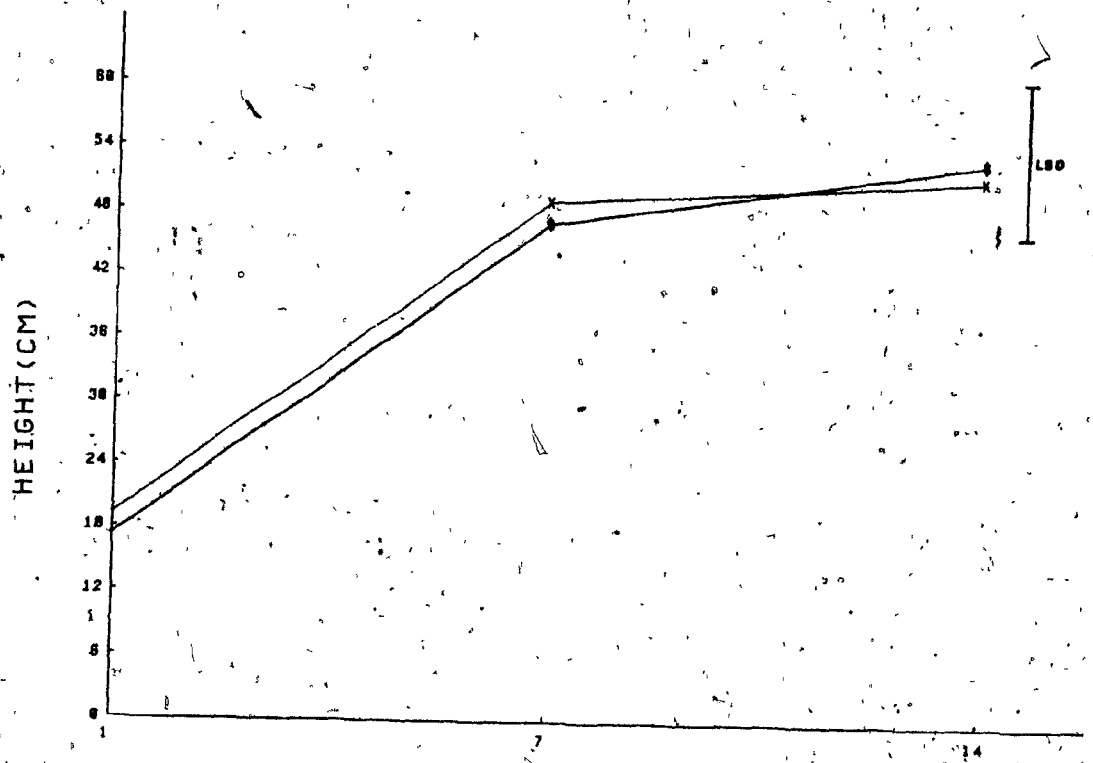


FIGURE 6 TIME(WEEKS)

measured (Table 16). Although no significant differences were found, there was a trend towards lower leaf number, root bud number and fresh and dry weight of the root for galled plants.

In similar experiments but with shorter thistles (4-7 cm, experiment 3 and 5-13 cm, experiment 4), the height (Figs. 7 and 10), leaf number (Figs. 8 and 11) and the number of ramets (Figs. 9 and 12) were lower for galled plants (mainshoot galls) than for nongalled plants. Significant differences were found in experiment 3 starting at approximately week seven for each parameter. For experiment 4 the diminished growth rate caused by gall formation (brackets on Figs. 10-12) was apparent (weeks 4 to 7), but recovery occurred. The effect on height by gall formation was also visibly noticeable (Figs. 13-18). Differences in other parameters included significantly higher sideshoot number for galled plants (Table 17), significantly lower root dry weight (Table 18) and root fresh weight (Table 17). The roots were also visibly reduced in size (Fig. 19). Also, the number of mainshoots (actually sideshoots on an unelongated stem) was significantly higher in galled plants. The shoot/root ratios for galled plants (3.5 and 1.2) were higher than those for nongalled plants (3.2 and 1.1).

Ramet production of roots collected was determined for roots from galled and nongalled plants from one experiment. Although root weight was decreased for galled plants, the ramet production was not greatly affected (Fig. 20).

Gall formation delayed and reduced flowering (Table 19), especially on plants with mainshoot galls. Flower production on floral sideshoots with galls was delayed when compared to sideshoots on the same plant without galls, but

Table 16. Effect of Gall Formation on Plant Parameters (Initial Height at Fly Introduction- 13 to 27 cm- Experiment 2)

Variable	Galled	Nongalled	t-test
Leaf number	40.3	42.8	ns
Ramet number	15.5	15.0	ns
Average ramet height/plant (cm)	12.8	13.0	ns
Root bud number	38.2	45.2	ns
Fresh weight (g)			
Root	37.9	45.2	ns
Plant	28.5	19.5	ns
Ramets	30.2	28.6	ns
Dry weight (g)			
Root	11.4	11.5	ns
Plant	11.1	8.0	ns
Ramets	6.9	8.4	ns
% Dry matter	41.2	48.1	ns

Table 17. Effect of Gall Formation on Plant Parameters (Initial Height 4 to 7 cm- Experiment 3)

Variable	Galled	Nongalled	F-test $
Sideshoot number #	5.1	5.2	ns
Number of tops	3.4	1.0	**
Average ramet height/plant (cm)	6.7	7.8	*
Root bud number	20.8	17.8	ns
Fresh weight (g)			
Root	17.3	23.3	*
Plant	37.1	35.2	ns
Ramets	12.1	17.5	ns
Dry weight (g)			
Root	4.1	4.8	ns
Plant	14.3	15.3	ns
Ramets	0.8	2.7	ns

$ * Significant at 0.05

** Significant at 0.01

ns not significant

actually the number of sideshoots on the unelongated stem which forms the gall region

Table 18. Effect of Gall Formation on Plant Parameters (Initial Height 7 to 13 cm- Experiment 4)

Parameter	Galled	Nongalled	F-test $
Sideshoot number #	5.8	1.4	*
Number of tops	1.6	1.0	ns
Root bud number	20.5	15.8	*
Dry weight (g)			
Plant	3.7	4.4	ns
Ramets	2.8	2.2	ns
Root	3.1	4.1	*
Fresh weight (g)			
Plant	14.8	18.9	ns
Ramets	9.4	4.3	ns
Root	7.9	10.0	ns
Average ramet height/plant (cm)	7.0	6.2	ns
Gall dry weight (g)	4.9		
Gall fresh weight (g)	1.0		

$ ns not significant

* Significant at the 0.05 level

\# actually the number of sideshoots on the unelongated region of stem which forms the gall

Table 19. Effect of Gall Formation on Flowering throughout the 1981 Season

Date	Nongalled	One Side-shoot Gall	Two Side-shoot galls	Mainshoot Gall
	----------	%	----------	
May 16	0	0	0	0
June 6	0	0	0	0
June 18	50	40	66	0
July 10	66	85	100	0
July 23	76	90	100	0
Aug 17	76	95	100	33

Figure 7:

Increase of height of galled and nongalled plants with initial height at fly introduction of 4 to 7 cm. X=galled and $=nongalled plants.

Figure 8:

Increase in leaf number of galled and nongalled plants with initial height at fly introduction of 4 to 7 cm. X=galled and $=nongalled plants.

Figure 9:

Ramet production per plant of galled and nongalled plants with initial heights at fly introduction between 4 and 7 cm. X=galled and $=nongalled plants.

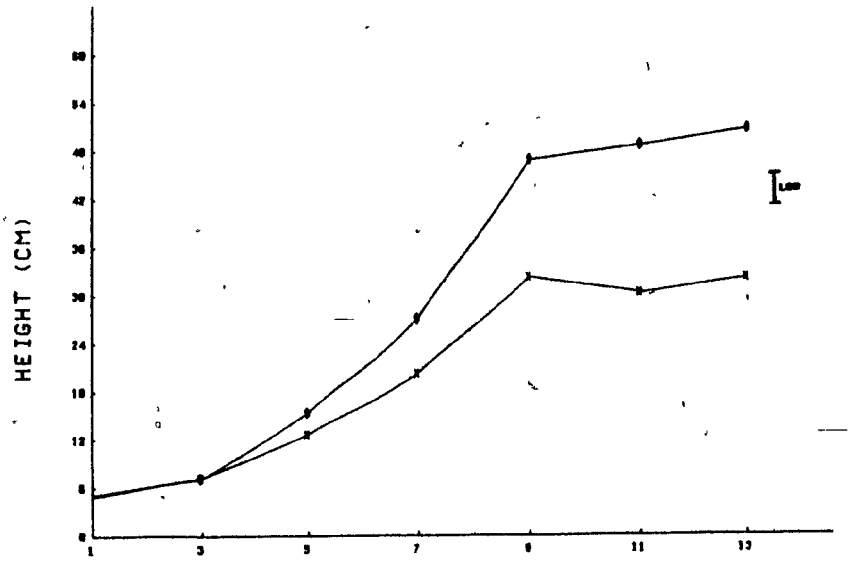


FIGURE 7 TIME(WEEKS)

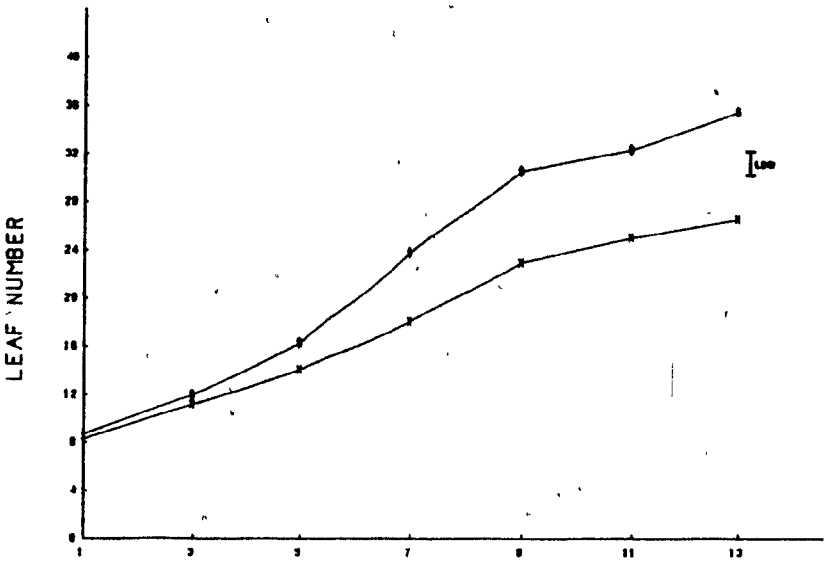


FIGURE 8 TIME(WEEKS)

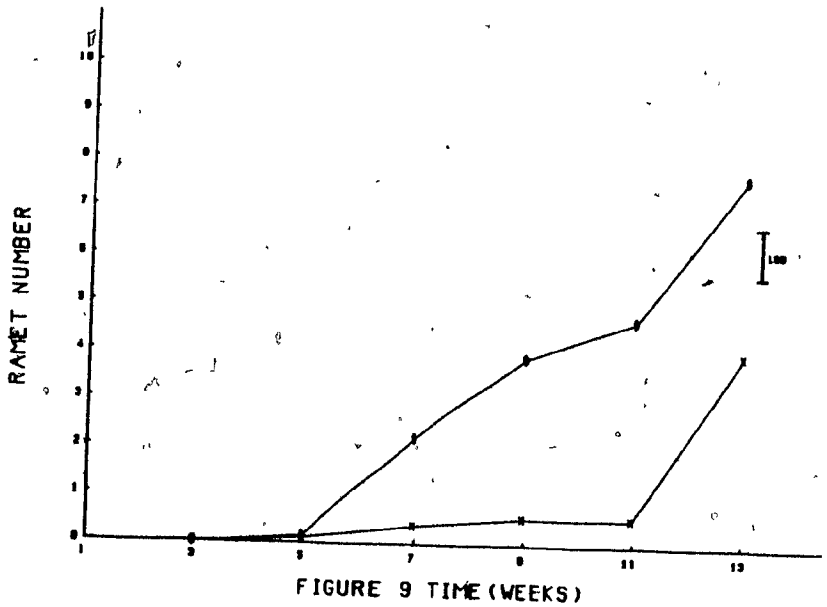


FIGURE 9 TIME(WEEKS)

Figures 10 to 12: Growth parameters for thistles with initial heights at fly introduction between 7 and 13 cm.

Figure 10:

Height (cm) increase of galled and nongalled plants. Note that the galled plants stopped increasing in height during gall growth and then recovery occurred. The bracket indicates the region of retardation caused by gall formation. X=galled and $=nongalled plants.

Figure 11:

Leaf number increase of galled and nongalled plants. Note a similar but less pronounced plateau region during gall growth as for height. The bracket indicates the region of retardation caused by gall formation. X=galled and $=nongalled plants.

Figure 12:

Number of ramets per plant of galled and nongalled plants. The bracket indicates the region of retardation caused by gall formation. X=galled and $=nongalled plants.

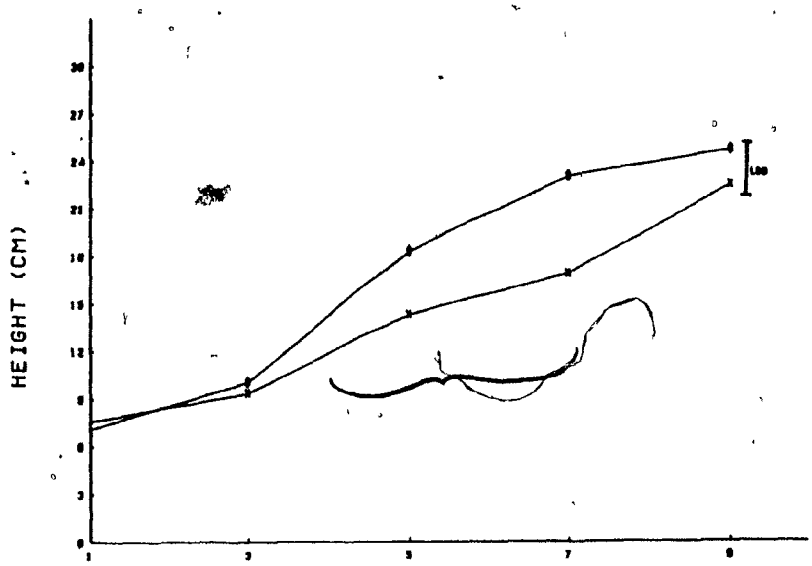


FIG. 10 TIME(WEEKS)

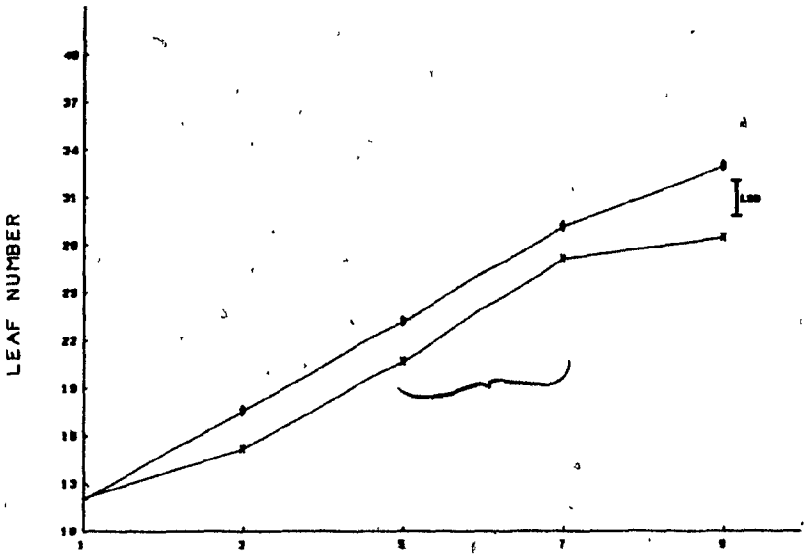


FIG. 11 TIME(WEEKS)

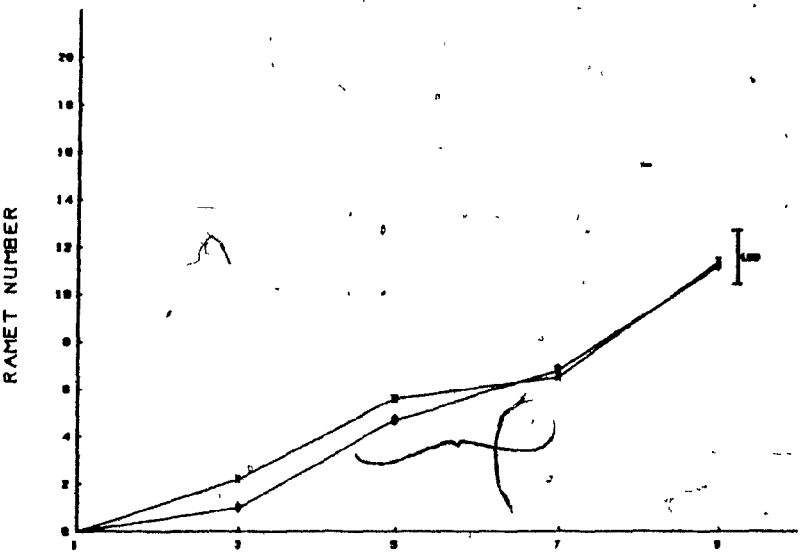


FIG. 12 TIME(WEEKS)

Figures 13 to 18:

Comparison of galled and nongalled plants 4, 6, and 8 weeks after fly introduction. Note the stunted and deformed appearance of the galled plants.

Figures 13 and 16:

Galled and non galled plants respectively 4 weeks after fly introduction.

Figures 14 and 17:

Galled and non galled plants respectively 6 weeks after fly introduction.

Figures 15 and 18:

Galled and nongalled plants respectively 8 weeks after fly introduction.

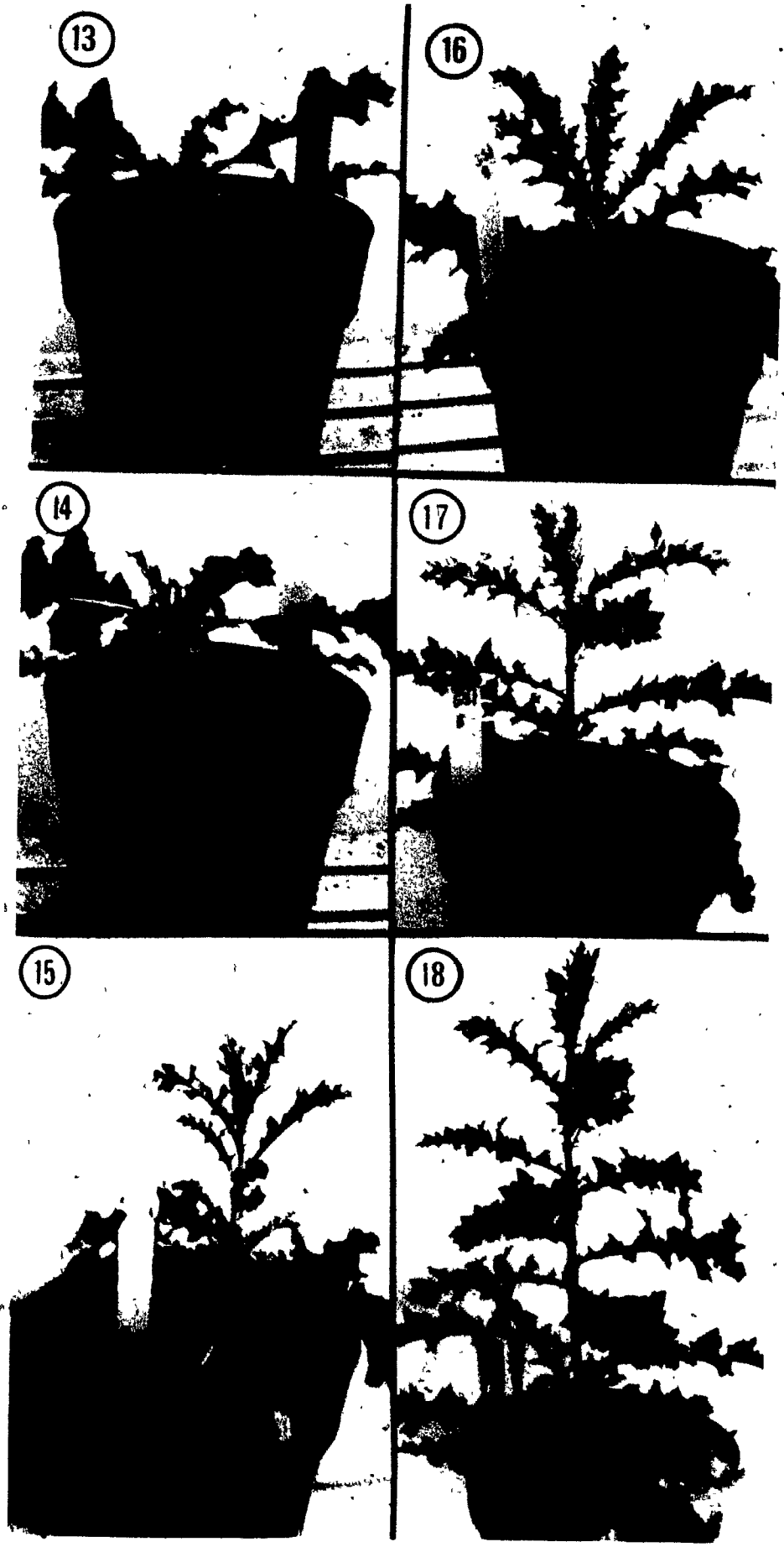

13
16
14
17
15
18

Figure 20:

Ramet production of roots collected from pots of nongalled (blue bars) and galled plants (red bars). Ramet production was very variable especially for galled plants and there was no significant difference between production of galled and nongalled plants.

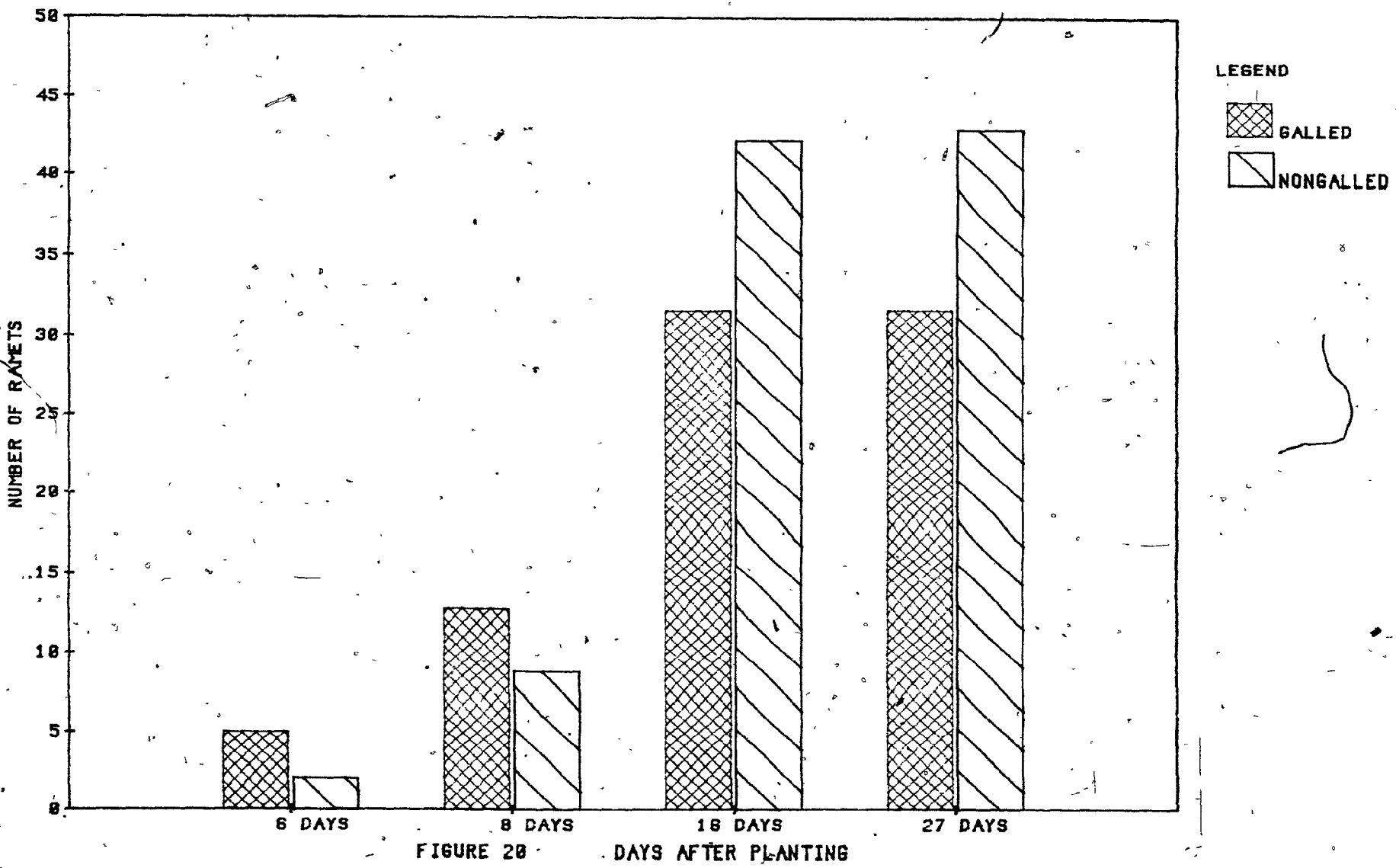

50
45
40
35
30
25
20
15
10
5
0
NUMBER OF RAMETS
6 DAYS
8 DAYS
16 DAYS
27 DAYS
DAYS AFTER PLANTING
LEGEND
GALLED
NONGALLED

FIGURE 20

overall plant flowering with sideshoot galls was neither delayed nor reduced.

B.3.3 Gall Morphology, Anatomy and Phenology.

Gall size (approximated by length X width) was positively correlated with larvae number ($r=0.53$, $n=23$) as were gall fresh weight ($r=0.72$, $n=15$) and dry weight ($r=0.70$, $n=4$). Mature gall size varied considerably from 1.8 cm^2 to 9.3 cm^2, as did the fresh weight (0.5 g to 15 g) and dry weight (0.6 g to 2.1 g) in the samples examined. Mainshoot galls tend to be larger than sideshoot galls (11.5$\pm$5.0 cm^2 versus 4.5$\pm$ 2.4 cm^2). Gall growth seemed to follow a logarithmic pattern (Fig. 21). Upon dissection of galls, the number of larvae per gall varied from 2 to 34 with an average of 9.0 $\pm$ 8.2 larvae. Exit channels similar to those described by Lalonde and Shorthouse (1982) were observed (Fig. 22). Inside the developing gall, each larvae formed its own chamber near the centre of the stem and all larvae faced acropetally (Fig 26). The variable size of the larvae and pupae (Figs. 23 and 24) affected fly emergence. Of larvae less than 3 mm in length 25% failed to develop pupae, 17% formed pupae but failed to emerge and 58% formed pupae and emerged, those 4 mm in length had 14%, 11% and 75% respectively and those 5 mm in length or larger , 1%, 0% and 99% respectively in the same categories.

Prior to visible swelling of the galls, the upper leaves of the plants appeared deformed as though some larval feeding had occurred (Fig. 25). The galls are green, spherical to subglobose, solid and smooth with occasional

Figure 21:

The change of gall size (cm^2) after fly introduction.

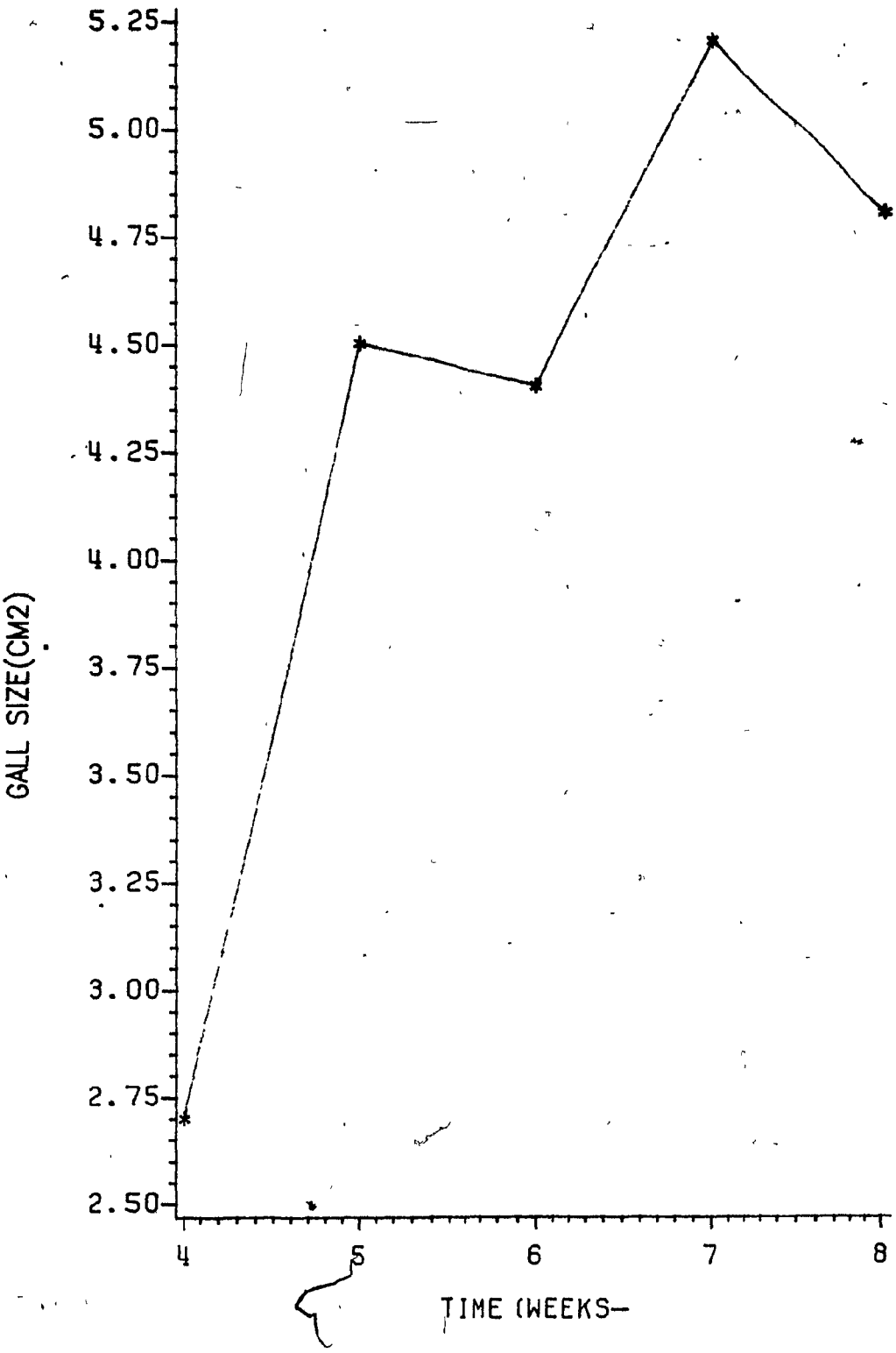

5.25
5.00
4.75
4.50
4.25
4.00
3.75
3.50
3.25
3.00
2.75
2.50
GALL SIZE(CM2)
4
5
6
7
8
TIME (WEEKS-

FIGURE 21

Figure 19:

Comparison of roots from nongalled plant (a) and galled plants (b). The galled plants roots were visibly smaller than the controls.

Figure 22:

A fresh young gall subdivided showing the larval chamber and the callus regions (arrowhead) leading to the exterior of the gall.

Figure 23:

Different sizes of Urophora cardui larvae. (Each small square on grid is 1 mm x 1 mm).

Figure 24:

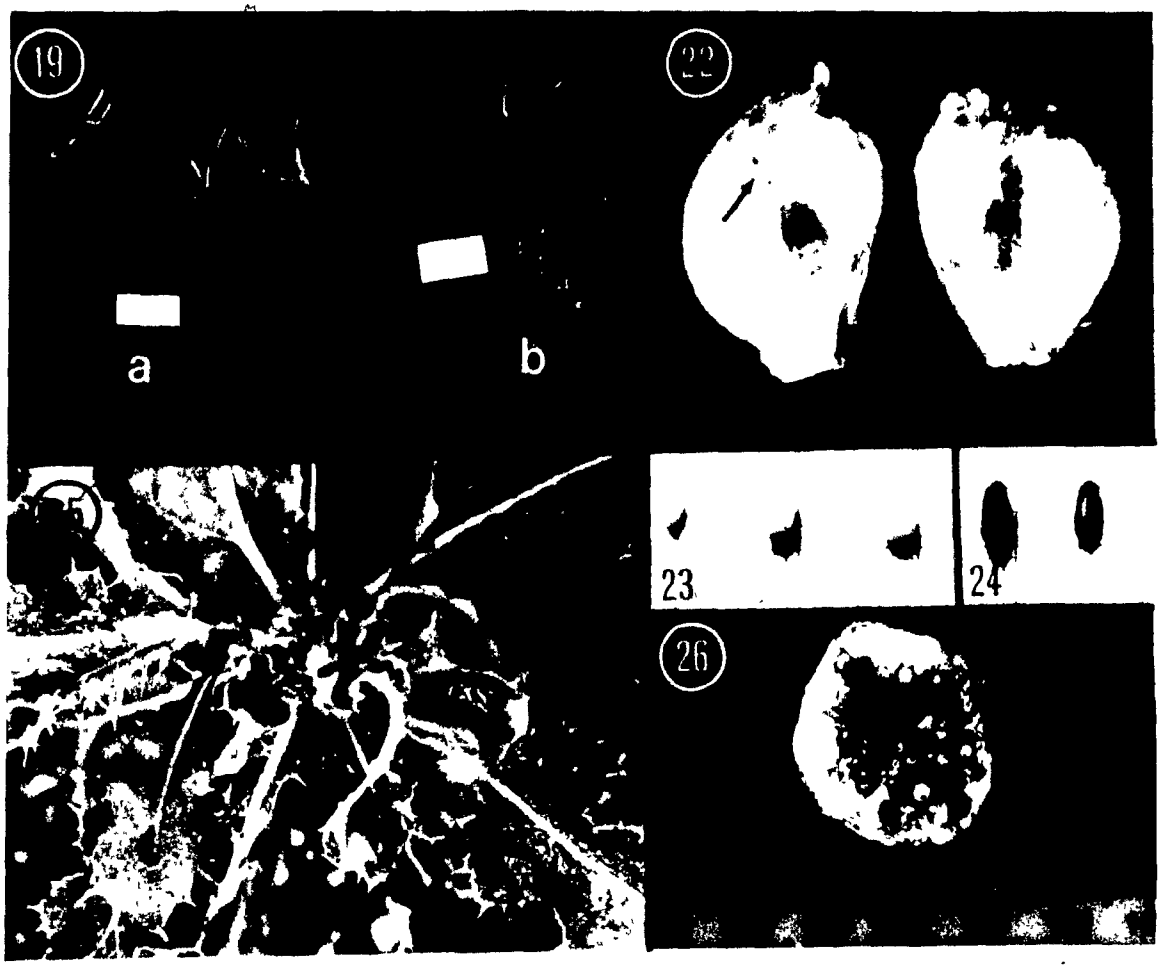
Different sizes of Urophora cardui pupae. (Each small square on the grid is 1 mm x 1 mm).

Figure 25:

Leaves which appear deformed and chewed shortly after fly introduction.

Figure 26:

Dissected gall showing a numerous (34 total) larvae facing acropetally in the centre of the stem tissue.


19
a
b
22
23
24
26

thorny areas where sideshoots were initiated.

In the field in 1981, adult U. cardui were seen as early as June 20th. In 1982, they were seen from June 1st to June 28th, but the majority were seen June 15th/16th. In 1981, the average height of all emerged thistles in the same pasture on June 20th was 54.4 cm which is 79% of mature height and 41% of the plants had initiated flower buds. In 1982, the average height on June 16th was 53.7 cm and the percent of plants in flower was 66%. Heights of plants analyzed for a summer season are shown in figure 27. In this case, the plants which bore mainshoot galls were significantly shorter than plants with one or more sideshoots and nongalled plants, except at the first and last date.

The larval cavities occurred primarily in the middle of the stem and were surrounded by nutritive tissues (Fig. 28). The cells were enlarged and irregular in shape. PAS positive material was deposited in the larval cavity. The nutritive zone did not stain with Sudan dyes suggesting that fats or lipids were not a major component of the nutritive zone. A vascular connection is an integral part of the nutritive tissue region. Fresh sections (Fig. 29) showed large, bulbous cells extending into the larval cavity.

Surrounding the nutritive tissue and closely abutted to it was a zone of sclerenchyma which did not stain with PAS but stained the typical turquoise for sclerenchyma with toluidine blue. The vascular fibers that ran through the sclerenchyma stained pink with toluidine blue, except for the sections that wound throughout the tissue (Fig. 30). Exterior to the sclerenchyma was thick-walled parenchyma through which the "liber fibers" continued to pass (Fig. 31). The vascular system, although intact was stretched radially in comparison to nongalled stem vascular tissue (Figs. 32 and 33). The epidermis was irregular in shape and just interior to the epidermis was a thick layer of tissue containing many chloroplasts.

Figure 27:

Heights of nongalled plants (black bars), plants with one sideshoot gall (blue bars), plants with two or more sideshoot galls (green bars) and plants with mainshoot galls (red bars) throughout the summer of 1981 (data from site 2 of field data). (Bars indicate LSD.)

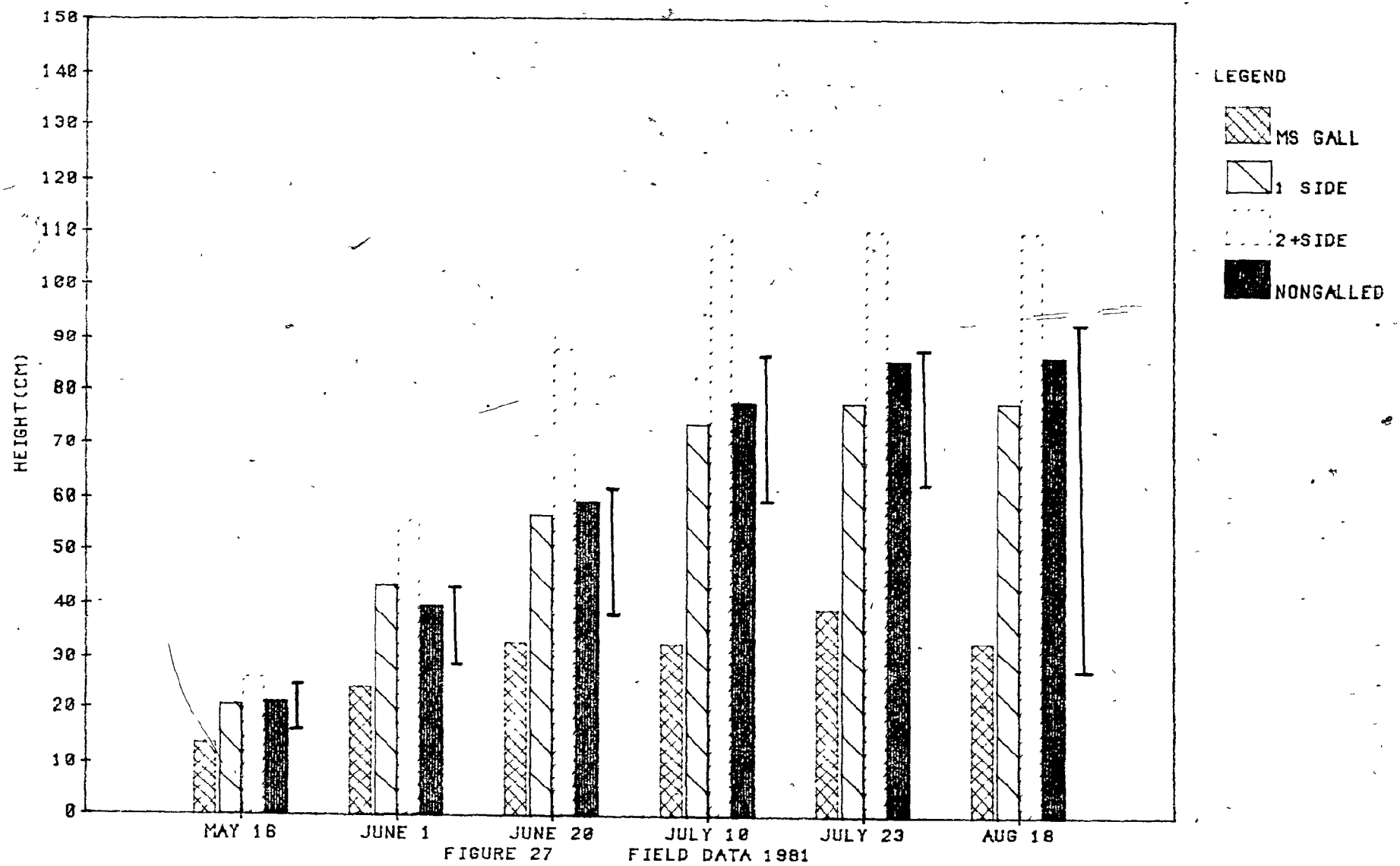


FIGURE 27 FIELD DATA 1981

Figures 28 to 33. Histological examination of U. cardui galls from plastic sectioning(unless otherwise indicated)

Figure 28.The nutritive tissue surrounding the larval cavity (a) with a large deposit of PAS positive material extending into the cavity (b). The cells are large but are not cytoplasmic. Vascular tissue (c) forms an integral part of the nutritive tissue (PAS reaction) (x130)

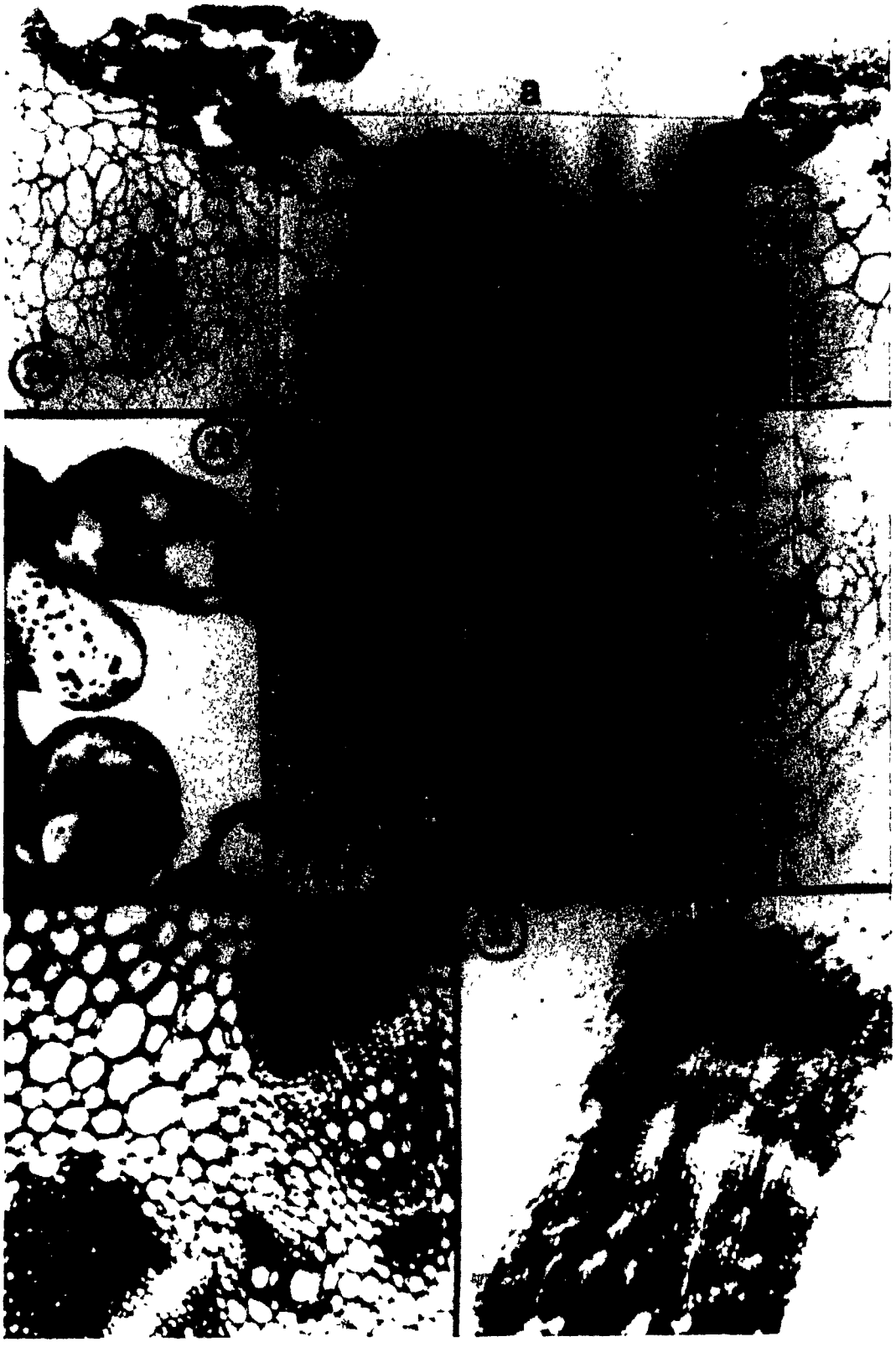
Figure 29.Hand section of the nutritive zone stained with toluidine blue showing large irregularly shaped cells extending into the larval cavity (x250)

Figure 30. Sclerenchyma region centrifugal to the nutritive tissue. Vascular tissue winds through the sclerencyma (arrowheads) (PAS, toluidine blue stain) (x250).

Figure 31. Vascular fibre in thick-walled parenchyma (x250).

Figure 32. Hand section of nongalled vascular tissue. (x120).

Figure 33. Hand section of galled vascular tissue. Although the vascular ring is complete, it is stretched and appears more diffuse than that of the nongalled stem (x90).



B.3.4 Protein and Sugar Content

Since the variances of the two repeats of the experiment were not significantly different, the data were pooled and analysis of variance and Duncan's multiple range test were performed.

Pooled results (Fig. 34) for water soluble protein content indicate that the leaves of galled plants had a higher level (mg/g fresh weight) of water soluble proteins than all other tested components.

Pooled results of the anthrone test (Fig. 35) indicate that the gall and the stem of nongalled plants had high water soluble sugar contents, whereas the level in leaves was low.

Total protein as performed by the microKjedahl analysis indicates that, as for water soluble protein, the leaves had the highest percentage of protein, the roots had the next lowest percentage, followed by the stems for both galled and nongalled plants. The gall had a low protein content in comparison to the root and the leaves, but was not significantly different from the stem (Table 20). There was, however, no significant difference due to high variability ($F=0.4$ with 6 and 35 degrees of freedom).

Although some contamination may have occurred during the storage period, it was probably low. However, the results are questionable and the experiment should be repeated with additional precautions.

B.3.5 Radioactive Tracer Experiments

The different sampling times (four, six and eight weeks after fly introduction) yielded different results. Four weeks after fly introduction

Table 20. Protein Content of Galled and Nongalled Plant Tissue Determined by the MicroKjedahl Method

Plant Condition	Gall	Leaves	Stem	Root
	----------%			
Galled Plant	7.9	14.5	8.4	11.3
Nongalled Plant	-	14.3	9.3	11.8

No significant difference was found ($F=0.40$ with 6 and 35 degrees of freedom)

Table 21. Accumulation of Radioactive Precursor (^{14}C-fructose) of Galled and Nongalled Plant Tissue Four Weeks after Fly Introduction

Tissue	Nongalled		Galled
	----disintegrations per minute----------		
Leaves	5864cd	*	3924cd
Stem	19709a		13621ab
Root	1075d		672d
Ramets	5671cd		5831cd
Gall	-		8232bc

* Values with the same letter are not significantly different (α=0.05, Duncan's multiple range test)

Figure 34:

Water soluble protein content (mg/g) of fresh tissue determined by the Lowry's reaction in galled and nongalled plant tissue; Gall=gall, GL= leaves from a galled plant, GS1= stem taken just basipetal to the gall of a galled plant, GS2= stem taken from 5 to 10 cm below the gall of a galled plant and GR= root from galled plant.

NL= leaves from nongalled plant, NS1= stem taken at a level comparable to that of a gall from a nongalled plant, NS2= stem taken from a postion 5 to 10 cm below that of S1 sample, NR= root of nongalled plant.

Bars with the same letter are not significantly different from each other (α=0.05, Duncan's multiple range test).

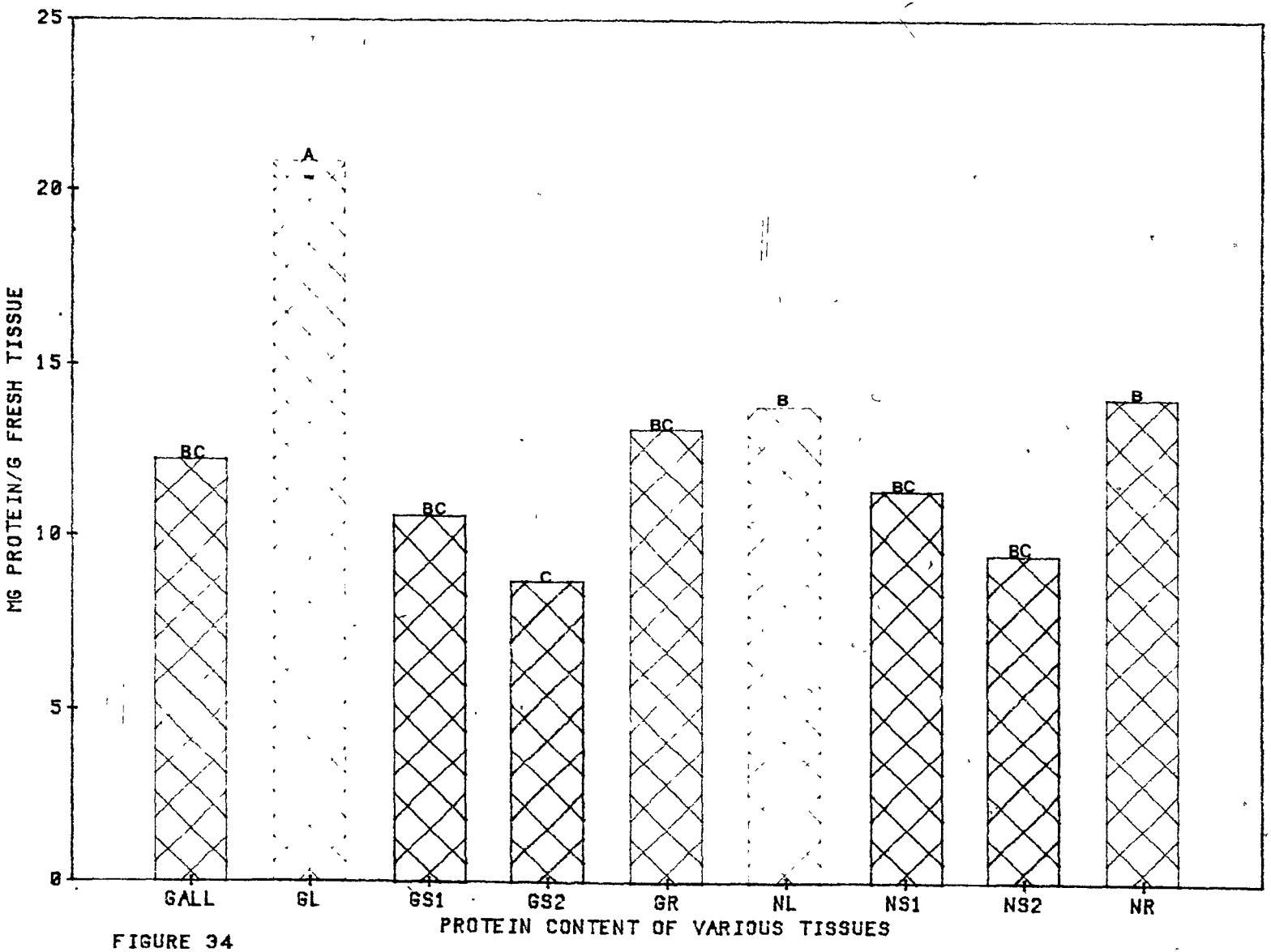

25
20
15
10
5
0
MG PROTEIN/G FRESH TISSUE
A
BC
BC
C
BC
B
BC
BC
B
GALL
GL
GS1
GS2
GR
NL
NS1
NS2
NR
PROTEIN CONTENT OF VARIOUS TISSUES

FIGURE 34

Figure 35:

Water soluble sugar content of fresh plant tissue (mg/g) from galled and nongalled plants. The categories are the same as for Figure 34. Bars with the same letters are not significantly different from each other (α =0.05, Duncan's multiple range test).

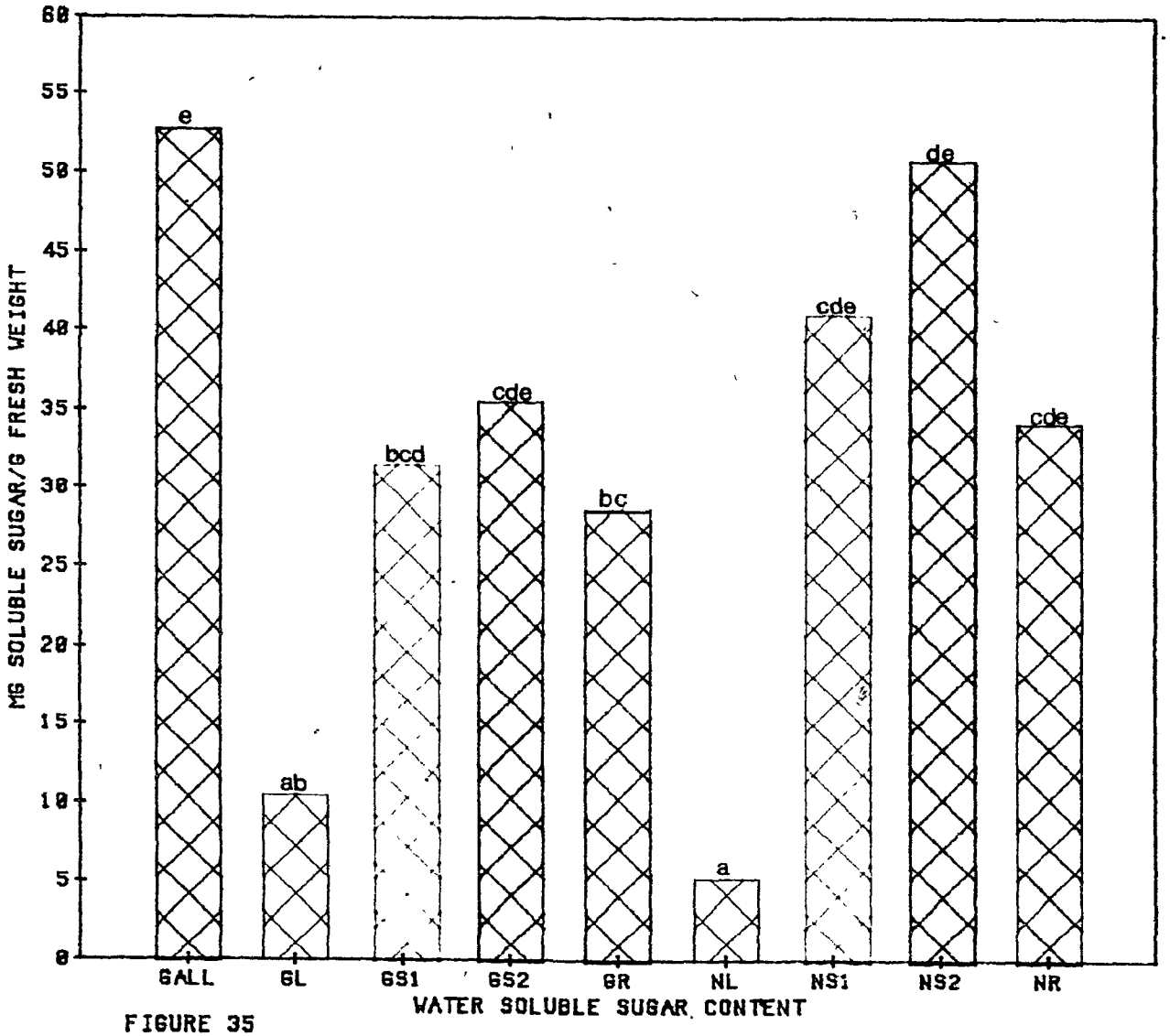

MG SOLUBLE SUGAR/G FRESH WEIGHT
60
55
50
45
40
35
30
25
20
15
10
5
0
e
ab
bcd
cde
bc
a
cde
de
cde
GALL
GL
GS1
GS2
GR
NL
NS1
NS2
NR
WATER SOLUBLE SUGAR CONTENT

FIGURE 35

(Table 21), the gall had accumulated a high level of radioactivity and leaf, stem and root tissue from galled plants accumulated only slightly less than the same tissue from the control. The roots did not accumulate radioactivity, whereas the stem accumulated the most of all tissues. The ramets were also sinks at this time period.

By six weeks (Table 22), the counts in the gall were much reduced in comparison to the rest of the tissue. Similar patterns of accumulation were found as for 4 weeks for galled and nongalled plants; the stems were high, the leaves intermediate and the roots low. The ramets at this point were not actively accumulating assimilates.

By week eight (Table 23), the galls were not accumulating radioactive material at all (almost as low as the roots). The ramets on the other hand were strong physiological sinks. At the two later dates, the values for the controls tended to be lower than the galled plants. This may have been due to the differential plant size, as the galled plants have been stunted by gall formation.

If the disintegrations per minute (dpm) were totalled for each sampling time for the galled and nongalled plants and a % for each sampling time calculated (Fig. 36), the percentage in the gall decreased, the percentage in the roots remained stable and the percentage in the leaves increased slightly over time. The percentage of radioactivity in the stem and ramets were mirror images (if the stem value was high, the ramet value was low and vice versa). The stem proportion increased from four to six weeks and then decreased again, whereas the ramet proportion decreased from four to six weeks and then increased again.

Table 22. Accumulation of Radioactive Precursor (C^{14}-Fructose) by Various Plant Organs of Galled and Nongalled Plants Six Weeks after Fly Introduction.

Tissue	Nongalled	Galled
	-----disintegrations per minute------	
Leaves	1124b *	3055b
Stem	2917b	10931a
Root	379b	388b
Ramets	642b	579b
Gall	-	1696b

* Values with the same letter are not significantly different (α=0.05, Duncan's multiple range test.

Table 23. Accumulation of Radioactive Precursor (^{14}C-Fructose) by Tissues of Galled and Nongalled Plants Eight Weeks after Fly Introduction.

Tissue	Nongalled	Galled
	------disintegrations per minute-------	
Leaves	1008cd *	2273bc
Stem	1573c	2908b
Root	302d	810cd
Ramets	2535bc	4464a
Gall	-	478cd

* Values with the same letter are not significantly different (α=0.05, Duncan's multiple range test)

Figure 36:

Change in accumulation patterns of % radioactivity in organs of galled and nongalled plants.

Green *- gall leaf, Green □ - nongalled leaf

Black *- gall stem, Black □ - nongalled stem

Blue *- gall root, Blue □ - nongalled root

Black ◆ gall ramet, Black × nongalled ramet

Red....- gall

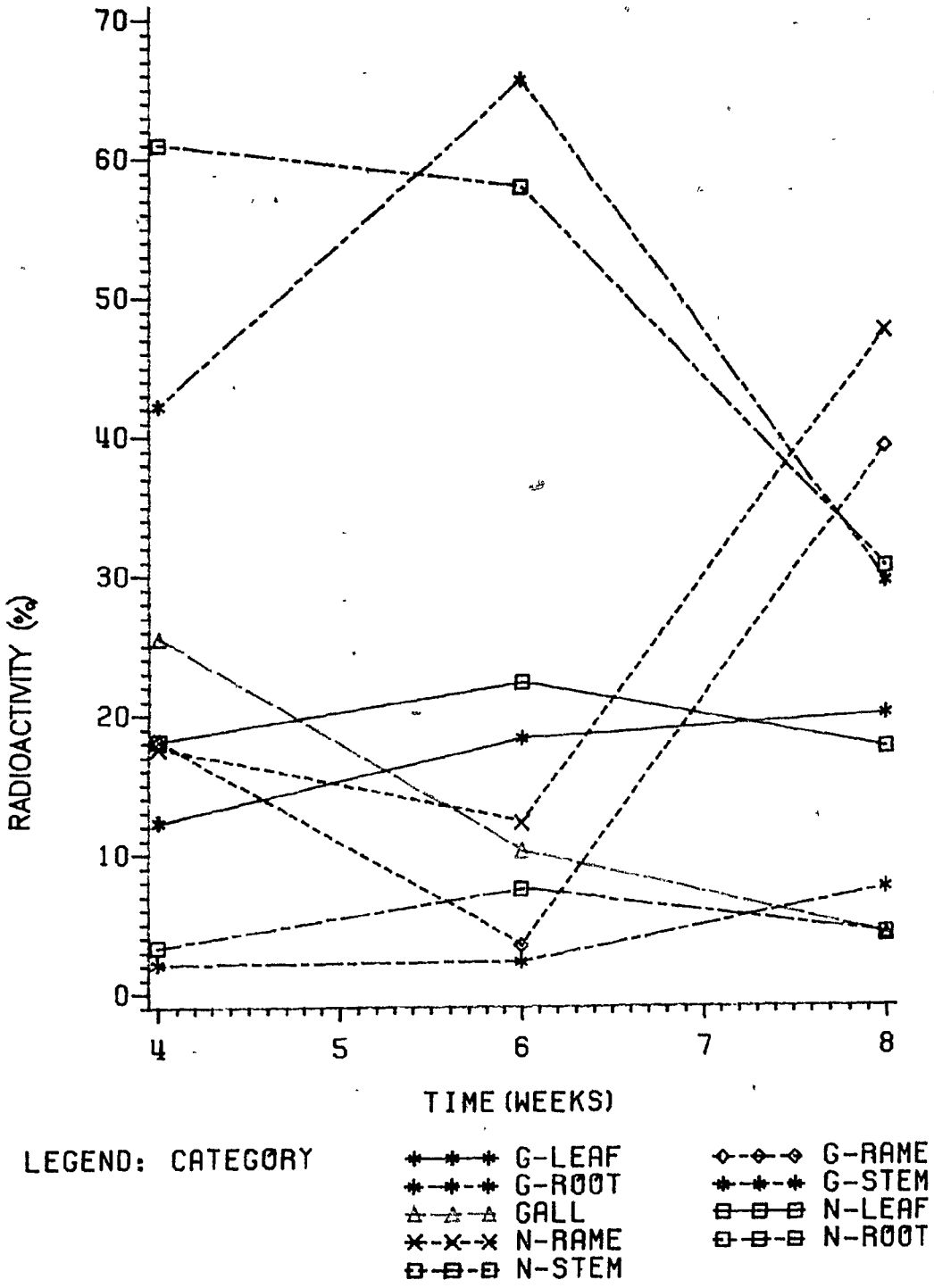

70
60
50
40
30
20
10
0
RADIOACTIVITY (%)
4
5
6
7
8
TIME (WEEKS)
LEGEND: CATEGORY
G-LEAF
G-ROOT
GALL
N-RAME
N-STEM
G-RAME
G-STEM
N-LEAF
N-ROOT

FIGURE 36

The injection procedure could be construed to result in the movement of the tracer primarily by the transpiration stream. However, since the accumulation occurred in other portions of the plant other than the major transpiration organs, it was assumed that the tracer was in general circulation.

B.3.6 Effect of Gall Size on Selected Parameters

Data extracted from experiments 3 and 4 were separated according to the gall size into three different categories: (1) those less than 5.0 cm^2, (2) those between 6 and 8.5 cm^2 and (3) those greater than 8.5 cm^2. With increasing gall size; height, leaf number, root bud number, fresh weight of the plant and the root, sideshoot number and dry weight of the plant and the root decreased. The number of mainshoots, however, increased with increasing gall size (Table 24).

Data taken from the first sampling date of the radioactive precursor experiment was categorised according to gall dry weight into two groups; galls with weight greater than 1 g and those less than 1 g. With increasing gall size, there was a significant decrease in the amount of radioactivity in the stem, the gall and the ramets, although the smaller galls had higher values for the ramets than the control (Fig. 37). There was also a reduction, although not significant in radioactive accumulation in the leaves and the roots. The larger galls, however, seemed to sequester less radioactivity than the smaller galls per unit dry weight.

Table 24. Effect of Gall Size on Growth and Yield Parameters

Parameter	Size 1*	Size 2$	Size 3#	LSD (0.05)
Height (cm)	36.5	28.9	25.0	5.5
Leaf number	31.5	26.4	23.7	5.0
Sideshoot number	5.3	6.4	4.7	4.0
Number of tops	1.5	2.8	3.3	1.5
Root bud number	28.0	19.6	11.0	10.4
Fresh Weight (g)				
Plant&	34.8	25.9	24.3	6.8
Root	19.3	11.7	15.5	3.4
Dry weight (g)				
Plant&	6.8	5.6	5.3	1.8
Root	4.2	2.8	3.6	1.0

* < 5 cm^2

$ between 6.0 and 8.5 cm^2

> 8.5 cm^2

& weight of remaining stem and the leaves after gall removal

Figure 37:

Radioactive precursor accumulation of tissue from plants with galls of different weights (greater than one g (large gall), less than one g (small gall)) and controls. (Bars with the same letter are not significantly different; α=0.05 Duncan's multiple range test)

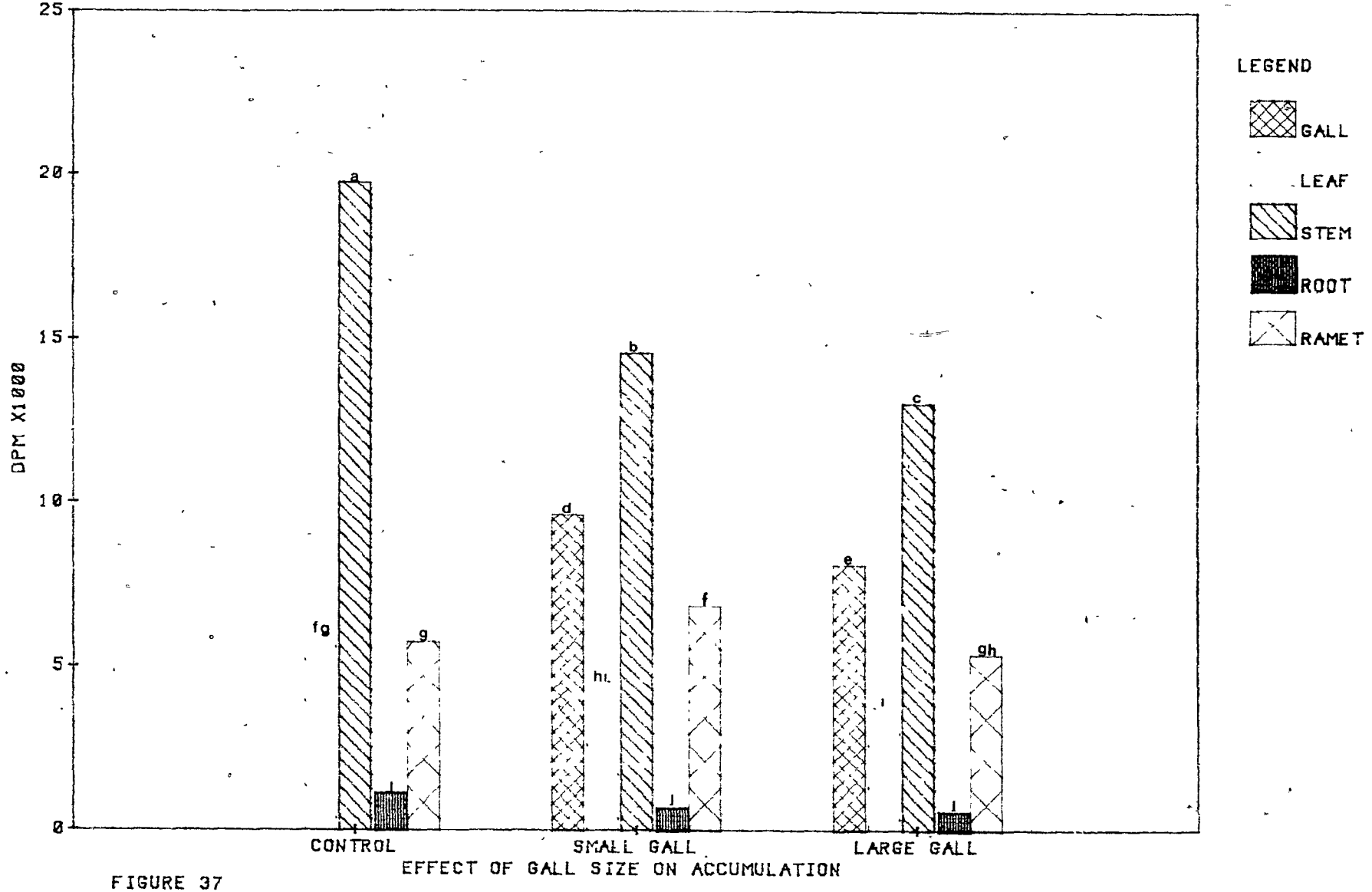


FIGURE 37

EFFECT OF GALL SIZE ON ACCUMULATION

8.4 Discussion

The population of U. cardui galls at the field release made in 1979 on the Macdonald College farm thrived and increased in density and frequency over the observation period (Table 14). The tendency to overdisperse noted in other cases (Peschken et al. 1982) was not found in this study, however, the fly did disperse gradually outward from the release site (Fig. 2). The total plant density appeared to decrease with time, but this was most probably due to the natural tendency of clonal Canada thistle populations to migrate away from the centre of the clone (Amor and Harris 1974), as can be seen by examination of Pasture A thistle clones over the years (Fig. 2).

If the gall occurred on the mainshoot, plant height was reduced (Table 15). This has been observed throughout Canada through the biological control evaluation program (Peschken et al. 1982). This has especially been noted since the data for nongalled plants were separated into plants emerged before and after fly emergence. This removes the bias found in the 1977-1979 data which indicated that galled plants were taller than nongalled plants. The separation into mainshoot and sideshoot galled plants allowed for the observation that gall orientation on the mainshoot seemed more detrimental than location on the sideshoot. The number of plants in natural populations with mainshoot galls, however, is small. Sideshoot galls, which are much more numerous, have little effect on the height of plants, indeed in some cases they appeared taller than nongalled plants (Table 15). This may be because the flies are naturally attracted to the taller plants. Attraction of insects to larger plants has been shown in other cases; Tyria jacobea on Senecio jacobaeae (Meijden 1976), Depressaria pastinacella on Pastinaca sativa and Papaipensna cataphrata

on Dipsacus sylvestris (Thompson 1978) and the gall causing insects on Solidago canadensis (Abrahamson et al. 1983).

There is some evidence that the reason plants with mainshoot galls are shorter is that they may have been shorter from the beginning of the season (Fig. 21), but later differences (June 20th, July 10th and 23rd) indicate that gall formation was also having an effect on reducing the height. Mainshoot galls also reduced and delayed flowering, whereas sideshoot galls did not (except for the particular sideshoot on which they occurred) (Table 19).

It is obvious from the field data that the plants were approaching maturity at fly emergence (i.e. almost 80% of mature height, up to 66% of the population with flowerbuds). This is no doubt the reason why (a) there are few mainshoot galls, because most of the mainshoots are in flower or at least initiating flowering and the flies cannot lay their eggs there, therefore they lay in the sideshoots and (b) galls seem to cause little stress in natural populations. In order to determine if the flies were more effective on shorter plants, laboratory experiments were performed.

For plants between 20 and 30 cm in height, a slight cessation of height increase was found in one case with mainshoot gall formation (Fig. 5). A similar observation was made by Peschken and Harris (1975). Although there was a trend toward lower leaf number, root bud number and root dry weight for galled plants, the differences were not significant (Table 16). With very short thistles (4 to 7 cm), there was a significant reduction in height, leaf number and ramet number when galled plants were compared with nongalled plants, whereas with slightly taller thistles (5 to 13 cm), these reductions did not occur (Figs. 10-12).

As reported by Peschken and Harris (1975), root development is restrained by gall development and the number of sideshoots was increased (Tables 17 and 18). The increase in sideshoots may be a problem since it could result in greater seed production. The higher shoot/root ratio indicated a smaller root system supporting the top, which is also an indication of stress. This may be important , because as in the case of Hypericum perforatum L., a decrease in root weight was the stress which made the biological program successful.

Studies on the effects of gall insects on Solidago canadensis indicated that the presence of a gall caused increased ramet production, decreased rhizome production and lowered seed allocation as measured by inflorescence production and propagule production (Hartnett and Abrahamson 1979). Stinner and Abrahamson (1979) in addition found that 7% of the ramet production was used on gall formation and support of the insects. This corresponds to the effects of mainshoot gall formation in this study, except that ramet production was neither significantly increased or decreased in most cases of U. cardui gall formation.

Gall formation deforms the host plant causing it to have many tops which are actually sideshoots on an unelongated stem. The gall also caused abnormal tissue differentiation in the stem. The gall form (nutritive tissue surrounded by sclerenchyma (inner gall) within thick-walled parenchyma) is one of the gall types described by Adler and Straton (1894) and the nutritive tissue of hypertrophied cells is one of the types described by Mani (1964). The nutritive zone was rich in starch, in an extracellular layer centripetally within the larval cavity (Fig. 28). The mechanical tissue in U. cardui galls occurred directly adjacent to the nutritive tissues and were the typical shape described by Mani (1964) (Fig. 30). The vascular system was modified, expanded and

stretched in comparison to nongalled tissue (Figs. 32 versus 33) and the epidermis had enlarged cells, two typical effects of galls. The tissue differentiation was not abnormal in the sense that unknown tissue types developed, but rather tissue type such as (nutritive tissues, sclerenchyma and thick-walled parenchyma) do not usually occur in Canada thistle stems.

Water-soluble protein content and total protein were highest in the leaves, especially of galled plants. This may be an indication of a higher mobilization of assimilates into a transportable form caused by gall formation. The high sugar content of the gall indicated that it probably accumulated carbohydrates.

The radioactive precursor experiments gave interesting insights into U. cardui gall development. When the gall was young (Table 21), it accumulated a high amount of the precursor, but as it aged (Tables 22 and 23), the quantity accumulated decreased gradually. Young galled plants also had slightly depressed accumulation in the leaves and roots as compared to nongalled plants, but there was no appreciable drain. This data indicates that the gall is a weak physiological sink when young and as it ages, it rapidly ceases to function as a physiological sink.

Ramet and stem accumulation patterns (mirror images- Fig. 36) indicated that the reallocation of resources for vegetative reproduction may come from the stem.

It has been postulated that the nutritive sink and stress on the plant increased with the number of larvae (Shorthouse 1980). Since the number of larvae is positively correlated with the gall size and gall weight, the data classified by size or weight (Table 24, Fig. 37), gave some positive evidence

to this theory for growth parameters and accumulation of a radioactive precursor.

In summary, the possible reason that gall formation appears to cause only minor stress in the field is related to poor synchrony of the insect to the phenology of the plant. By the time the flies have emerged, many of the plants already have flower buds and the fly lays its eggs in the sideshoots which are beginning to elongate. Laboratory tests indicated that more detrimental effects were found for smaller thistles. It has been shown that sideshoot galls caused much less stress on plant height, flowering and root growth than mainshoot galls. Therefore, theoretically, if the fly emerged earlier or if plant development was delayed so that the fly could lay its eggs in the mainshoot of small plants, a greater detrimental effect would occur.

The gall is a weak sink when young with a high concentration of sugar and accumulation of the radioactive sugar precursor, but as it matures, it accumulates less radioactivity. The type of nutritive tissue, large hypertrophied, but empty cells may not be as great a sink as the large cytoplasmic type and therefore this may also contribute to the low level of stress caused to the plant by this particular gall causer. There is also some evidence to support the theory that an increase in nutritive sink and stress parallels with the number of larvae.

C. PREDISPERSAL SEED PREDATION: *ORELLIA RUFICAUDA*

C.1 Introduction

C.1.1 Predispersal Seed Predation

Seeds of all plant families are the principal food of many animals and are attacked by pathogens worldwide (Harper et al. 1970, Janzen 1971). Predation on immature fruit is common and not only reduces seed yield, but may introduce fungal and bacterial diseases into the mother plant (Janzen 1970).

Predispersal seed predation has been recorded for several weedy species including *Ambrosia artemisiifolia* L. (Reed and Stephenson 1972), *Arctium lappa* L. (Hawthorn and Hayne 1978), *A. minus* (Hill) Bernh. (Reed and Stephenson 1972, Hawthorn and Hayne 1978), *Asclepias syriaca* L. (Willson and Rathcke 1974), *Asclepias viridis* Walt. (Evans 1983), *Astragalus canadensis* L. (Platt et al. 1974), *Astragalus cibarus* Sheld. and *Astragalus utahensis* Gand. (Green and Palmbald 1975), *Cirsium canascens* Nutt (Lamp and McCarty 1981, 1982), *Cirsium palustre* (L.) Scop. and *Cirsium vulgare* (Savi) Ten. (van Leeuwen 1983) and also *Cirsium arvense* (L.) Scop (Harris 1971, Watson et al. 1980).

Seed predation affects plant population dynamics and plant-animal interactions (Janzen 1970, Borchert and Jain 1978, Heithaus 1981). Aspects of plant demography affected by seed predation include species diversity and density (Halligan 1974), spatial juxtaposition (Janzen 1971), competitive

abilities (Brown and Davidson 1977) and timing of seed production (Janzen 1975). There is controversy concerning the effects on dispersal; Janzen (1969, 1970, 1971) and Wilson and Janzen (1972) state that dispersal is enhanced by predation whereas Platt et al. (1974) and Hubell (1979) claim that clumping is enhanced.

The rate of seed predation is a function of the proportion of previous seed predation (Vandermeer 1975) and may also be affected by the search abilities of the predator (Janzen 1971b). Variation of predation is common (Platt et al. 1974, Marshall and Jain 1970) and is considered as important to population dynamics as the degree of losses (Janzen 1971a, Harper 1977). Low predation may be a function of poor and sporatic synchrony (Beattie et al. 1973).

Seed head predators have been used in several biological weed control programs including Lantana camara L., Ulex europeaus L. and Senecio jacobaea L. (Huffaker 1957, Holloway and Huffaker 1957, Julien 1982). Seed head gall insects, Urophora affinis Frld. and U. quadrifasciata Meigen are increasing in North America on Centaurea diffusa Lam. and C. maculosa Lam. populations but with little regulatory impact (Harris 1980).

In natural habitats, seed predation ranges from less than 1% to 100% with usual recorded intensities between 10 and 90% (Janzen 1969). Losses between 50 and 80% are common for perennial plants (Breedlove and Ehrlich 1968, 1972, Willson and Rathcke 1974, Platt et al. 1974, Green and Palmbald 1975) and also for annuals and biennials (Hawthorne and Hayne 1978, Willson and Rathcke 1974). In some cases seed predation is low (7-25%) (Hawthorne and Hayne 1978, Beattie et al. 1973).

The concept that a plant produces sufficient seed relegating seed mortality as unimportant since only one seed is required for population renewal is in dispute (Janzen 1969). The effectiveness of the predation is not necessarily positively correlated with severity, but is complicated by the type of plant, seed germination and whether it takes a large or small annual seed production for population maintenance.

C.1.2 Case Study Organism : Orellia ruficauda (Diptera:Tephritidae)

Description

Orellia ruficauda (Fab.) is a small fly that oviposits into developing flower heads of Canada thistle (Detmers 1927, McFadden and Foote 1961). The species is distinguished in the adult stage from other members of the genus by a wing pattern composed of spots as opposed to bands (McFadden and Foote 1961). The adult is between 5-6 mm long with a wing expanse of 6 mm. The head is small , yellowish green, with vivid green compound eyes; the thorax is dorsally black with a lighter marginal line and a wedge shaped light area at the posterior end. The larvae are footless, white and between 5-6 mm in length (Detmers 1927).

Host and Geographical Range

O. ruficauda has been reared from Cirsium arvense heads collected throughout Europe, northern and central United States and southern Canada (McFadden and Foote 1961) and from Taraxacum officinale and Cirsium palustre (Europe) (Maw 1976). Hendel (1905) also reports Cirsium palustre as a host plant. Adults have been observed resting on carrots and raspberry plants (McFadden and Foote 1961) and on alfalfa and wheat (Maw 1976). The fly has a

wide geographical range including middle and north Europe, central Asia (Hendel 1905), North America from British Columbia to Newfoundland and south to California (USDA 1965). It was accidentally introduced into Canada and has been reported in British Columbia, Manitoba, Ontario, New Brunswick and Newfoundland (McFadden and Foote 1976, Maw 1976) and in Quebec (Virly and Watson 1977).

Life History

The adult female fly oviposits into developing flower heads of Canada thistle in the middle to late summer (Detmers 1927, McFadden and Foote 1961). Damage is caused by young larvae which enter the achenes by eating through the pericarp and exit after consuming the interior of the seed. In late July, after completing their development, the mature larvae construct a cocoon in the seedhead composed of pappus hairs glued together by larvae secretions. The larvae diapause in the cocoons and pupate in the flower head in their larvae skins the following spring. Without a cold treatment, adults can emerge in 2 to 3 months (Phillips 1923).

Stress Caused by Orellia ruficauda

Reported values of occurrence of O. ruficauda in the literature vary by site; 32% in grazed pastures, 44% at right-of ways and 63% in an ungrazed pasture (Watson et al. 1980). It is capable of attacking up to 70% of thistle heads (Harris 1971, Virly and Watson 1977), ranging from 27-70%.

The average number of larvae per head has been found to be slightly over one per head with a maximum of 8 per head (Watson et al. 1980). The seed damage ranged from 5-30% (Virly and Watson 1977).

There is some controversy concerning the type of damage caused to the seed

heads. Lund and Rostrup (cited from Detmers 1927) described damage as ruptured involucres and also twisted heads. Detmers (1927) also observed similar heads but they were not infested with O. ruficauda, rather by the Canada thistle midge Dasyneura gibsoni Felt. The mature midge larvae are small (3 mm long), orange red and footless with sucking mouthparts. They suck the juices from the base of the florets and young achenes, preventing their development (Detmers 1927).

The objective of this portion of the study was to examine the stress physiology of the predispersal seed predator Orellia ruficauda on Canada thistle.

C.2 Materials and Methods

During 1980 to 1982, female flower heads of Canada thistle were sampled at random from field populations on the Macdonald College farm. For each head, the number of larvae, the number of damaged seeds and the number of undamaged seeds were counted. Eighty-two heads were examined in 1980, and 127 in 1982. Only 41 heads were examined in 1981 due to an extremely low population of O. ruficauda.

Samples of damaged and undamaged seeds from attacked Canada thistle heads were fixed in 3.0% glutaraldehyde in 0.025 M phosphate buffer (pH 6.8) (O'Brien and McCully 1981), dehydrated in a methyl cellosolve, ethanol, n-propanol, n-butanol series and infiltrated with and embedded in glycol methacrylate (GMA) monomer mixture (Feder and O'Brien 1968) for plastic sectioning. O. ruficauda cocoons were similarly fixed and dehydrated but embedded in wax for sectioning (Jensen 1961). Sections (1-2μ) of the seeds were cut and stained with periodic acid Schiff's (PAS) reaction alone or counterstained with toluidine blue O (O'Brien and McCully 1981). Thick sections were cut of the cocoon and left unstained.

Correlation coefficients were determined between the number of larvae and the number of damaged seeds, the percentage of damaged seeds, the number of viable seeds for attacked heads and the number of damaged and viable seeds for all heads examined for data from years 1980 and 1982. Correlations were not performed for 1981 data due to low predation level.

C.3 Results

The portion of heads attacked by O. ruficauda in this study was low with 21 to 37% for 1980 and 1982 (Table 25) and was only 2% in 1981. Damaged heads sometimes appeared blighted and twisted (Fig. 38). Blasted heads sometimes contained O. ruficauda or Dasyneura gibsonii (Canada thistle midge) or both. Both insects were also found in heads which were not disfigured. Therefore no association between seed head morphology and insect presence could be drawn. Seed heads infested with O. ruficauda were disrupted by cocoon formation. The larvae was surrounded by a clear zone probably due to shrinkage of the larvae during fixation and the cocoon was formed from pappus hairs (Fig. 39). Damaged seeds were easily distinguished by the entrance/exit holes in the pericarp or by the presence of fragmented seed coats (Fig. 40). The location of the holes in the seed varied; with 45% near the base, 27% in the middle and 28% near the apex of the seed (n=130).

The seed coat of Canada thistle seeds has many layers (Figs. 41 to 44) including an epidermis and sclereid layer. O. ruficauda predation did not affect the seed coat layers except at the entrance/exit hole. The layers just inside the testa, the cotyledonary tissue and the embryo were however severely disrupted. In intact seeds (Figs. 41 and 42), the cells of the cotyledons were regular in shape with easily distinguishable cell walls and were highly cytoplasmic with many small vacuoles. In some of the O. ruficauda damaged seeds (Figs. 43 and 44), the layer just beneath the pericarp was torn away. In all cases, the cotyledon and embryo areas were almost completely obliterated and the remaining tissues were amorphous collections (PAS positive) with few distinguishable cell walls or intact cells.

Figure 38. Canada thistle seed heads (a) not infested and (b) containing an Orellia rufucauda larva

Figure 39. Thick section through an O. rufucauda larva and cocoon. (a) larva; (b) larval skin (c) clear region around the larva and (d) cocoon formed of pappus hairs

Figure 40. Seeds damaged by O. rufucauda larvae. Some seeds were entirely fragmented (far right) while others were easily distinguished by entrance/exit holes

Figure 41. Plastic section made through an undamaged thistle seed showing layers of the testa (t), the sclereids (s), the nutritive tissue (n) and the cotyledon (c). The cells are regularly shaped, highly cytoplasmic and have many small vacuoles. (PAS/toluidine blue stain) (x250)

Figure 42. Plastic section of an undamaged seed stained with PAS. The cell walls are easily distinguished by this stain. (x250)

Figure 43. Plastic section of a damaged seed stained with PAS/toluidine blue. Some of the nutritive tissue has been torn away from the seed coat and the area is generally empty as most of the cotyledonary and embryo tissue has been eaten. (X250)

Figure 44. Plastic section of a damaged seed stained with PAS. Much of the material remaining is PAS positive, amorphous with few distinguishable cells (X250)

43
44

The average number of larvae per head was slightly over one (Table 25) and the percent damaged seeds was 21.5%. Correlations between the number of larvae and the percent damaged seeds, the number of damaged seeds and the number of viable seeds for attacked heads were low (0.03 to 0.37). Correlations for data inclusive of heads without larvae between the number of larvae and the number of damaged seeds were higher (0.6 and 0.84).

TABLE 25. Orellia ruficauda Predation of Canada Thistle

Parameter	Year * 1980	1982	Combined
Heads sampled (n)	59	127	186
Heads damaged %	37.3	14.2	21.5
Seeds/head $ x+S.D. (range)	42.1±22.0 (4-84)	28.2±30.1 (1-104)	28.7±25.6 (1-104)
No. larvae/head # x+S.D. (range)	1.1±0.4 (1-2)	1.2±0.5 (1-3)	1.2±0.4 (1-3)
Damaged seeds # per head x+S.D. (range)	8.7±4.8 (2-17)	3.8±3.2 (0-11)	6.5±4.8 (0-17)
Viable seeds/head # x+S.D. (range)	40.3±16.8 (6-84)	16.6±13.4 (1-54)	29.6±19.3 (0-17)
Predation level # (%) x+S.D. (range)	17.9±7.9 (5.1-33.3)	24.1±21.2 (0-87.5)	20.4±15.8 (0-87.5)

* Data for 1981 omitted because of low O. ruficauda predation in the study area

$ Unattacked heads

\# Values presented on data for attacked heads only

C.4 Discussion

Seed predation can affect plant population dynamics, plant demography and plant-animal interactions (Janzen 1969, 1970, 1971, 1974, Brown and Davidson 1977, Harper 1977, Borchert and Jain 1978, Heithaus 1981).

In natural habitats, seed predation can range from less than 1% to 100% with usual recorded levels between 10 and 90% (Janzen 1969). Values for O. ruficauda predation of Canada thistle heads range from 27 to 70% (Harris 1971; Virly and Watson 1977) and vary by site (32% for grazed pastures, 44% for right-of-ways and 63% for ungrazed pastures). (Watson et al. 1980). In this study, the percent of heads attacked was much lower at 21.5%. The average number of larvae per head (1.2) and the percentage of seeds damaged (20.4) were similar to previously reported values (Watson et al. 1980).

The low correlation value between the number of larvae and the number of viable seeds, the number of damaged seeds per head and the % of damaged seeds per head indicates either that these factors are not affected by the number of larvae present, or that the range of 0 to 3 larvae is not sufficient for accurate correlation analysis. The latter is more probably the case, since intuitively an increase in the number of larvae should result in an increase in the damage.

The low number of seeds eaten per head indicates that predator satiation occurred in this study. The variation in the predation level (from almost 40% in 1980, near 0% in 1981 and 14% in 1982) in this study and the range reported in other studies indicate that the effect of O. ruficauda as a natural enemy or a biotic stress on Canada thistle is unreliable. However, variation in

predation is common (Marshall and Jain 1970, Platt et al. 1974, Heithaus 1981) and is considered important to plant population dynamics (Janzen 1971, Harper 1977).

Unpredictability of seed production is considered a defense against predation (Harper 1977). In this study, in 1982, when seed production was low (20 seeds per head) the percentage of heads attacked was less than half of those attacked in 1980 when approximately twice the number of seeds per head was produced (50 seeds per head). The number of damaged seeds per head in 1980 (8.7) was more than double that of 1982 (3.8). Therefore, the proportion of damaged seeds was the same for both years and the proportional contribution to the seed bank was similar.

Seed head predators have been used in several biological control programs with little regulatory effect (Harris 1980). With annuals and biennials, for which seed production is the only method of reproduction, seed predation can reduce the local population and also dispersal to new habitats. However, the effect of the reduction of the seed bank is dependent on the demography of the seed crop (Harper 1977, Borchert and Jain 1978). In some cases, for example, where density-dependent mortality of seedlings and competition is high, a reduction in the number of viable seeds produced may have little effect or may even cause an increase in the population. On the other hand, in cases where the density-dependent seedling mortality is insignificant, a reduction in seed production may cause a depression in the population level (Lamp and McCarty 1982, van Leeuwen 1983).

Success in biological weed control programs using seed predators on perennial weeds has been limited and the efficacy of a seed predator in the

case of Canada thistle is uncertain. Huffaker (1957) considers that the suitability of seed predators for the control of perennials is questionable and control by seed reduction would likely be a long, slow process, especially if vegetative reproduction occurs. Within pasture habitats Canada thistle seedlings do not survive (Amor and Harris 1974), but dispersal to new habitats is still possible and a seed predator may be effective in reducing weed spread, since a decrease in seed production decreases the average distance of seed dispersal (Harper 1977). Chancellor (1972) noted that although perennial weeds have less need for seed production since it is not essential for them to reproduce by seed within a single or even many seasons, that seed production should not be neglected, as it may negate the effectiveness of other control measures.

The implications for biological control by predispersal seed predators are complexed by several factors including post-dispersal predation, the number of seeds required for population maintenance, seed survival in the soil, seedling mortality and so forth. It is known that seed germination of Canada thistle is highly variable (0-100% depending on the conditions) (Hayden 1934, Bakker 1960, Kumar and Irvine 1971) and seedling establishment is low especially under low light intensity (Bakker 1960) and competition (Hodgson 1968). However, many of the values required for accurately predicting the effect of predispersal seed predation on the population dynamics of Canada thistle are not known. Although O. ruficauda may not be a severe predator; complexed with low seed germination, short distance dispersal and low seedling survival, intuitively seed predation of this perennial weed may be an important factor for the regulation of the density and spread of the weed population.

D. ROOT CROWN DAMAGE: CLEONUS PIGER

D.1. Introduction

D.1.1 Root Crown Damage

According to Ross and Hedicke (1902) and Mani (1964), Cleonus piger (Scop.) initiates gall formation in the root crown of Canada thistle. The gall is described as occurring in the lower part of the shoot, being spindle in shape and approximately 40 mm long by 10 mm wide. The various aspects of gall formation have been described in section B.

The damage caused by root crown feeding insects in general, can interfere with (1) the transport of minerals, water and so forth from the roots to the plant or (2) the translocation of storage products to and from the roots. The extent of the damage to the vascular system no doubt determines the stress to the plant. If damage is sufficient, water supply transport may be reduced below the required capacity and the plant may wilt and die.

D.1.2 Case Study Organism: Cleonus piger

Cleonus piger (Coleoptera:Curculionidae) is a large weevil (snout beetle) whose larvae feed in the root crown area of thistles. It occurs from Scotland (Cawthra 1958), through the dry region of Lapland, south to Italy and Corsica and Elba in the Mediterranean (LaFerla 1939 cited from Peschken unpub.). It was first found in North America in New York in 1929 and in Quebec in 1933 (Brown 1940) and spread into Ontario and Michigan (Peschken unpub.). It

attacks species in the genera Cirsium, Carduus, Cynara, Onopordum, Arctium and Silybum in the field (Scherf 1964 cited from Peschken unpub., Zwolfer 1965). In laboratory tests, adult weevils also fed on Inula, Zinnia, Rudbeckia, Cnicus, Carthamus, Centaurea, Taraxacum, Sonchus, Leontodon and Lactuca species (Zwolfer 1963). It was frequently reported as a pest of sugarbeets (Beta vulgaris L.), but this may have been misidentification but it does develop on artichoke roots (LaFerla 1939 cited from Peschken unpub.).

Life History Adult weevils overwinter near the soil surface and become active in May in New York State and in late April in Scotland. According to Peschken (unpub.), the female lays single eggs in the lower portion of the stems by chewing a cavity in the stem, depositing the eggs and plugging the hole with excrement. Cawthra (1958) on the other hand observed females digging and forming holes in the soil near the base of the stem with the rostra, then laying their eggs in the hole and covering it with soil. LaFerla (1939) also found eggs at the base of the ribs of the leaves or on the adaxial leaf surfaces of artichokes. The larvae hatch within 8 to 12 days, enter the stem and mine to the soil level. By the second instar, a swelling or spindle shaped gall is visible just below the soil level. Pupation occurs 30 days later. Usually only one larvae is found per Canada thistle plant (Scherf 1964, Cawthra 1958), but up to three have been found (Cawthra 1958). In artichoke roots, three larvae are common in 2 to 3 year old roots and up to seven in older roots (Peschken unpub). Adults emerge in August or early September in New York State. In North America and most of Europe, C. piger is univoltine, but in Italy, it can have two generations per year on artichoke (LaFerla 1939 cited from Peschken unpub.). Mortality factors reported in Germany (Urban 1967 cited from Peschken unpub.) include dipterous larvae and nematodes in Scotland

(Cawthra 1958).

Stress Effects of Cleonus piger In 1959, Cleonus piger was rated as the most important insect pest on Canada thistle at the Belleville Ontario Research Station, occurring on 26-38% of the plants and killing plants on poor soils (Anon. 1959). Watson et al. (1980) found up to 60% of Canada thistle plants sampled in Quebec were attacked by C. piger and that attacked plants were wilted, often failed to produce flower buds and usually perished. Harris and Zwolfer (1971) reported that both Cleonus piger and Cassida rubiginosa were confined to eastern Canada and that infested plants showed few deleterious effects. Cawthra (1958) reported that plants containing live C. piger larvae were non-flowering, whereas Peschken (unpub.) found larvae in both flowering and non-flowering plants in Canada. Other Canadian results indicate that the height, stem diameter at ground level and the number of flowers are greater for plants associated with C. piger inhabitation than for "clean" thistles. A conclusion from this is that either C. piger selects larger plants or there is a stimulation of height, diameter growth and flower bud production and that the effect is not injurious.

The purpose of this study was:

(1) to determine the field population levels of Cleonus piger

(2) to examine the type and level of mechanical damage caused by the insect and

(3) by simulating the damage caused (i.e. damage to the vascular tissue) to determine the response of the plant to complete stele severance.

D.2 Materials and Methods

Field Studies. Random samples of field grown plants were collected from the sites mentioned in Section A and were examined for the presence of C. piger. Numbers of plants with and without damage were recorded.

Microscopal Studies. Healthy and damaged tissue was fixed, dehydrated and embedded and sections prepared according to procedures in section B.2.5. Some damaged tissue was handsectioned and placed in 70% lactic acid and heated for clearing. Plastic sections were stained with either the PAS reaction or toluidine blue alone or the PAS reaction counterstained with toluidine blue as per Section B.2.5.

Simulation Experiments. Plants at the 4 to 6 leaf stage grown from root pieces in Promix (grown under conditions outlined in Section B.2.2) were notched with a razor blade and placed in a moist chamber until sampling. Plants were removed after 2, 4, 8, 12 and 24 hours and 2, 4, 5 and 7 days (3 replicates at each time period). Control plants were notched and sectioned immediately. At each sampling time the notched area was isolated and sectioned by hand longitudinally. The sections were cleared in 70% lactic acid. The experiment was repeated.

D.3. Results

In the years 1981 to 1982, Cleonus piger attacked a maximum of 27% of Canada thistle plants randomly chosen from field populations (Table 26). Plants attacked by C. piger tended to be taller than the average plant (72.2 cm versus 59.9 in late August (1982 field data)) and flowering was not usually affected by the presence of the larva.

Of 17 dead plants examined in late August, (at site 3) seven contained signs of Cleonus piger habitation (41%). Therefore, C. piger can kill plants, however, the only dead plants observed containing C. piger from samples collected from the four field sites (Fig. 1) were from site 3 with the poorest soil.

Plants containing C. piger often had a swelling a few centimeters below the soil line, some swellings were as large as 3 to 4 cm in diameter (regular root crown region diameter is 1 to 1.5 cm). Upon dissection of plants containing C. piger larvae, the larval cavity was found approximately 7.0 cm below the soil line, but a head sheath made of chewed plant material (Fig. 45) extended in the middle of the stem/root crown region up to 5.5 cm below the soil line. The larval cavity lining was blackened indicating necrotic tissue.

Cleared hand sections of the larval cavity showed a thick layer of necrotic tissue lining the interior. Regenerated tissue from the cortex (parenchyma) grew between the vascular tissue (Figs. 46-49) into the cavity. In undamaged tissue, vascular tissue filled the entire zone which would be eaten by C. piger larvae, therefore the occurrence of the larval cavity (between 0.5 to 1+ cm in diameter) eliminated a large portion of the xylem

Table 26. Occurrence of Cleonus piger Larvae in the Root Crown Region of Canada Thistle in Field Populations in 1981 and 1982.

Year and Site*	------Total Number of Canada Thistle Plants ------- With Cleonus	Without Cleonus	% attacked
1981			
Site 3	0	22	0
Site 4	0	18	0
1982			
Site 3	4	10	27
Site 1	1	16	6

* location and description of sites in Section A.

Figure 45: A split stem of Canada thistle showing the larval cavity and a head sheath of Cleonus piger larvae (Arrowhead at larvae)

Figures 46-49: Hand sections cut through a C. piger cavity and cleared in lactic acid.

46: Section showing a region of regenerated tissue pushed through the xylem which has necrotic tissue on its surface. (X 50).

47: An enlargement of figure 46. The regenerated tissue has many small, parenchyma-like cells (X 100).

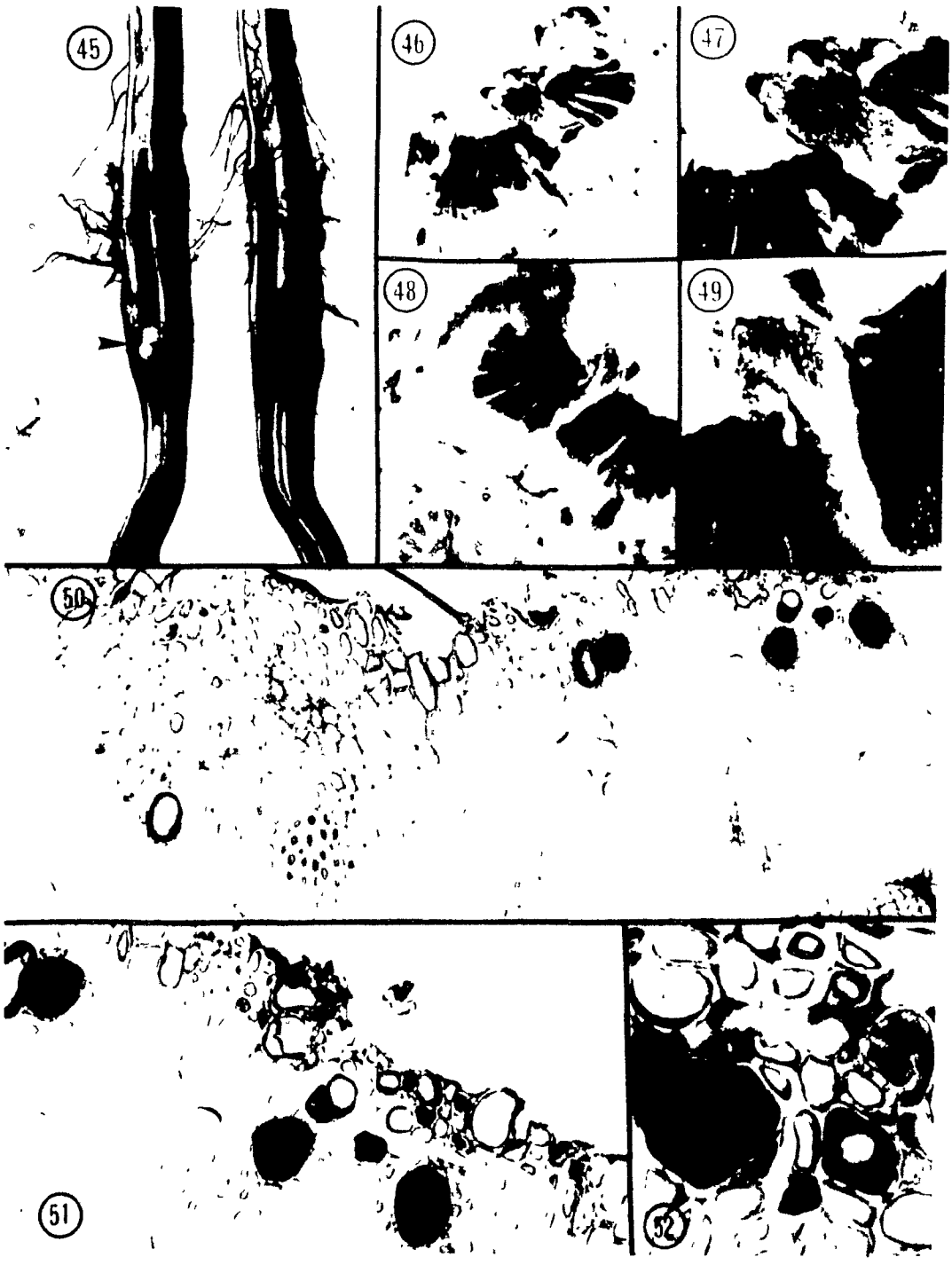
48: Section through the larval cavity also showing the regenerated tissue and the damage to the vascular system (X 50).

49: Closeup of the regenerated tissue and the surrounding thick necrotic region (X 100).

Figure 50: Radial section through the edge of the larval cavity. Note the irregular edge and the variety of cell types. Note also the cells which stain densely with toluidine blue (Plastic section stained with toluidine blue) (X 220).

Figure 51: Region containing many necrotic deposits and densely stained cells. Note the dark deposits between the cells and the number of dead cells on the edge. (Plastic section stained with toluidine blue) (X 440).

Figure 52: A closeup of the edge with several large densely stained cells, a wide variety of cell sizes and shapes and irregularly shaped deposits between the cells. (Plastic section stained with toluidine blue) (X 1000).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tissue. Higher magnification of the lining of the cavity indicated enlarged cells, many of which were dead. There were also dark deposits between cells (Figs. 50-52). The edge of the cavity was rough and cell types of many different sizes and shapes occurred directly along the edge (Figs. 51, 52).

In the simulation of damage (Figs. 53-57), the wound was not greatly modified by the plant even 7 days after the notch was made (no callus formation or phelloderm production). Regeneration of vascular tissue (xylem) was first observed as early as 12 hours after notching in the form of a simple connection between a cut vascular strand with an uncut one (Fig. 54, 56). After 4 days, the vascular connection had grown around the end of the notched area resulting in the rejoining of the vascular tissue (Figs. 55, 57).

Figures 53 to 58: Simulation (Notching) Experiments

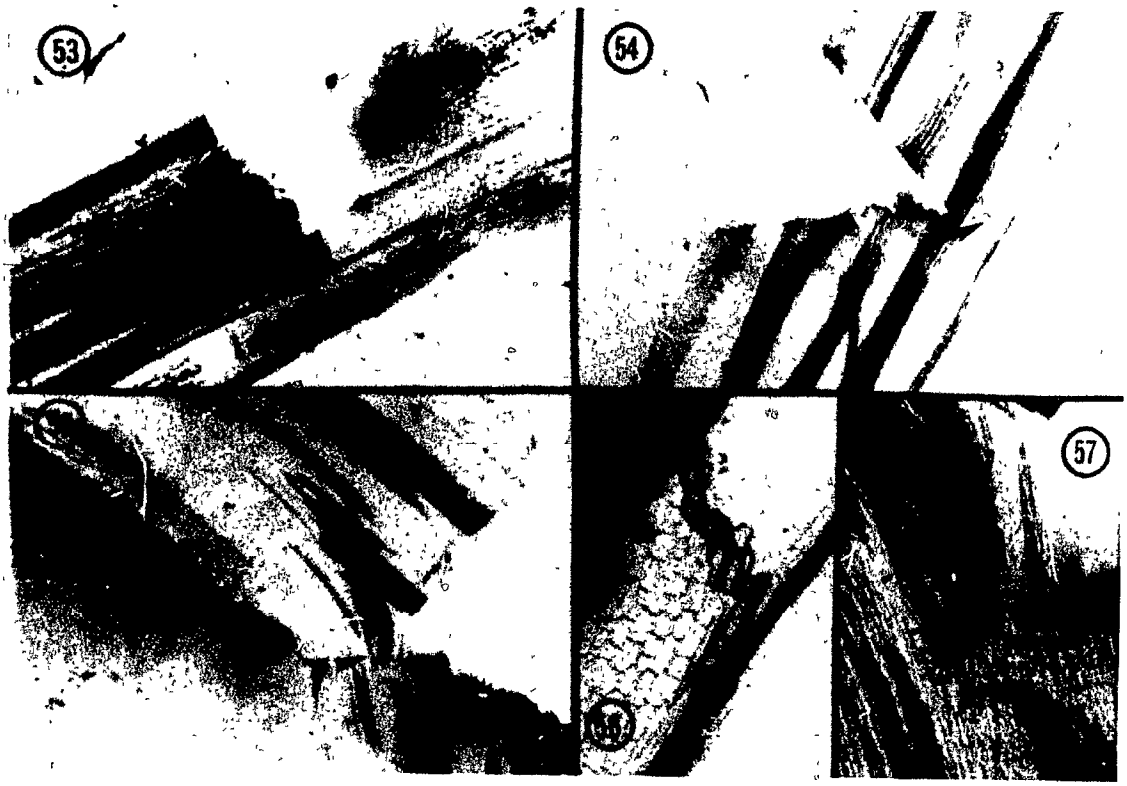
Figure 53: Control (sectioned soon after the notch was cut) showing the extent of the notching which occurred (X 125).

Figure 54: Twelve hours after notching, a simple connecting strand ran from a severed strand to an unsevered strand (Arrowhead) (X 125).

Figure 55: Four days after notching, a bridge of vascular tissue ran around the end of the notch rejoining the vascular tissue from the two ends (Arrowhead) (X 125).

Figure 56: An enlargement of the vascular strand from figure 54 (X 300).

Figure 57: An enlargement of the vascular bridge (arrowheads) from figure 55 (X 300).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54
57

D.4 Discussion

Cleonus piger is a low level predator, (affecting a maximum of 27% of the plants in the field situation in this study). Watson et al. (1980) found higher values for the same region (maximum 60%) and Anon. (1959) found up to 38% in Belleville, Ontario. Even at a low level, it no doubt contributes to the overall regulation of the Canada thistle population.

If a plant is severely damaged by C. piger larval feeding, the plant will die. Microscopal examination has shown that this is due to the removal of large quantities of vascular tissue. Plant wilt is one of the first symptoms of C. piger presence in the root crown area. The plant combats the damage by regeneration of tissue into the larval cavity. Results from the simulation (notching) experiment indicated that even with complete stele severance, the vascular tissue could regenerate by differentiation of parenchyma tissue. Such regenerated vascular strands are believed to be functional (Robbertse and McCully 1979).

The fact that Canada thistle can withstand removal of a large amount of vascular tissue and still grow and produce flowers and root buds indicates that C. piger causes at best only a minor stress to the plant. But, in the overall scheme, it probably asserts a sufficient stress to cause some degree of regulation of the population especially during periods of water stress.

At one time, it was proposed that C. piger and Cassida rubiginosa be moved into the western provinces, since Canada thistle is much less prevalent in the st and since these two natural enemies were present in the east, it was deduced that they were contributing to the control of the weed. Due, however

to these insects feeding on economic crops, the transfer was not made (Peschken, unpub.)

E. DEFOLIATION. CASSIDA RUBIGINOSA

E.1. Introduction

E.1.1 Defoliation

Defoliation has been defined as the, "deprivation of leaves, especially prematurely" and as the, "severing or removal of part or all of herbage by animals (grazing) or cutting machines (harvesting)" (Thomas 1980). A more comprehensive and general definition is; the loss of photosynthetic area (or capacity) by any means including grazing, mowing, foliage diseases, herbivory. The effects of defoliation have been extensively examined in natural grasslands and in the forages. Total or partial defoliation can result from grazing by animals, harvesting, feeding by phytophagous insects, plant pathogens, fire, hail or wind damage. A decrease, an increase or no change in plant growth, yield or reproductive capacity can result from defoliation (Harris 1972, 1973a).

Decrease in Plant Growth. Leaf removal can cause, (i) lower seed production, (ii) a decrease in the growth rate, (iii) increased mortality and (iv) other indirect effects including structural adaptations. A reduction in reproductive capacity is a common effect of defoliation in many crop plants. Leaf removal often results in reduced grain yields in cereals (Archibold 1942, Last 1955, Pauli and Laude 1959, Stickler and Pauli 1961, Walpole and Morgan 1974, Simmons et al. 1982). Defoliation reduces soybean yield and also affects the oil and protein content of the seed (McAlister and Krober 1958, Begum and

Eden 1965, Thomas et al. 1976, Caviness and Thomas 1980). Corn (Hanway 1969, Remison and Omuetti 1982) and sunflower (Sackston 1959) seed yields can be reduced by defoliation.

For forages, defoliation at some points during the season reduces seed yield for timothy, perennial ryegrass and cocksfoot (Roberts 1958) and crimson clover (Knight and Hollowell 1962). Tree seed production may also be reduced by defoliation (Kulman 1971, Stephenson 1980). For some Central American deciduous trees, defoliation resulted in a decrease in fruit number and weight and at defoliation levels greater that 80% no fruit was produced.

There are also examples of reduced reproductive capacity caused by defoliation in weed species. The fruit and seed weight per panicle were reduced for Rumex crispus L. upon removal of the cauline leaves (Maun and Cavers 1971). A 50% mechanical grazing of wild ginger (Asarium caudatum) decreased seed production and dry weight by 50% (Cates 1975). With 45% defoliation of Austolodria reticulata by Battus philenor , there was increased plant mortality, decreased plant growth rate and a decrease in seed production (Rausher and Feeny 1980). Similar results have been reported for Ambrosia artemisiifolia L. and Artium minus Hill(Bernh). (Reed and Stephenson 1972). For Abutilon theophratis Medic., seed production decreased proportionately with increased defoliation (Lee and Bazazz 1980).

Decreases in yield (biomass, rather than seed) of forages and other crops also occur as a result of defoliation. For forages, grazing and mowing, especially if frequent can reduce forage yield (Madison 1962, Griffith and Teel 1965, Watson and Watson 1982), and also stand longevity (Knight and Hollowell 1962). In cocksfoot (Dactylis glomerata , defoliation decreased leaf initiation

and expansion (Davidson and Milthorpe 1966a, Ryle and Powell 1975). Dry matter yield of grain sorghum was also reduced with defoliation (Pauli and Stickler 1961). For trees, Kulman (1971) found growth reduction was proportional to the quantity of foliage removed. Williams (1967) found that spruce budworm feeding reduced the radial growth of grand fir, Douglas fir and Engelmann spruce and Piene (1980) found that defoliation reduced volume growth in balsam fir.

Defoliation can also cause a reduction in root growth and regrowth and root carbohydrate reserves (Sampson 1931, Archibold 1942, Jameson 1963, Alcock 1964, Marshall and Sagar 1965, Davidson and Milthorpe 1966b, Sosebee and Wilke 1971, Ryle and Powell 1975, Grant et al. 1981). Roots can be shorter and thinner and root initiation and growth rate may decline after defoliation (Marshall and Sagar 1965, Dunn and Engall 1971). Other effects recorded include; reduction of root respiration, carbon dioxide exchange and phosphorus and other nutrient uptake (Davidson and Milthorpe 1966b, Jameson 1963), increased root exudation (Dyer and Bokhari 1976, Whittaker 1979) and decreased root weight (Buwai and Taylor 1977). Vance et al. (1980) found that defoliation caused temporary senescence of alfalfa root nodules, whereas Lynd et al. (1980) found an increase in nodulation for hairy vetch. Several workers have noted that clipping affected translocation (Sosebee and Wilke 1971, Ryle and Powell 1975, Marshall and Sagar 1965).

Several structural changes have been noted. For big sagebrush (Artemisia indentata), clipping redistributed the major portion of dry matter to a lower position on the plant (Willms and Bailey 1980). Similar effects occurred with heather (Calluna vulgaris) (Mohamed and Gimingham 1970, Grant and Hunter 1966). Grasses and forages also can be structurally stunted and an alteration of leaf shape and size can occur (Grant et al. 1981, Detling and Painter 1983).

Changes in community structure and species composition, can result from defoliation (Tansley and Adams 1925, Hope-Simpson 1940, Davis 1958, Jameson 1963, Brougham and Harris 1967, Smith 1979,, Whittaker 1979, Coppoch et al. 1983, Detling and Painter 1983, Lubchenko 1983, Herchell and Tunen 1983, McBrien et al. 1983).

Other effects of defoliation include: decrease in phloem area (Sanders et al. 1977), decrease in nitrogen content (Pauli and Stickler 1961), decrease in root competitiveness (Remison and Snaydon 1980), decrease in photosynthetic rate (Poston et al. 1976) and alteration of competitive interactions (Grime 1979, Windle and Franz 1979).

Effects of defoliation on the plant are modified by several factors including (1) stage of plant development, (2) plant species, (3) type of leaves removed, (4) severity of the defoliation and (5) compensatory effects.

Stage of Plant Development Decreases in seed yield are generally greatest if the defoliation occurs during reproductive stages as opposed to vegetative or post-reproductive stages. This occurs in a wide variety of plants; corn (Hanway 1969, Allison et al 1975, Egharreba et al. 1976), soybeans (Begum and Eden 1975, Turnipseed 1972, Tergin and Vorst 1975, Fehr et al. 1977, Caviness and Thomas 1980), potato (Bereford 1967, Hare 1980), various tropical legumes (Enyi 1975), peanut (Boote et al. 1980, Santos and Sutton 1982) and lima beans (Coggin and Dively 1980a,b).

Type of Plant Species. Different plants have different responses to defoliation depending on the species, the mode of growth and resistance to defoliation. Differential cultivar response to defoliation has been found in

potatoes (Hare 1980) and soybeans (Fehr et al. 1977).

Types of Leaves Removed. In general, leaf removal from the upper portion of the plant is more detrimental than from the lower portion as reported for grain sorghum (Stickler and Pauli 1961), sunflower (Sackston 1959), potatoes (Hare 1980) and soybeans (Johnston and Pendleton 1968). Damage to leaves exporting carbohydrates (so-called 'feeder leaves')is more detrimental than damage to other leaves as exemplified by the effect of the removal of cereal flag leaves (Walpole and Morgan 1974, James et al. 1968).

Severity of Damage. In general, as the defoliation level increases, the greater is the detrimental effect to yield and other parameters (Begum and Eden 1965, Hanway 1968, Taylor and Bardner 1968, Caviness and Thomas 1980, Coggin and Dively 1980, Jackson 1980, Fick 1982, Rees et al. 1982). The effect, however, is not proportional to the amount of damage (i.e. 30% defoliation results in less than 30% reduction in yield et cetera).

Compensatory Effects. In most plants, a portion of foliage is dispensible and there is little yield reduction until a threshold is exceeded. Some examples of defoliation levels that various crops can withstand without detrimental effects include (at a stage of low susceptibility):

50%	soybean	Weber 1955 Tergin and Vorst 1975 Caviness and Thomas 1980
25%	sunflower	Sackston 1959
50%	snapbean	Guene and Minch 1967 cited from Rockwood 1973
30%	cucumber	Hussey and Parr 1963
50%	sugarbeet	Hodkinson and Hughes 1982

The determination of threshold damage levels are complexed by the growth stage of the plant. In general, crop plants can endure much higher levels of defoliation in the vegetative phase than at the reproductive stage.

Increase in Response:

It has long been known that some plants (apples and blueberries for example) require and benefit from clipping for maintenance of optimum yield (Janzen 1976). Forage crop productivity can also be increased by judicious clipping (Jameson 1963, Willms and Bailey 1980).

Harris (1973a) stated, "It is a fallacy that the consumption of leaves, flowers and other plant tissue by insects necessarily reduced plant vigour or reproductive capacity,... these factors may be increased." There are several cases where stimulation of plant growth and yield occurs after defoliation (Taylor and Bardner 1968, Hanway 1969, Kumar and Joshi 1972, Vickery 1972, Harris 1972, 1974, Dyer 1975, McNaughton 1976, 1979, Dyer and Bokhari 1976, Owen and Wiegert 1976, Hilbert et al. 1981, McNaughton 1983). For most of these examples cited in the literature, the defoliation pressure generally occurred early in the growing season, the damage was of limited extent and duration and the plants were not under intense competition (Harris 1972).

Ways in which defoliation may be stimulatory to plant growth include (1) an increase in photosynthetic rates of residual tissue (Vickery 1972, Gifford and Marshall 1973, Detling et al. 1979, Painter and Detling 1981); (2) reallocation of photosynthates and other substrates to new leaves (Ryle and

Powell 1975, Detling et al 1979); (3) the removal of leaves past their physiological prime (physiologically parasitic leaves); (4) increase of light intensity on all leaves (elimination of shade) and readaptation of shaded leaves to the sun; (5) a delay in leaf senescence; (6) hormonal redistribution promoting cell division and elongation and (7) enhanced conservation of soil moisture (McNaughton 1979).

No effect:

Harris (1972) hypothesizes that, "most insect species, most of the time, have little effect on plant abundance." Many plants require a high level of defoliation or a high threshold value (at certain periods of the year) before any detrimental effect occurs (Caviness and Thomas 1980 and references listed before).

The effect of defoliation can often be nullified by compensatory growth (Detling et al. 1979, Jackson 1980, McNaughton 1983). Removal of vegetative tissues rarely results in a proportional reduction of yield or growth measures. For most plants, roots and leaves operate below maximum potential as an ecological necessity to provide a buffer against accidents or environmental fluctuations (Maggs 1964). Therefore, most plants can suffer some defoliation without any apparent adverse effect.

Overall, the effect of a defoliator appears simple; a reduction of leaf area should intuitively adversely affect the plant. However, the relationships between leaf damage and plant productivity are complex and can be modified by several factors including the time and level of the damage, the type of leaf

damaged and the environment. However, although defoliators frequently consume only a small portion of available plant material they may have an important effect on ecosystem structure and function.

E.1.2 Defoliators and Biological Control:

In the early days of biological control, emphasis was placed on insects with specialized feeding habits, however, experience has shown that defoliators are also equally as safe and effective (Huffaker 1957). Consequently, many of the insects selected for the biological control programs have been defoliators. Indeed, several defoliators have been successful biological control agents including Chrysolina quadrigemina Suffr. and C. hyperii (Forst) on Hypericum perforatum L. and Tyria jacobeae L. on Senecio jacobaeae L.. In both cases, the co-action of the environment specifically winter frost in the tansy ragwort case and dry summers for St. John's wort were necessary for high success rates.

E.1.3 Case Study Organism: Cassida rubiginosa (Coleoptera: Chyrsomelidae)

Cassida rubiginosa is a tortoise beetle which defoliates plants and has a host range restricted principally to the Cirsium-Silybum- Carduus genera group of the Carduinae tribe of the Asteraceae. It has a marked preference for Canada thistle (Zwölfer and Eichorn 1966). As part of the biological control program, Zwölfer and Eichorn (1966) examined the host range of Cassida spp. in Europe

that attacked Cynareae. Their decision concerning C. rubiginosa was that it could not be recommended for introduction to foreign countries, because of the possibility of attack on artichoke (Cynara scolymus L.). However, the beetle was accidentally introduced into North America and was first reported on burdock in 1902 in Quebec (Fyles 1902, Barker 1916). The present North American geographical range is as far west as south Michigan and Ohio and south to north Virginia. It has been collected in Canada in New Brunswick, Quebec and Ontario (Brown 1940, Ward and Pienkowski 1978a) and the author observed it on Prince Edward Island.

As a defoliator, Cassida rubiginosa fills a niche which is relatively void on several thistles including Canada thistle (Ward 1976).

Life History. In Switzerland, beetles could be collected from early April until early September, adults being the most abundant from early May until late June and eggs and larvae from May to August (Zwölfer and Eichorn 1966). In Virginia, overwintering adults were active from mid March to early September and the larvae were active from April to early August. Oviposition occurred from mid March to early July. The oöthecae were laid 90% of the time on the abaxial leaf surface, most commonly at the leaf tip. Eclosion occurs in mid April. All five instars occurred from April to early August. The larvae carry their excrement over their backs on the caudal furca as a "parasol". Young larvae (1 to 3 instars) occur primarily on the abaxial leaf surface and older larvae (4 and 5 instars) occur frequently on the adaxial surface. After feeding is completed, the larvae usually discard the parasol and attach themselves by the last two or three sternites to the adaxial leaf surface, usually on a midrib or on the stem for pupation. New adults emerge from mid June to early August in Virginia and upon temperature induced quiescence move

to overwinter in the forest floor litter in September to October (Ward and Pienkowski 1978a).

Seasonal occurrence and phenology of the beetle are dependent on the host and possibly also on the microclimate (Ward 1976). Life cycle duration is only 25 weeks on Canada thistle, whereas it is 37 weeks on musk thistle (Carduus nutans).

The importance of the damage caused by Cassida rubiginosa has been noted in Russia and in Canada (Harris and Zwölfer 1971). Adult females feed more than the males and the preferred feeding site for both sexes in on the abaxial surface. Increased feeding occurs during the ovipositional periods of the cycle. Developmental rates of eggs, larvae and pupae, eclosion of eggs and weight of newly hatched adults are all temperature dependent (Ward and Pienkowski 1978a).

High parasitism prevents the buildup of Cassida rubiginosa populations (Harris and Zwölfer 1971). Ward and Pienkowski (1978b) found total parasitism to be about 20% with the major parasites being Tetrastickus rhosaces (Walker) (Hymenoptera: Eulophidae) and Eucelatoriopsis dimmocki (Aldrich) (Diptera: Tachinidae).

The objectives of this study were:

(a) to determine the effect of insect defoliation on yield and growth parameters of Canada thistle using insect defoliation, simulation of defoliation in the greenhouse and in the field and by examining the relative

importance of the upper versus the lower leaves.

(b) to examine the life history of Cassida rubiginosa in Quebec.

E.2 Materials and Methods

E.2.1 Defoliation Experiment with Insects

Field collected larvae were placed on individual plants at densities of 1, 5, 10 and 20 larvae per plant. The insects were confined to the plants by wire mesh cages taped to the top of the pot. Controls were also covered by mesh cages. Plants (at similar stage as outside when first attacked) were grown on a growth bench (20^{o} C day, 14 hours, 300 microEinsteins m^{-2} sec^{-1}; 15^{o} C 10 hour nights). The cages were removed after two weeks and height and leaf number recorded. The plants were harvested seven weeks later (9 weeks after commencement of the experiment). Visual ratings of percent leaf area removed were made. (Appendix F). Other data recorded at harvest included height, number of ramets, leaf number, fresh and dry weight of plant, root and ramets, heights of the ramets and number of visible root buds.

E.2.2. Simulation of Defoliation

Plants were started from 1-2 cm root pieces planted in Promix in flats. When the plants were at the three to four leaf stage, they were potted in 155 mm diameter plastic pots in a soil mixture of 3:1:1=pasturized soil:sand:peat moss and placed in the greenhouse. They were watered and fertilized regularly.

Plants were defoliated at 25, 50, 75 and 100% levels at various leaf stages. Control plants were not defoliated. The level of defoliation was obtained by removing the required percentage of each leaf on the plant (i.e. 25% by removing 1/4 of each leaf on the plant). The blade of the leaf only was removed, leaving the midrib intact and no sideshoot leaves were defoliated.

E.2.2.1 Preliminary Experiments

Plants were defoliated at the 7-leaf stage (2 repeats- experiment 1 (3 replicates per treatment) and 2 (4 replicates)) and at the 9 leaf stage (2 repeats- experiments 3 (3 replicates) and 4 (4 replicates)). The plants were placed randomly on the bench. Data taken periodically included; height, leaf number, ramet number and ramet heights and the number of flowers produced. When the plants were harvested (after 18 weeks, experiments 1 and 3 and 12 weeks- experiments 2 and 9 weeks experiment 4), data taken included; height, leaf number, ramet heights and number, sideshoot number, flower number, root bud number. Dry weights of the plant (mother), root and ramets were determined. Data were analyzed as a completely random design.

E.2.2.2 One-Time Defoliation

This experiment was repeated once. The plants from the two repeats were grown under similar conditions as those in section E.2.2.1, except that the lighting regime for repeat one was at environmental levels (without supplemental lighting) and for repeat 2 was under lights (450-500 microEinsteins m^{-2} sec^{-1} with a 14 hour day. Plants were defoliated at 5-, 10-, 15- and 20-leaf stages (4 replicates of each). The experimental design was randomized complete block with age/defoliation combinations applied to each plant. Data taken throughout the growth period included; height, leaf number and the number of ramets at weekly intervals. The times of flower bud formation and flowering were recorded. The plants were harvested after 11 weeks (week 12) and similar data as per section E.2.2.1 were taken as well as dry weights. Analysis of variance (split plot design) was performed on the data.

E.2.2.3 Repeated Defoliation

The same methods and procedures were followed as described for the one-time defoliation experiments (E.2.2.2), except that the plants were defoliated each week at the designated level for 9 weeks. Similar data were taken throughout the growing period and at the harvest (week 12) as for E.2.2.2. The light regimes were also the same (i.e., environmental levels for repeat one and supplemented lighting for repeat two). Analysis of variance was performed as per section E.2.2.3.

E.2.2.4 Field Defoliation

Plants in the field at two sites (site 3 and 4- Fig 1) were artificially defoliated (similar technique as per sections E.2.2.1-3) at 50% and 100% levels (6 of each per site) early in the season and controls were not defoliated. Measurement of height and leaf number were made periodically and dry weight was determined late in the season. Flower number was also determined.

E.2.2.5 Examination of Removal of Upper and Lower Leaves of Canada Thistle

Plants were grown in the greenhouse under similar conditions as described for repeat 2 of sections E.2.2.2 and E.2.2.3. Plants were defoliated one time at ages 4-, 8-, 12-, 16- leaf stages by either removing all of the leaf blade of leaves at the top or at the bottom of the plant (4 replicates of each) depending on the treatment (i.e. if the treatment was 4-leaf stage, bottom, all of the leaf blade without cutting the midrib of the lower 2 leaves would be removed). Heights were recorded throughout the growing period and at harvest (11 weeks after initiation of the experiment) data taken and data analysis were the same as for E.2.2.2. and E.2.2.3.

E 2.3 Microtechnique

Samples of defoliated leaves were fixed, dehydrated, infiltrated and embedded for plastic sectioning following the procedure outlined in section B.2.5.

E.3 Results

E.3.1 Cassida rubiginosa Phenology and Damage

In Quebec, the overwintering adults become active in early spring and oviposition occurred from mid April to early July (Fig. 58). As noted by Ward and Pienkowski (1978a) most oöthecae were deposited on the abaxial leaf surface commonly at the leaf tip (Fig. 59). Oöthecae were quite commonly associated with plants systemically infected with Puccinia punctiformis. Larval eclosion occurred from June to August and pupation from July until late August. Newly emerged adults were seen until mid September.

Adults and larvae skeletonized the leaves, generally leaving one epidermis intact. Adult and older larval feeding were indistinguishable (Figs. 60 and 61), but early larval instar feeding scars were smaller. Microscopy of damaged leaves shows the ragged edge of the feeding area (Fig. 62) which maintains an intact epidermis, but the rest of the tissue has been eaten. In the field the majority of the damage occurred to the lower and mid-stem leaves and rarely on the upper leaves. Plant damage was low, only occasionally were plants highly damaged. Those plants with high damage levels (50% or greater) were stunted, rarely flowered and often died before the season was completed, but the incidence of plants with this level of damage was very low (less than 1%). The time of major feeding occurred in June and July (larvae) and corresponded with the flowering stage of the plant (Fig. 63).

E.3.2 Insect Defoliation under Controlled Environment Conditions

In cage experiments with increased damage (based on ratings I to IV, Appendix F), at harvest, the leaf number, the dry weight of the parent plant,

Figure 58. Phenology of Cassida rubiginosa over the year in Quebec. The diagonal break in the adult bar indicates the time period where there is an overlap of the generations, the right half of the bar being the new generation. The period when the adults are visible above ground is indicated by the area between the arrows.

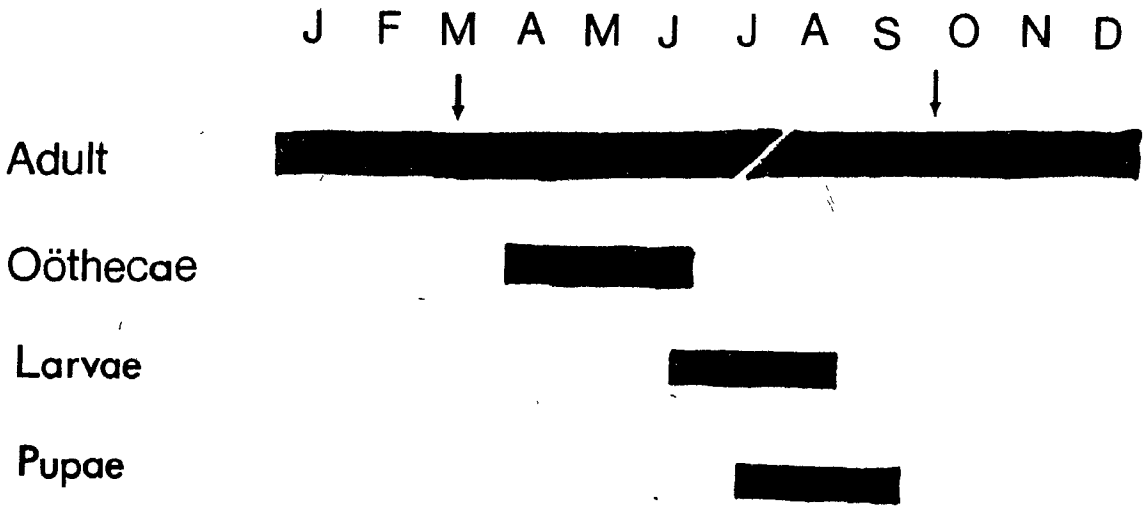


Figure 58

Figure 59. Two Cassida rubiginosa oöthecae (arrowheads) on the abaxial surface of a Canada thistle leaf (X 2)

Figure 60. Skeletonization damage by older larval instars of C. rubiginosa

Figure 61. Leaf damage caused by adult C. rubiginosa

Figure 62. Plastic section through the edge of a feeding scar (arrowhead indicates the beginning of the hole). Note that only the epidermis remains where feeding has occurred (X 120).

60
LARVAL
61
ADULT
62

Figure 63. Zone of maximum feeding (dotted region) in relation to initiation of flowering (arrowhead), plant height and the time of year.

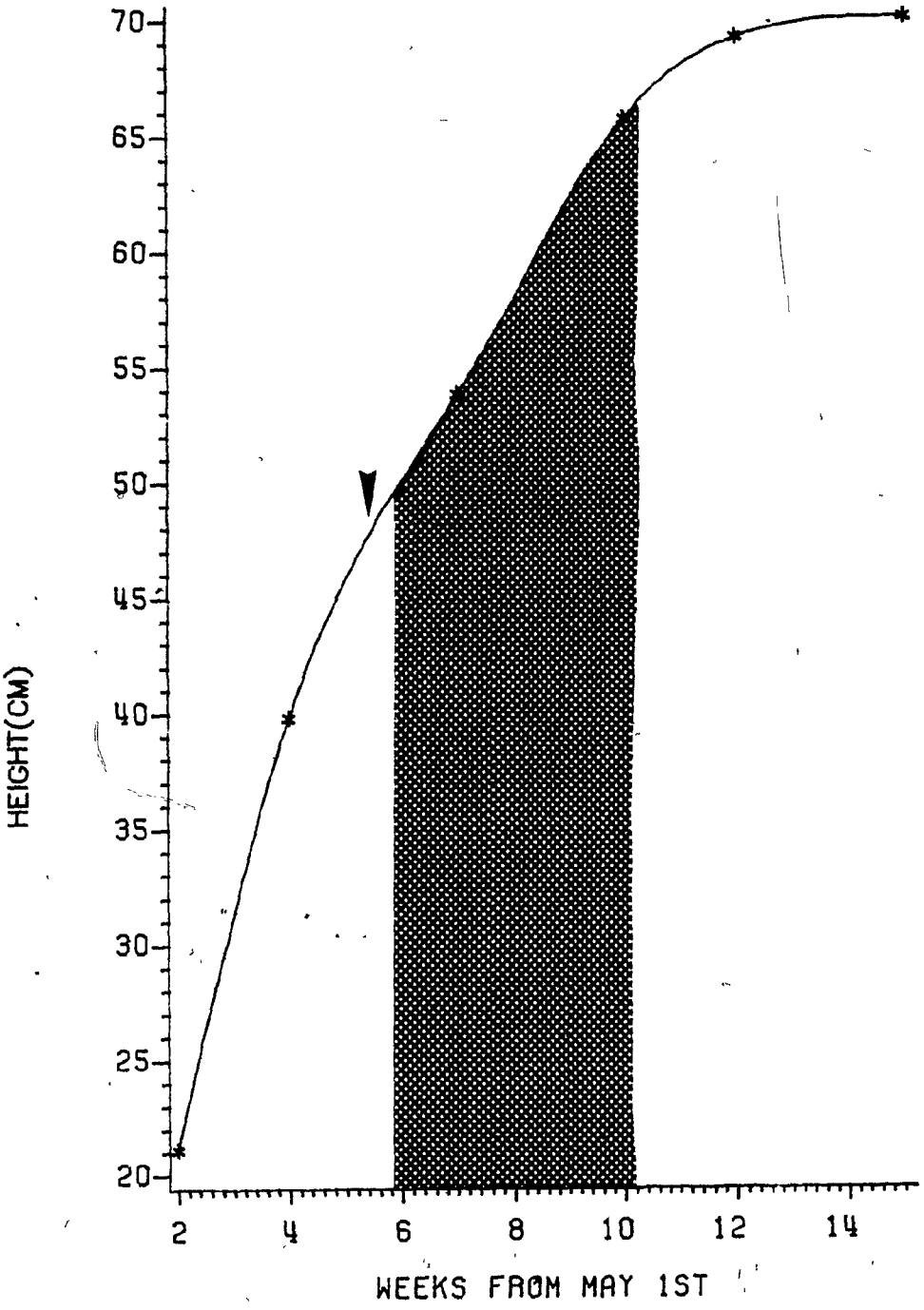

70
65
60
55
50
45
40
35
30
25
20
HEIGHT(CM)
2
4
6
8
10
12
14
WEEKS FROM MAY 1ST

FIGURE 63

the dry weight of the root, the number of root buds and fresh and dry weights of the total declined (Table 27). Leaf number for group IV (highest level of defoliation) was 88% of the control (Group I), the ramet number was 82%, dry weights of the plant, root , ramets and total were 69%, 62%, 44% and 77% respectively, the total fresh weight, 81% and the root bud number 65%, but there were no statistically significant reductions.

E.3.3. Simulation Experiments: Single Leaf Stage Examinations

Experiments (one to four) with 7- and 9-leaf stage plants indicated that with increasing defoliation levels, there was a gradual reduction of height over time. Reductions in height were greatest at the 75% and 100% defoliation levels (Figs. 64 to 67). Growth and yield parameters which were significantly lower at higher defoliation levels included height, ramet number, dry weight of the plant and total. There were also trends toward decreased dry weight for the plant, root, ramet and total and an increase of the shoot/root ratio with increasing defoliation levels (Tables 28 to 31).

E.3.4 Simulation Experiments: One-Time Defoliation

Results from pairs of similar experiments for this section (Experiment 5 and 6) and for the next section (E.3.5- Experiments 7 and 8) indicated similar trends and differences, but the data from the different experiments were not analyzed together because of differential rates of seasonal growth and differential lighting regimes.

Figures 68 to 75 and figures 76 to 79 illustrate that for all ages, the greater the defoliation pressure, the greater the depression of height over time. Upon analysis of variance, it was found that in some cases, there was an

Table 27. Effects of Insect Defoliation on Growth and Yield Parameters of Canada Thistle (Controlled Environment)

Parameter	------Damage Rating* ----------				
	I	II	III	IV	LSD (0.05)
Leaf number	46.4	44.4	46.3	40.7	9.1
Height(cm)	47.4	51.5	53.1	53.0	15.5
Ramet Number	13.7	10.3	14.1	11.3	7.3
Root Bud Number	40.3	35.9	31.9	26.3	22.0
Dry Weight (g) Plant	11.1	9.6	9.0	7.7	4.7
Root	12.2	8.7	9.4	7.6	4.6
Ramets	5.2	5.1	5.1	7.5	7.3
Total	28.5	23.5	23.5	22.8	7.6
Fresh Weight (g) Total	102.8	96.0	93.0	83.1	32.1

*- Ratings of % average leaf removals by Cassida rubiginosa larvae (Appendix F).

I 0 (Control) (n=8)

II 0.1-5% (n=13)

III 5.1-10% (n=8)

IV 10.1+% (n=3)

Figures 64 to 67. Effect of defoliation of height over time for one-time defoliation experiments (preliminary).

Figure 64. 7-leaf stage Experiment 1
Figure 65. 7-leaf stage Experiment 2
Figure 66. 9-leaf stage Experiment 3
Figure 67. 9-leaf stage Experiment 4

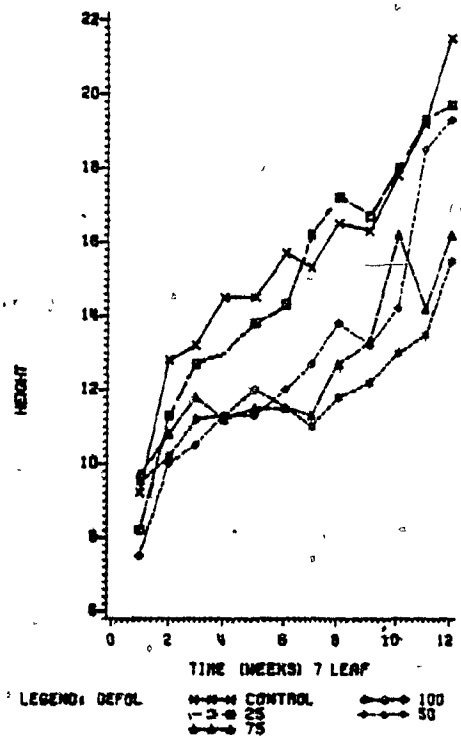


FIGURE 64

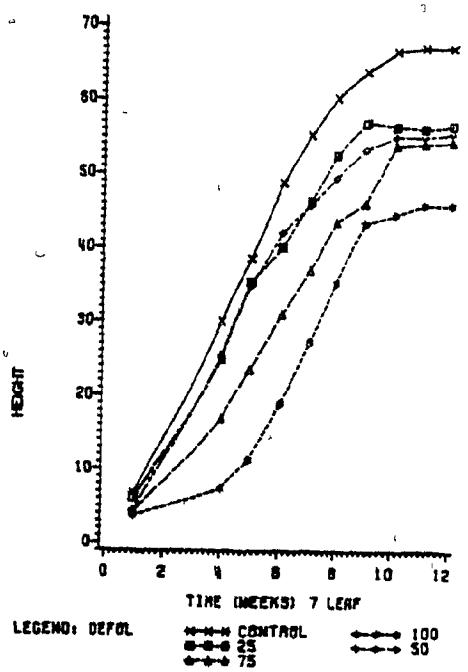


FIGURE 65

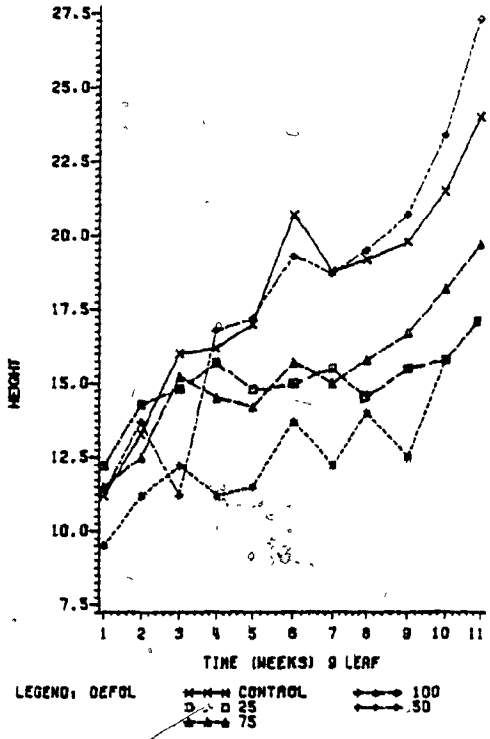


FIGURE 66

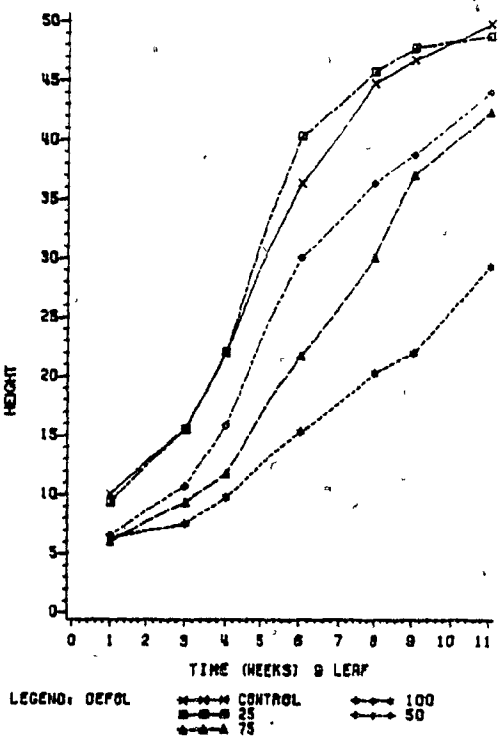


FIGURE 67

Table 28. Defoliation Simulation Experiment One: 7-Leaf Stage
Effect of Defoliation on Growth and Yield Parameters

Parameter	% Defoliation					LSD (0.05)
	Cont.	25	50	75	100	
Height(cm)	40.3	38.3	17.7	19.7	24.7	25.9
Leaf number	17.5	15.5	14.4	15.7	15.3	9.1
Ramet number	7.0	3.7	5.3	2.0	4.0	5.9
Sideshoot number	3.0	0.3	0	2.0	0	5.1
Flower number	0	0	0	0	0	-
Root bud number	9.7	8.3	5.3	4.3	4.3	10.0
Average ramet height (cm)	20.3	32.3	20.0	33.7	22.0	22.3
Dry weight (g)						
Plant	1.6	1.8	1.5	1.3	1.2	1.1
Root	2.1	2.1	2.6	1.8	1.4	1.7
Ramets	2.5	1.9	3.3	2.2	1.8	1.9
Total	6.1	5.7	7.3	4.5	4.4	2.7

Table 29. Defoliation Experiment 2. 7-leaf stage.
Effect of Defoliation on Growth and Yield Parameters

Parameter	% Defoliation					LSD (0.05)
	0	25	50	75	100	
Height (cm)	64.3	55.5	55.5	52.0	45.8	11.3
Leaf number	20.8	20.0	20.8	23.5	20.8	4.5
Ramet number	4.5	3.5	2.5	2.5	1.3	2.4
Ramet height Average (cm)	23.0	31.8	20.3	26.5	32.3	16.3
Flower number	3.5	1.5	2.3	1.8	0	3.6
Sideshoot number	10.3	9.8	2.3	6.5	3.5	8.8
Dry weight (g) Plant	7.0	6.3	5.6	5.9	4.3	2.4
Root	4.5	4.3	4.9	5.7	2.6	2.1
Ramets	3.0	4.4	2.6	3.0	1.6	2.0
Total	14.5	13.9	13.0	15.6	8.5	4.9
Shoot/Root	2.4	2.6	1.8	1.6	1.3	1.3

Table 30. Defoliation Simulation Experiment 3. The Effect of Defoliation on Growth and Yield Parameters (9-leaf Stage)

Parameters	% Defoliation					LSD (0.05)
	0	25	50	75	100	
Height(cm)	34.3	19.3	42.7	24.0	19.3	24.5
Leaf number	13.3	8.3	13.7	-	11.7	13.3
Ramet number	9.7	4.3	5.7	2.0	3.0	6.2
Average ramet height (cm)	20.3	32.3	20.0	33.7	22.0	23.3
Sideshoot number	2.0	1.0	0.3	0	0.3	3.1
Root bud number	9.7	8.3	5.3	4.3	7.5	3.1
Dry weight (g)						
Plant	1.5	1.1	1.9	1.9	1.3	0.4
Root	3.0	2.7	2.0	2.4	2.1	0.5
Ramets	3.7	3.9	2.8	3.5	3.0	0.9
Total	8.2	7.6	6.6	7.3	6.4	1.2

Table 31. Defoliation Simulation Experiment 4 (9-leaf Stage. One-time Defoliation: Yield and Growth Parameters.

Parameters	% Defoliation					LSD (0.05)
	0	25	50	75	100	
Height (cm)	49.7	48.7	44.0	42.3	29.3	21.9
Leaf number	17.0	15.0	20.0	17.0	14.0	3.4
Flower number	3.0	3.7	3.3	2.3	0	4.9
Ramet number	3.3	3.0	3.0	3.0	1.0	5.9
Average Ramet Height (cm)	12.5	21.7	10.7	7.5	14.0	4.1
Sideshoot number	4.7	3.0	2.7	1.7	2.7	8.2
Root bud number	1.0	2.0	3.8	2.0	1.7	2.5
Dry weight (g) Plant	3.5	3.2	3.6	3.0	2.4	0.7
Root	3.7	3.0	2.6	2.2	1.4	0.6
Ramet	1.2	2.1	1.1	0.6	0.5	0.6
Total	8.4	8.3	7.3	5.8	4.3	1.3

Figures 68 to 71. Effect of defoliation on height over time. Experiment 5. One-time defoliation

Figure 68. 5-leaf stage
Figure 69. 10-leaf stage
Figure 70. 15-leaf stage
Figure 71. 20-leaf stage

Note that in general the height is lower for higher levels of defoliation. Note that for the 15- and 20-leaf stages (Figs. 70 and 71), that defoliation seems to stimulate the height.

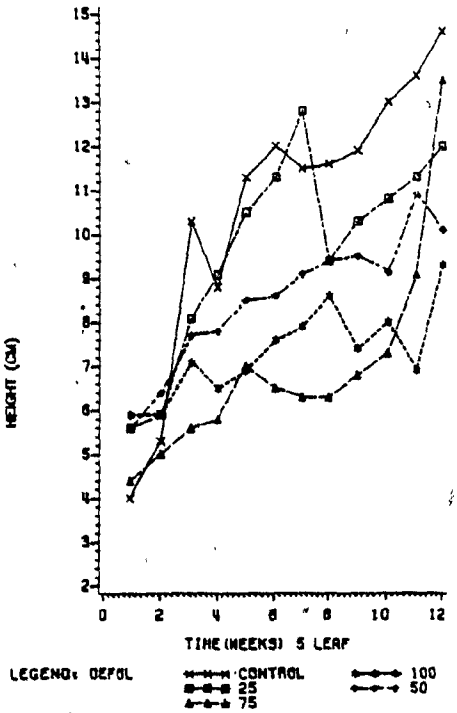


FIGURE 68

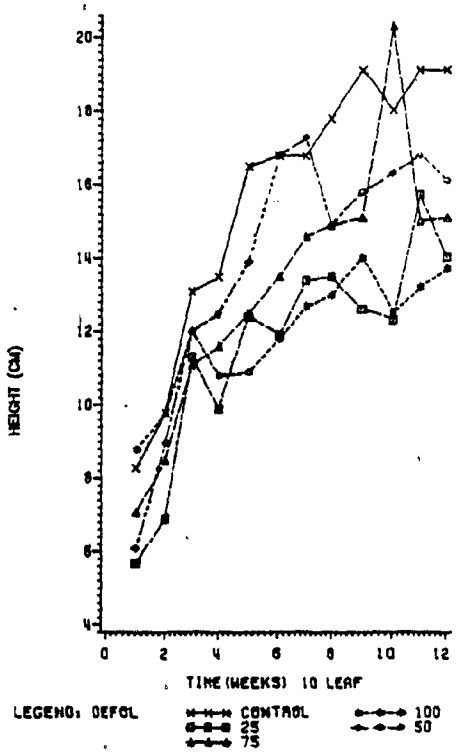


FIGURE 69

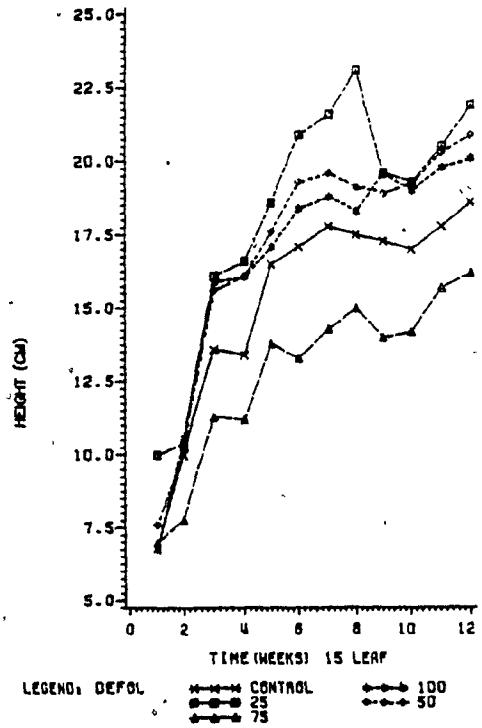


FIGURE 70

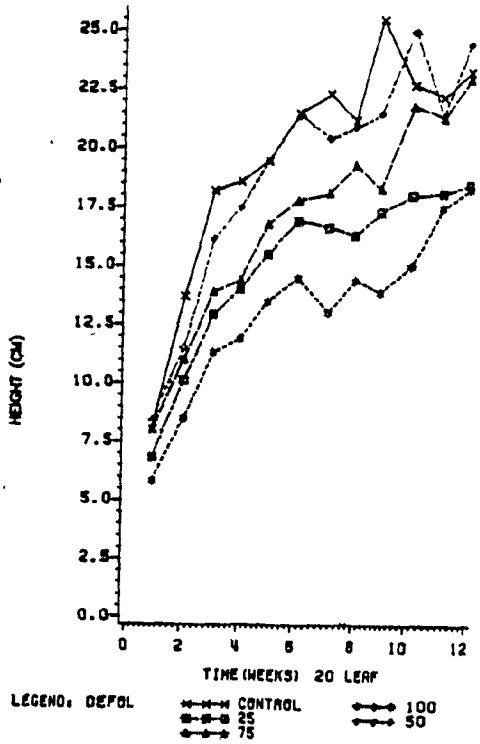


FIGURE 71

Figures 72 to 75. Effect of defoliation on height over time. Experiment 6. One time defoliation.

Figure 72. 5-leaf stage
Figure 73. 10-leaf stage
Figure 74. 15-leaf stage
Figure 75. 20-leaf stage

Note that there is little difference between all the levels of all the treatments. Even though this is the same experiment as experiment 5, the better lighting regime seems to have resulted in greater height increase and a better compensation for the defoliation.

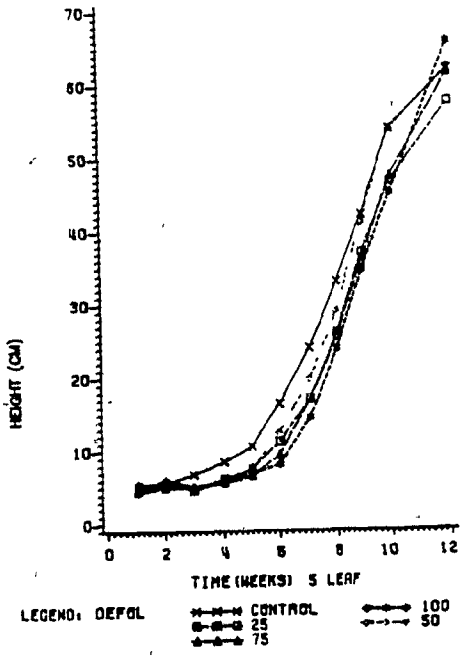


FIGURE 72

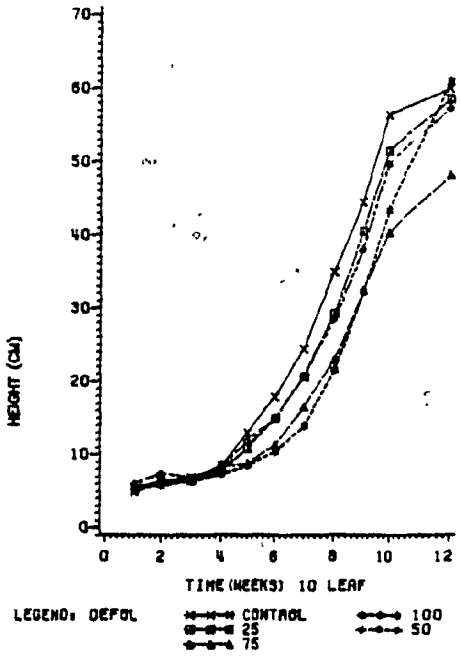


FIGURE 73

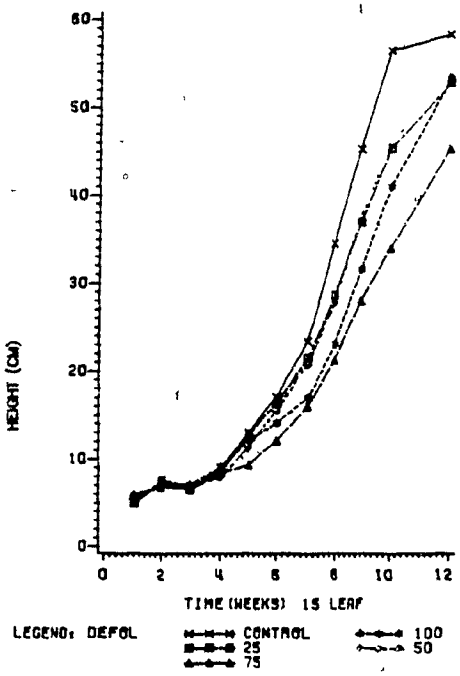


FIGURE 74

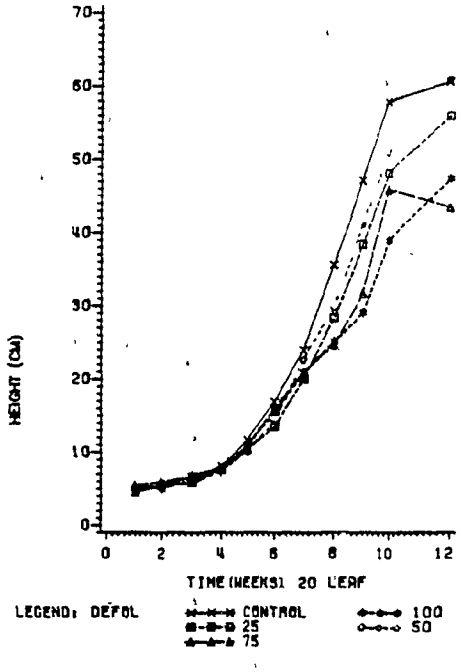
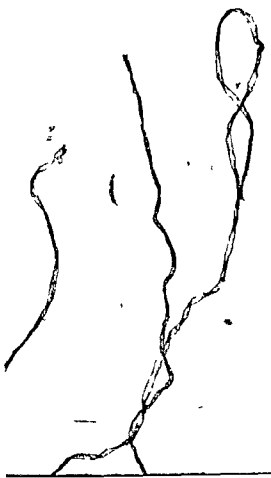


FIGURE 75



effect of the block, usually associated with ramet production which generally has a large variability and is probably not due to a gradient in the block. In experiment 6, there was some significant difference between the blocks for height and total dry weight. There was a significant interaction between age and defoliation for dry weight of the roots and in one case for the shoot/root ratio. This indicated that the age and defoliation have some effect on the expression of the simple effect of the other parameter. When differences in various parameters are considered for the four age groups averaged over all defoliation levels for experiments 5 and 6 (Tables 32 and 33), there were significant differences among the ages for the following parameters; height, dry weight of the plant and total dry weight for experiment 5 and also for dry weight of ramets , root and the shoot/root ratio. In experiment 5, at younger ages, there were lower heights, dry weight of the parent plant, the root and total and also the number of ramets, sideshoot number, root bud number (latter three trends only) and also higher shoot/root ratio. In experiment 6, on the other hand, the reverse seemed to have occurred for the height, leaf number, flower number, root bud number and dry weight of the plant and total (i.e. with increasing age, decreasing parameter value). The general conclusion from experiment 5 was that defoliation had a more detrimental effect at the younger stages (especially the 5-leaf stage) and the reverse or no effect in the case of experiment 6.

When defoliation levels were considered over all ages (Tables 34 and 35) there were significant differences for experiment 5 and 6 in height, shoot/root ratio, dry weight of the plant, root and total and also average ramet height; for experiment 5 alone the sideshoot number and for experiment 6 alone, flower production. In both cases, there was a decrease in height, ramet number, root

Table 32. Effect on Yield and Growth Parameters. One-Time Defoliation. Age Averaged over Defoliation. Experiment 5.

Parameter [#]	Age (Leaf Number) [$]			
	5	10	15	20
Height(cm) [**]	13.2c	16.1b	19.9a	21.0a
Ramet number [ns]	4.3b	5.4ab	4.8ab	6.4a
Root bud number [ns]	12.3b	15.2ab	21.2a	19.1ab
Sideshoot number [*]	1.1b	2.1ab	3.1ab	4.2a
Average ramet height (cm) [ns]	10.3a	8.3ab	7.3b	8.1ab
Dry weight (g)				
Plant [**]	1.5b	2.1a	2.5a	2.1a
Root [**]	1.9b	3.3a	3.1a	3.5a
Ramets [*]	1.7ab	2.0ab	1.5b	2.2a
Total [**]	5.1b	7.4a	7.2a	7.6a
Shoot/root [**]	1.8a	1.4bc	1.6ab	1.3c

[#] ** F-test significant at 0.01 level
* F-test significant at 0.05 level
ns F-test not significant
(F-test takes precedence)

[$] Means with the same letter in the same row are not significantly different at the 0.05 level (Duncan's multiple range test)

Table 33. Effect of Defoliation among Ages Averaged over Defoliation Levels. One-Time Defoliation. Experiment 6.

Parameter [$]	Age (Leaf Number) [#]			
	5	10	15	20
Height (cm) *	59.9a	56.8ab	52.8b	51.8b
Leaf number ns	29.7a	30.7a	28.9a	27.7a
Ramet number ns	9.4ab	11.5a	10.1ab	8.0b
Average ramet height(cm) **	21.6a	17.7b	22.7a	22.5a
Flower number ns	4.4a	3.5a	3.6a	4.1a
Sideshoot number ns	12.0b	15.5ab	15.8ab	16.4a
Root bud number ns	12.3ab	14.6a	13.0ab	9.9b
Dry weight (g)				
Plant **	7.0a	7.0a	5.8b	5.2b
Root ns	4.7a	4.8a	4.7a	4.3a
Ramet *	6.3ab	5.4b	6.7a	6.3ab
Total **	18.1a	17.2ab	17.2ab	16.0b
Shoot/root ns	2.9a	2.7a	2.7a	2.8a

Means with the same letter in the same row are not significantly different (α =0.05, Duncan's multiple range test)

$ F-test ** significant at the 0.01 level
* significant at the 0.05 level
ns not significantly different

Note: The Duncan's test sometimes finds differences where the F-test does not. F-test takes precedence.

Table 34. Effect of Defoliation on Defoliation Level Averaged over all Ages. One-Time Defoliation Experiment 5.

Parameter $	Defoliation Level (%) #				
	0	25	50	75	100
Height (cm) **	20.4a	16.9b	17.9ab	16.6b	14.8b
Leaf number ns	26.4a	26.3a	27.1a	27.9a	26.0a
Ramet number *	6.9a	5.2ab	5.5ab	4.3b	3.9b
Average ramet height (cm) *	5.8b	8.8a	9.0a	10.0a	9.3a
Root bud number *	22.8a	18.4ab	14.9ab	16.0ab	11.5b
Sideshoot number **	4.5a	3.0ab	2.7abc	1.9bc	0.4c
Dry Weight (g)					
Plant **	2.7a	2.3ab	2.0b	1.9b	1.3c
Root **	4.1a	3.6a	2.9b	2.3b	1.6c
Ramet ns	1.4b	2.1a	1.9ab	1.8ab	2.0ab
Total **	7.9a	7.9a	7.0ab	6.1b	4.9c
Shoot/root **	1.1d	1.3cd	1.5c	1.7b	2.3a

Means of the same letter in the same row are not significantly different (α =0.05, Duncan's multiple range test.

$ F-test ** Significant at 0.01 level
* Significant at 0.05 level
ns Not significant

Note: F-test takes precedence over the Duncan's Multiple range test.

Table 35. Effect Of Defoliation among Defoliation Levels Averaged over Age. One-Time Defoliation. Experiment 6.

Parameter	$	Defoliation Level (%)				
		0	25	50	75	100
Height (cm)	**	60.1a	51.7ab	58.4a	49.4c	56.8ab
Leaf number	ns	26.6a	30.0a	28.6a	30.5a	30.4a
Ramet number	ns	11.2a	10.0ab	8.6ab	11.0ab	8.0b
Average ramet height (cm)	*	18.6b	23.2a	21.6ab	19.1b	23.0a
Flower number	**	6.1a	4.9a	4.6ab	1.7b	1.9b
Sideshoot number	ns	17.6a	14.5ab	15.6ab	11.8b	15.2ab
Root bud number	ns	13.0a	13.0a	12.4a	12.1a	11.9a
Dry weight (g)						
Plant	**	7.4a	6.4ab	6.3ab	5.2b	6.0b
Root	**	5.3a	4.8ab	4.7ab	4.3c	4.1c
Ramet	ns	5.6a	6.3a	6.1a	6.4a	6.5a
Total	**	18.4a	17.4ab	17.1ab	16.1b	16.6b
Shoot/root	*	2.5b	2.7b	2.8ab	2.7b	3.2a

\# Means of the same letter in the same row are not significantly different (α =0.05, Duncan's multiple range test)

$ F-test ** significant at the 0.01 level
* significant at the 0.05 level
ns not significant

Note: the F-test takes precedence over the Duncan's multiple range test.

bud number, sideshoot number and dry weight of the plant, root and total with an increase in the shoot/root ratio with increasing levels of defoliation.

When combinations of leaf stage at defoliation and defoliation level (experimental unit) were considered, for experiment 5 (Table 36), the greatest detrimental effect on height, sideshoot number, dry weight of the plant, root, ramets and total and the shoot/root ratio occurred at the 5-leaf stage at high defoliation levels. Generally for all leaf stages, there was a decrease in the parameter value (increase for shoot/root ratio) with increasing defoliation (especially 75% and 100%) and the detrimental effect is greater for younger plants. For experiment 6, on the other hand, the same trends did not occur and little real differences were observed (Table 37).

E.3.5 Simulation Experiments: Repeated Defoliation Pressure

Figures 80 to 87 indicate that the rate of height increase was restrained at the higher defoliation levels, especially for the younger ages. Visual results also indicate this (Figs. 88 to 91).

Analysis of variance indicated that there were some significant effects of the block especially for experiment 8, which could have resulted from a slighty uneven lighting regime. When results for age were averaged over defoliation (Tables 38 and 39), there was a significant difference among the ages for height for experiment 7 and height, ramet number, flower and sideshoot number and also dry weight of the parent, root and the shoot/root ratio for experiment 8. In general, with decreased age of first leaf removal, there was lower height, dry weight of total, plant, ramets and root and also lower flower number, sideshoot number and ramet number.

Table 36. Effect on Yield and Growth Parameters. Age-Defoliation Combinations. One-Time Defoliation. Experiment 5.

Defoliation Level(%)	Age (Leaf Stage) 5	10	15	20
	Height(cm)			
0	21.0abcd	19.4abcdefg	18.8abcdef	23.3ab
25	12.3fgh	15.3cdefgh	21.4abc	18.8abcdef
50	10.3gh	16.3bcdefg	22.0abc	24.7a
75	13.5efgh	15.1cdefgh	16.2bcdefg	23.0ab
100	8.9h	13.8defgh	20.3abcde	16.1bcdefg
	Sideshoot number			
0	2.5bc	4.3abc	4.0abc	8.0a
25	3.0abc	2.0bc	4.3abc	2.8abc
50	0c	1.5bc	5.0abc	4.7abc
75	0c	2.0bc	0.3c	6.0bc
100	0c	0.3c	1.0c	0c
	Dry Weight (g)			
0	1.9bcd	3.0ab	3.3a	2.8abc
25	1.4cd	2.1abcd	3.3a	2.4abc
50	1.6bcd	2.1abcd	2.3abc	2.0abcd
75	1.6bcd	1.7bcd	1.9bcd	2.5abc
100	0.9d	1.7bcd	1.8bcd	0.7d

Table 36 cont'd

Defoliation Level (%)	Age (Leaf stage) 5	10	15	20
	Dry Weight Root (g)			
0	2.2fghij	5.1a	4.5abcd	4.8ab
25	2.7fghi	3.2efg	4.7abc	3.7bcde
50	2.0ghij	3.5cdef	3.0efgh	3.1efgh
75	1.9hij	2.6efghi	1.5ij	3.4def
100	1.0j	2.1ghij	1.4ij	2.2fghij
	Dry Weight Ramet (g)			
0	1.3bc	1.2bc	1.0c	2.3abc
25	2.3abc	2.4ab	2.0abc	1.8abc
50	1.5bc	1.8abc	2.0abc	2.4ab
75	2.0abc	2.1abc	1.5bc	1.8abc
100	1.4bc	2.6ab	1.2bc	3.1a
	Dry Weight Total (g)			
0	5.4fghi	9.2ab	8.7abc	8.7abc
25	6.4cdefgh	7.7abcdef	9.8a	7.8abcde
50	5.1ghi	8.1abcd	7.3bcdefg	7.5abcdef
75	5.5efghi	6.3cdefgh	4.9ghi	7.7abcdef
100	3.2i	6.0defgh	4.7hi	6.1defgh

Table 36 cont'd

Defoliation Level(%)	Age (Leaf stage) 5	10	15	20
	Shoot/root *			
0	1.4bcde	0.9e	1.0e	1.1de
25	1.4bcde	1.5bcde	1.1de	1.0e
50	1.6bcde	1.4bcde	1.5bcde	1.4bcde
75	2.1ab	1.5bcde	2.0abc	1.3cde
100	2.6a	1.9abc	2.6a	1.8bcd

* Means with the same letter by parameter are not significantly different. (α =0.05, Duncan's multiple range test)

Table 37. Effect on Yield and Growth Parameters by Age-Defoliation Combinations for One-Time Defoliation Experiment 6.

Defoliation Level(%)	Age (Leaf Number) 5	10	15	20
	Height (cm) *			
0	61.8ab	59.8abc	58.3abc	60.5ab
25	48.8bcd	58.3abc	52.8abcd	47.0bcd
50	62.0ab	57.0abcd	54.0abcd	60.8ab
75	61.3ab	48.0bcd	45.3cd	43.3d
100	65.5a	60.8ab	53.5abcd	47.3bcd
	Ramet Number *			
0	11.0abc	13.3ab	12.0abc	8.5abc
25	14.8a	8.5abc	9.3abc	7.5bc
50	5.3c	12.8ab	9.3abc	7.0bc
75	8.8abc	15.3a	12.3ab	7.8bc
100	7.3bc	7.8bc	7.8bc	9.3abc
	Dry Weight Parent (g) *			
0	7.7a	7.9a	7.4a	6.9ab
25	6.6ab	6.9ab	5.9abc	6.1abc
50	7.1ab	6.6ab	5.5abcd	5.9abc
75	6.6ab	5.6abcd	4.6bcd	3.9cd
100	7.2ab	8.0a	5.7abcd	3.2d

Table 37 cont'd

Defoliation Level (%)	Age (Leaf stage) 5	10	15	20
	Dry Weight Root (g) *			
0	4.9abcde	5.4abc	6.0a	4.7abcde
25	3.9de	5.2abcd	5.4abc	4.7abcde
50	5.9ab	5.2abcd	4.0de	3.8e
75	4.8cde	4.4cde	4.2cde	3.9de
100	4.1cde	3.9de	4.0de	4.5cde
	Dry Weight Total (g) *			
0	18.6abc	18.1abc	19.5a	17.4abcd
25	16.3abcd	18.2abc	18.4abc	16.6abcd
50	19.3ab	17.7abc	15.5cd	16.0cd
75	17.7abc	16.4abcd	15.8cd	14.4d
100	18.5abc	15.6cd	16.7abcd	15.7cd
	Shoot/Root *			
0	2.6bcd	2.4bcd	2.1d	2.9abcd
25	3.2abc	2.5bcd	2.5bcd	2.6bcd
50	2.4cd	2.5bcd	2.9abcd	3.3abc
75	2.7abcd	2.7abcd	3.0abcd	2.5bcd
100	3.6a	3.2abc	3.3ab	2.5bcd

* Means with the same letter by parameter are not significantly different ($\alpha = 0.05$, Duncan's multiple range test)

Figures 80 to 83. Effect of repeated defoliation on height (Experiment 7), environmental light regime.

Figure 80. 5-leaf stage

Figure 81. 10-leaf stage

Note that the increasing defoliation for the 5- and 10-leaf stages resulted in greatly decreased height (especially for 50 to 100%). The 100% lines stop at 8 weeks because the plants had all died by the next sampling date.

Figure 82. 15-leaf stage. 25% defoliation results in a stimulation of height over that of the control for this leaf stage. 100% defoliation has the greatest detrimental effect.

Figure 83. 20-leaf stage. No differences occurred between all defoliation treatments.

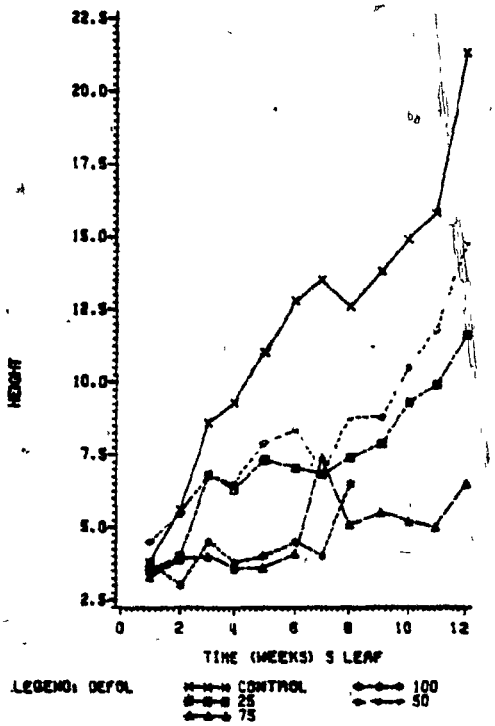


FIGURE 80

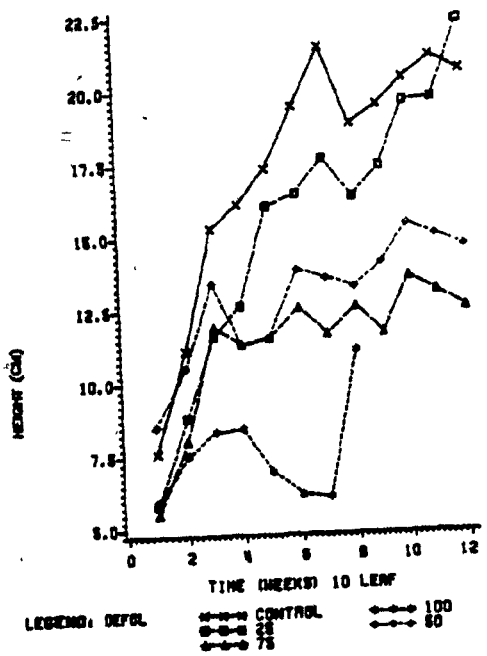


FIGURE 81

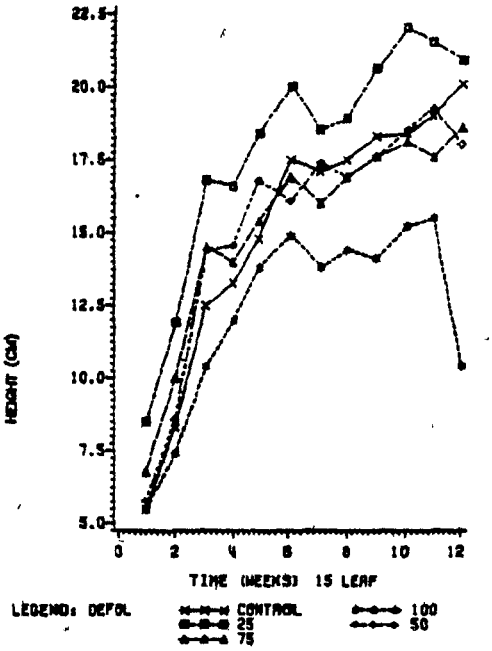


FIGURE 82

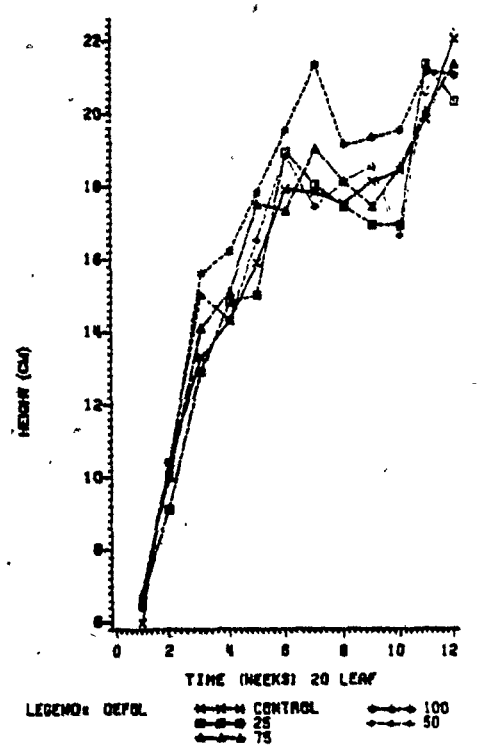


FIGURE 83

Figures 84 to 87. Effect of repeated defoliation of height (Experiment 8) with an enhanced lighting regime. The better lighting resulted in greater heights when compared to experiment 7 and the differences between the treatments were easier to distinguish. As with experiment 7, increasing defoliation resulted in lower heights.

Figure 84. 5-leaf stage

Figure 85. 10-leaf stage. Note that the 75% and 100% defoliation levels result in much lower heights.

Figure 86. 15-leaf stage

Figure 87. 20-leaf stage.

For the 15- and 20 leaf stages, although the 75% and 100% defoliation levels are still depressed, the differences are not so noticable as for the 5- and 10 leaf stages.

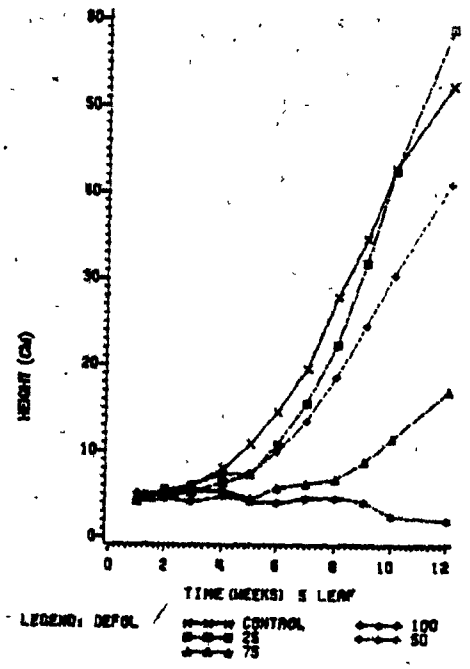


FIGURE 84

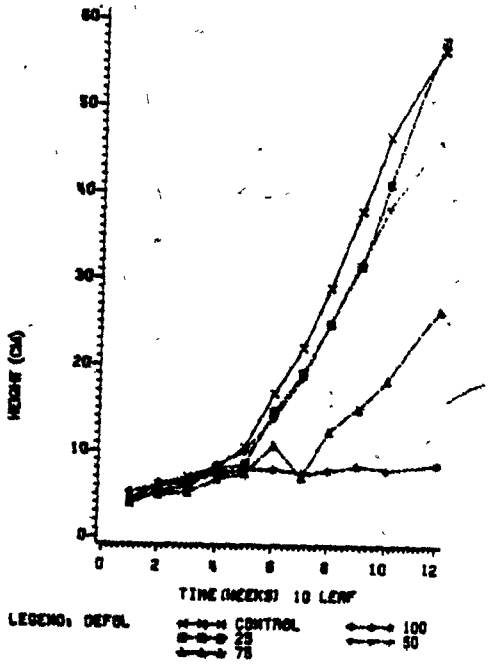


FIGURE 85

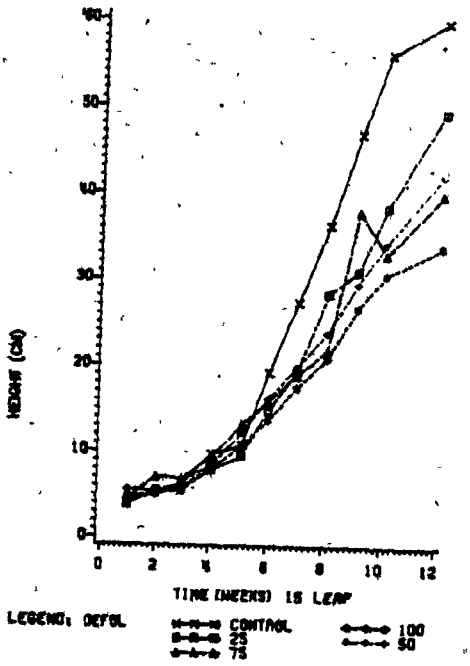


FIGURE 86

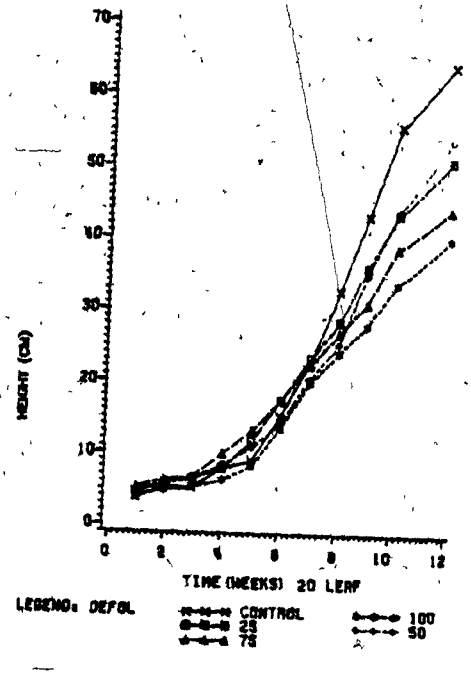


FIGURE 87

Photographic record of the effects of defoliation on height taken on the harvest day. In each case the pots are arranged from left to right in the following sequence: control, 25%, 50%, 75% and 100% defoliation treatments.

Figures 76-79. One time defolation. Note there is little difference in most causes for all defoliation levels.

Figure 76. 5-leaf stage Figure 77. 10-leaf stage Figure 78. 15-leaf stage Figure 79. 20-leaf stage

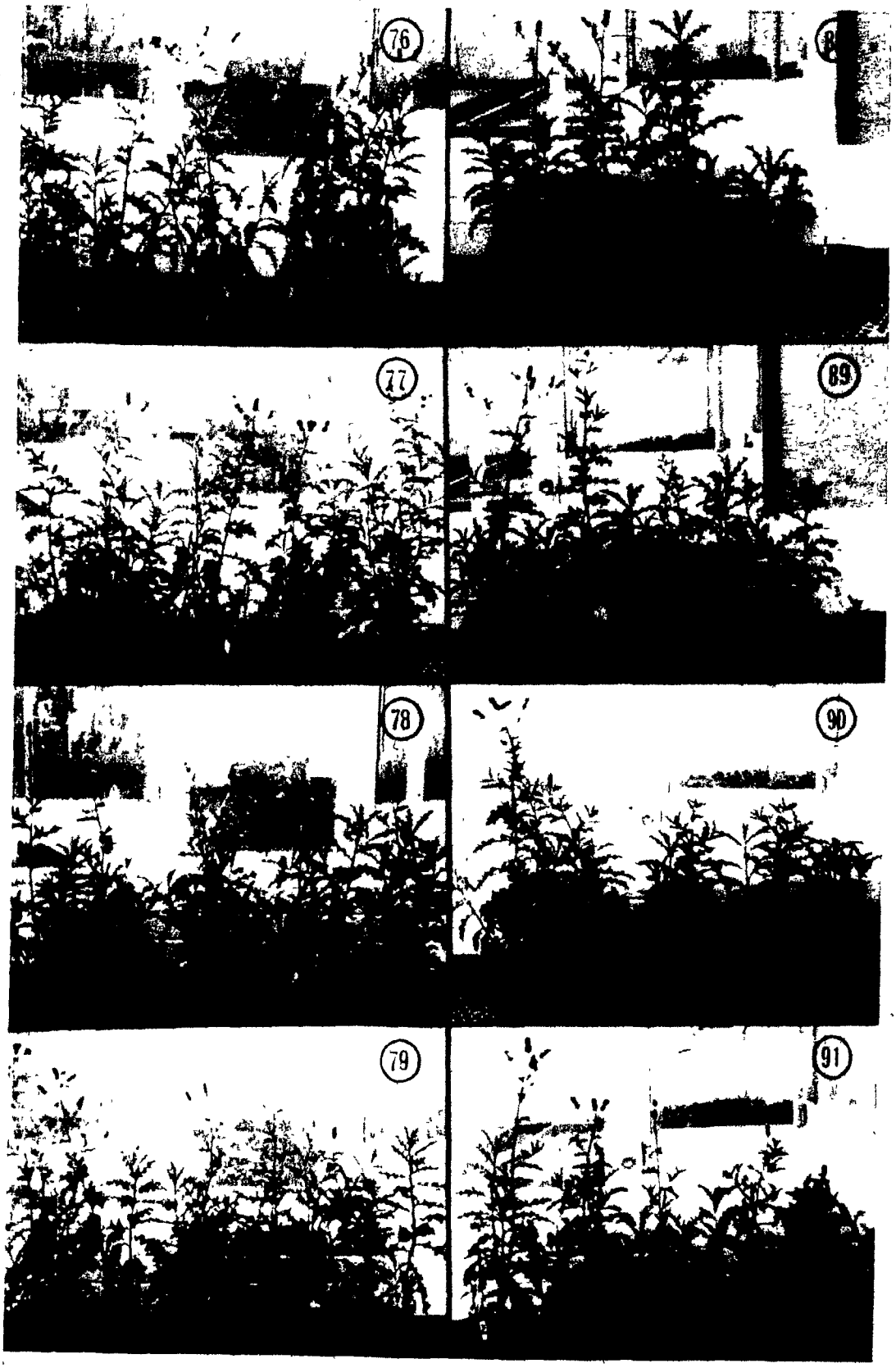
Figures 88 to 91. Repeated defoliation

Figure 88. 5-leaf stage. Note the large difference in height (100% plant is dead) with increasing defoliation. Note also the effects on flowering, only the control and 25% defoliation treatment have flowered

Figure 89. 10-leaf stage. Note again the large differential in height.

Figure 90. 15-leaf stage. The height difference is not so great as in the younger stages of defoliation.

Figure 91. 20-leafstage. Although the decrease in height with increasing defoliation still occurred, the differences were not large.


76
88
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89
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90
79
91

Table 38. Effect of Age Averaged over all Defoliation Levels on Yield and Growth Parameters. Repeated Defoliation Experiment 7.

Parameter [$]	Age (Leaf Stage) [#] 5	10	15	20
Height(cm) **	14.4c	16.4bc	18.6ab	20.0a
Ramet number ns	4.0b	6.2a	4.2b	4.6ab
Root bud number ns	11.9a	16.6a	15.6a	11.9a
Sideshoot number ns	1.1a	1.8a	2.2a	2.4a
Average ramet height (cm) ns	8.8a	8.8a	9.5a	10.6a
Dry Weight(g)				
Plant ns	1.4b	1.9a	1.6ab	1.5ab
Root ns	1.3b	2.3a	2.2a	2.2a
Ramet ns	1.2a	1.6a	2.0a	2.0a
Total ns	3.9b	5.9a	5.6a	5.8a
Shoot/root	2.1a	2.0a	1.9a	1.8a

#- Means with the same letter in the same row are not significantly different (α= Duncan's multiple range test)

$ F-test ** significant at 0.01 level
ns not significant

Note: F-test takes precedence over multiple range test.

Table 39. Effect on Yield and Growth Parameters by Age Averaged over all Defoliation Levels. Repeated Defoliation Experiment 8.

Parameter [$]	Age (Leaf Stage) [#] 5	10	15	20
Height(cm) **	39.9a	40.6b	45.2ab	50.8a
Leaf number ns	31.3a	31.2a	30.4a	29.9a
Ramet number **	6.4c	7.4bc	9.5ab	10.4a
Average ramet height(cm) ns	20.3a	18.8a	20.1a	20.1a
Flower number **	0.8b	1.1b	1.3b	2.3a
Sideshoot no. **	7.0b	9.4b	16.8a	18.4a
Root bud no. ns	8.4a	9.4a	11.6a	12.4a
Dry weight (g)				
Plant **	4.4a	4.4a	4.8a	5.1a
Root **	3.9b	4.5ab	4.8a	4.0b
Ramet **	5.1a	4.2b	6.4a	5.6a
Total **	13.3b	13.3b	16.1a	14.7a
Shoot/root ns	2.7a	1.8b	2.4a	2.7a

Means with the same letter in each row are not significantly different (α = Duncans' multiple range test)

$ F-test ** Significant at the 0.01 level
ns Not significant
F- test takes precedence over Duncan's multiple range test.

When results for defoliation were averaged over all ages, there were significant differences among the different defoliation levels for height, ramet, sideshoot and root bud numbers, dry weights of the plant, root, ramets and total for both experiments and also flower number and leaf number for experiment 8 (Tables 40 and 41). In general, with increasing defoliation, most yield and growth parameters decreased with the exception of the shoot/root ratio which increased slightly.

For defoliation-age combinations, there was a general decrease of leaf number, height, ramet number, dry weight of the plant, root and total, sideshoot number and an increase in the shoot/root ratio with lower leaf ages and with higher defoliation levels. Therefore, the combinations with the greatest detrimental effect on Canada thistle were those of 75% and 100% defoliation levels at the 5- and 10- leaf stages (Tables 42 and 43).

Continuous defoliation also affects plant survival (Table 44), especially at the higher defoliation levels. The 5- and 10- leaf plants were most adversely affected. The detrimental effect of continuous defoliation is greater than that of a one-time defoliation (Table 45). Sometimes, low levels of defoliation actually stimulated the plant (Table 45).

E.3.6 Comparison of Effects of the Removal of Bottom versus Top Leaves of Canada Thistle Plants

In general, it was more detrimental for the plant to lose the upper leaves than the lower leaves. Parameters differentially affected included; height, ramet number, flower number, sideshoot number and the dry weights of parent, root, ramets and total and shoot/root ratio (Tables 46 and 47).

Table 40. Effect on Yield and Growth Parameters by Defoliation Levels Averaged over all Ages. Repeated Defoliation Experiment 7.

Parameters [$]	Defoliation Level(%) [#]				
	0	25	50	75	100
Height(cm) **	20.8a	18.0ab	16.2b	16.0b	17.5ab
Ramet number **	7.1a	4.5bc	5.5ab	3.1c	2.5c
Average ramet height (cm) ns	7.2a	10.3a	9.1a	11.8a	9.1a
Root bud no. **	21.2a	16.8ab	12.1bc	8.5c	10.3bc
Sideshoot no. **	3.4a	1.7a	1.3a	1.3a	2.6a
Dry weight (g)					
Plant **	2.7a	2.0b	1.5b	1.0c	0.5c
Root **	3.3a	2.5b	1.7bc	1.4c	1.4c
Ramet ns	1.9a	1.8a	1.5a	1.6a	2.2a
Total **	7.9a	6.3ab	4.8bc	4.0c	3.8c
Shoot/root **	1.5b	1.7b	2.0ab	2.2ab	2.6a

Means with the same letter in the same row are not significantly different

$ F-test ** Significant at the 0.01 level
ns Not significant

Table 41. Effect on Yield and Growth Parameters Among Defoliation Levels Averaged over all Ages. Repeated Defoliation Experiment 8.

Parameters [$]	Defoliation Level (%) [#]				
	0	25	50	75	100
Height (cm) **	58.3a	54.2a	45.8b	31.9c	27.1c
Leaf number **	28.6b	31.7a	33.3a	31.9a	26.9b
Ramet number **	11.7a	9.4a	9.9a	6.2b	4.8b
Average ramet height(CM) ns	18.0a	18.4a	19.6a	22.9a	20.7a
Flower number **	3.6a	1.2b	1.2b	0.4b	0.5b
Sideshoot no. **	16.8a	16.0a	12.3b	9.4b	10.0b
Root bud no. **	11.7ab	14.9a	9.6b	10.8ab	4.9c
Dry Weight (g)					
Plant **	7.3a	5.8b	4.8c	3.0d	2.0e
Root **	6.0a	5.0b	4.6b	3.2c	2.1d
Ramet **	5.4a	5.5a	6.0a	5.4a	4.0a
Total **	18.9a	16.3b	15.8b	11.7c	7.8d
Shoot/root ns	2.3a	2.3a	2.3a	2.7a	2.3a

Means with the same letter in the same row are not significantly different

$ F-test ** significant at the 0.01 level
ns not significant

Table 42. Effect of Age-Defoliation Combinations on Yield and Growth Parameters under Repeated Defoliation Pressure. Experiment 7.

Defoliation Level(%)	Age (Leaf Stage) 5	10	15	20
	Height(cm) *			
0	21.3a	20.4ab	20.1abc	22.0a
25	11.7cd	18.8abc	20.9ab	20.3ab
50	14.9abc	14.3abcd	18.0abc	18.3abc
75	6.5d	12.5bcd	18.6abc	19.4abc
100	_**	_ **	13.8abcd	21.2a
	Ramet Number *			
0	5.0abcd	7.5abc	6.3abcd	9.0a
25	3.3bcde	5.8abcd	4.3bcde	4.0bcde
50	5.5abcd	8.0ab	5.3abcd	3.0cde
75	0.0e	3.5bcde	1.8de	4.8abcd
100	2.0de	_**	3.0cde	2.3de
	Root bud number *			
0	12.5bcde	30.3a	18.3abcde	19.0abcd
25	14.7abcde	15.8abcde	20.5abc	14.5abcde
50	14.5abcde	13.0abcde	25.0ab	2.5de
75	1.0e	7.3cde	7.0cde	13.0abcde
100	3.0cde	_ **	8.5bcde	14.0abcde

Table 42 cont'd

Defoliation Level (%)	Age (Leaf stage) 5	10	15	20
			*	
	---------Dry weight Plant (g) ----------			
0	2.5ab	2.8ab	2.3abc	3.0a
25	1.1def	2.3abcd	2.5ab	1.9abcde
50	1.5bcdef	1.8bcde	1.6bcde	1.3cdef
75	0.6ef	1.0ef	1.0ef	1.1ef
100	-**	-**	0.3f	0.7ef
			*	
	----------Dry weight Root(g) ----------			
0	2.4abcdef	4.2a	2.8abcde	3.2abc
25	1.2defg	2.5abcdef	3.3ab	2.9abcd
50	1.4cdefg	1.6bcdefg	2.4abcdef	1.7bcdefg
75	0.5g	0.9g	1.3defg	2.3abcdefg
100	-**	-**	1.0efg	1.5bcdefg
			*	
	----------Dry weight Total ----------			
0	6.2abcde	8.8a	7.1abcd	8.2ab
25	3.7def	6.7abcde	7.9abc	6.4abcde
50	4.2cdef	4.6bcdef	6.3abcde	4.3cdef
75	1.1f	3.5def	3.3def	6.2abcde
100	-**	-**	3.1ef	4.5bcdef

Table 42 cont'd

Defoliation Level (%)	Age (Leaf stage) 5	10	15	20
	Shoot/root *			
0	1.6b	1.1b	1.6b	1.8b
25	2.1ab	1.7b	1.5b	1.7b
50	2.4ab	2.0ab	1.5b	1.9ab
75	1.2b	3.3a	1.7b	1.7b
100	_**	_**	3.3a	1.8ab

* Means with the same letter by parameter are not significantly different (α =0.05 Duncan's multiple range test)

** - All plants with this treatment died.

Table 43. Effect of Age-Defoliation Combinations on Yield and Growth Parameters under Repeated Defoliation Pressure. Experiment 8.

Defoliation Level(%)	Age(Leaf Stage)			
	5	10	15	20
	Height(cm)			
0	52.3abcdef	56.5abcde	60.0ab	64.3a
25	58.8abc	57.5acde	49.5abcdef	51.0abcdef
50	41.0efgh	46.0bcdefg	42.3defg	54.0abcdef
75	17.0ij	26.5hi	40.0fgh	44.3cdefg
100	2.0k	8.7jk	34.0gh	40.3fgh
	Leaf Number			
0	28.0bcd	29.3bcd	29.8bcd	27.5cd
25	33.3abc	31.3bcd	30.0bcd	32.3abcd
50	38.3a	33.5abc	32.3abcd	29.3bcd
75	29.3bcd	35.0ab	33.3abcd	31.0bcd
100	18.0e	25.7d	27.8cd	29.3bcd
	Ramet Number			
0	14.3a	11.0abcd	11.0abcd	10.5abcde
25	9.0abcdef	7.8bcdef	12.0abc	8.8abcdef
50	5.5defg	13.8ab	10.5abcde	10.0abcde
75	3.3fg	4.3efg	6.8cdef	10.5abcde
100	0.0g	0.0g	7.0cdef	12.0abc

Table 43 cont'd

Defoliation Level(%)	Age (Leaf stage) 5	10	15	20
		Flower Number *		
0	2.0cde	2.8bc	4.5ab	5.0a
25	2.5bcd	0.3de	0.5cde	1.5cde
50	1.0cde	1.0cde	0.3de	2.5bcd
75	0.0e	0.0e	0.5cde	1.3cde
100	0.0e	0.0e	0.8cde	1.3cde
		Sideshoot Number *		
0	12.3a	15.8a	19.5a	19.5a
25	17.0a	16.3a	14.3a	16.5a
50	5.5b	12.5a	13.8a	17.5a
75	0.0b	0.0b	18.3a	19.3a
100	0.0b	0.0b	18.3a	19.3a
		Root Bud Number *		
0	1.5ab	10.5abc	8.6bc	12.8ab
25	20.8a	11.0ab	13.0ab	15.0ab
50	10.8ab	11.0ab	7.5bc	9.0bc
75	11.5ab	7.3bc	9.3bc	15.3ab
100	0.0c	0.0c	8.8bc	9.8bc

Table 43 cont'd

Defoliation Level (%)	Age (Leaf stage) 5	10	15	20
	Dry Weight Plant (g)			
0	5.9bcd	7.1abc	8.1ab	8.3a
25	6.9abc	6.0abcd	5.0defg	5.3cdef
50	4.7cdefg	5.0cdefg	4.2defg	5.4cde
75	1.4hi	2.6gh	4.2defg	3.9defg
100	0.1i	0.1i	2.9fgh	3.0efgh
	Dry Weight Root (g)			
0	5.7abc	6.8a	6.8a	4.7bcde
25	4.8bcde	5.8ab	5.1bcd	4.4bcdef
50	4.0def	5.2bcd	5.0bcd	4.2cdef
75	2.2g	3.3efg	3.8def	3.8def
100	0.1h	0.1h	3.2efg	3.0fg
	Dry Weight Ramets (g)			
0	5.8ab	4.5ab	6.2ab	5.2ab
25	5.0ab	5.5ab	5.9ab	5.7ab
50	6.1ab	5.7ab	7.0a	5.4ab
75	3.7b	4.5ab	7.2a	6.3ab
100	0.0c	0.0c	5.5ab	5.4ab

Table 43 cont'd.

Defoliation Level (%)	Age (Leaf stage) 5	10	15	20
	Dry Weight Total (g) *			
0	17.8abc	18.4ab	21.1a	18.2ab
25	16.7bc	17.3bc	16.0bc	15.1bcd
50	14.8bcde	17.1bc	16.4bc	14.9bcde
75	7.2g	10.3fg	15.2bcd	13.9cde
100	0.2h	0.3h	11.7def	11.3ef
	Shoot/root Ratio *			
0	2.4abcd	1.8cde	2.1bcd	2.9ab
25	2.7abcd	2.0bcd	2.1bcd	2.5abcd
50	2.7abcd	1.7de	2.3abcd	2.6abcd
75	3.1a	2.1bcd	3.1a	2.5abcd
100	-	1.2e	2.7abcd	2.8ab

* Means with the same letter by parameter are not significantly different (α =0.05, Duncan's multiple range test)

Table 44. Effect of Repeated Defoliation on Percent Survival

Leaf Stage (Age)	% Defoliation	Time (Weeks)			
		1	4	8	12
		% Survival			
5	0	100	100	100	100
	25	100	100	100	100
	50	100	100	100	100
	75	100	100	100	50
	100	100	75/100	25/100	0/25
10	0	100	100	100	100
	25	100	100	100	100
	50	100	100	100	100
	75	100	100	100	100
	100	100	100	25/100	0/75
15	0	100	100	100	100
	25	100	100	100	100
	50	100	100	100	100
	75	100	100	100	100
	100	100	100	100	50/100
20	0-100	100	100	100	100

* Experiment 7/Experiment 8 or if only one entry then both the same

Table 45. Comparison of One-Time (OT) and Repeated (DC) Defoliation Pressure for Two Parameters: Height and Root Dry Weight

Defoliation Level (%)	Age and Defoliation Pressure							
	5		10		15		20	
	DT	DC	DT	DC	DT	DC	DT	DC
	% of Control							
	Height (cm)							
0	100	100	100	100	100	100	100	100
25	59	55	79	92	114	104	81	93
50	49	70	84	70	117	90	106	83
75	64	3	78	61	86	90	99	88
100	42	0	71	0	108	69	69	96
	Root Dry Weight							
0	100	100	100	100	100	100	100	100
25	123	50	63	60	104	118	77	91
50	91	58	69	38	67	86	65	53
75	86	21	51	21	33	46	71	72
100	45	0	41	0	31	36	46	47

Table 46. Differential Effects of Lower and Upper Leaf Removal on Yield and Growth Parameters. Repeat One.

Parameter [&]	Age (Leaf Number) [#] 4		8		12		16	
	T	B	T	B	T	B	T	B
Height(cm)	45.8	64.8	37.3	62.0	34.3	42.0	37.0	43.0
Ramet number	4.0	0.8*	5.5	4.0	6.8	5.5	7.5	7.0
Average ramet height (cm)	25.1	12.0	24.4	15.1	22.2	31.5	21.5	27.7
Flower number	0	7.5	3.5	12.8	1.3	1.8	2.0	3.3
Sideshoot number	0.8	7.8	4.0	17.5	6.8	5.3	5.8	13.0
Root bud number	4.0	2.8	4.8	7.0	8.5	6.5	9.3	5.5
Dry weight (g) Plant	3.7	6.5	4.2	9.0	3.3	4.3	3.7	4.2
Root	1.4	1.7**	2.2	2.4	2.8	2.5	3.0	2.8
Ramet	1.5	1.3	4.1	1.2	5.8	5.5	5.1	5.5
Total	7.6	9.2	10.3	12.3	11.5	12.3	12.3	12.4
Shoot/root	2.3	5.6*	4.8	4.8	3.6	4.2	3.0	3.9

&- T-tests were performed for each parameter at each age between top and bottom defoliation treatments. The t-test was not significant unless indicated by **-0.01 level or *-0.05 level after the second number of the pair.

#- T- Top defoliation.

B- Bottom defoliation.

Table 47. Differential Effect of Lower and Upper Leaf Removal on Yield and Growth Parameters. Repeat 2.

Parameter	Age (Leaf Number) 4		8		12		16	
	T	B	T	B	T	B	T	B
Height (cm)	54.5	41.5	55.8	43.5	48.5	47.3	36.8	37.0
Ramet number	3.3	2.8	3.8	5.8	3.8	6.0	6.0	6.0
Average ramet height (cm)	11.8	16.2	7.2	19.9	14.1	19.5	24.6	19.8
Flower number	4.0	2.0	3.0	3.3	3.3	2.0	3.0	2.3
Sideshoot number	6.0	2.0	5.0	6.5	11.3	8.5	7.8	4.5
Root bud number	6.0	10.5	4.3	6.0	6.0	5.0	6.5	7.0
Dry weight (g)								
Plant	6.3	4.2	6.2	5.3	5.7	4.7	4.4	3.8
Root	2.0	2.5	2.2	2.7	2.5	3.3	3.9	3.8
Ramet	1.1	1.3	1.1	2.3	2.6	3.8	5.2	3.6
Total	9.4	7.8	9.2	10.3	11.1	11.8	13.4	10.0*
Shoot/root	4.6	2.6	4.3	3.1	3.7	2.6	2.9	3.3

$ T-tests were performed for each parameter at each age between top and bottom defoliation treatments. No significant difference was found unless indicated after the second number of the pair.
* 0.05 level.

T Top defoliation treatment
B Bottom defoliation treatment

E.3.7 Simulation of Defoliation in the Field

Defoliation of young plants in the field at sites 3 and 4 (Fig. 1) resulted in differential effects with different levels of defoliation. With increasing defoliation, the height was reduced over time and also in the case of site 3, it was noted that delaying the defoliation reduced the detrimental effect (Site 3- 1 versus 2) (Table 48). Dry weights and flower production were also reduced with increased defoliation (Table 49).

Table 48. Effect of Defoliation on Field Grown Plants

Site 3 &

% Defoliation	Date 5/6	15/6	30/6	24/8
	Height(cm) *			
0	26.9±9.6	36.2±9.6	49.3±14.5	69.5±17.6
50	26.7±11.7	31.6±15.9	44.7±29.6	77.0±33.0
100	29.2±12.4	33.7±12.7	46.1±15.2	61.5±20.8

Site 4 &#

% Defoliation		Date 26/5	9/6	24/6	22/7	24/8
		Height(cm) *				
0	1	19.7±4.3	31.2±10.3	51.0±9.8	61.8±9.1	64.3±8.3
	2	-	40.8±10.7	51.8±19.2	66.0±24.7	67.7±23.8
50	1	18.2±4.4	29.8±7.2	40.0±10.3	51.7±15.9	52.0±15.6
	2	-	41.0±9.6	55.9±13.6	65.3±14.2	66.9±10.7
100	1	17.7±8.9	28.2±4.2	37.8±6.4	43.3±10.8	50.0±7.1
	2	-	40.4±8.7	53.4±13.7	59.3±15.3	60.9±14.8

* ± S.D.

& Sites from Section A

Group 1 defoliated on 26/5
Group 2 defoliated on 9/6

Table 49. Effect of Defoliation on Yield and Growth Parameters on Field Grown Plants

Site	% Defoliation		
	0	50	100
	Dry Weight (g) Plant		
Site 3	14.4±9.1	11.7±8.3	8.8±7.0
Site 4	9.1±6.3	10.2±9.5	6.6±4.9
	Flower Production		
Site 1	13.7±8.9	4.6±3.1	1.0±1.4
Site 2	31.7±30.4	26.7±17.8	6.0±5.6

E.4 Discussion

The effects of defoliation at first may appear simple; there is a measurable leaf area loss and there should be an equal decrease in the photosynthetic capacity and productivity of the plant. In reality, the relationship between the damage and plant productivity is complex and dependent on several factors, including plant age and type, predisposition to damage, compensatory growth capacity and climate. Defoliation can cause (i) lowered seed production (ii) reduced growth rate (iii) increased mortality and (iv) several other indirect effects. All these effects were observed in this study.

Damage caused by Cassida rubiginosa in the field is usually minimal and also late in the season, when Canada thistle was already near mature height and flowering initiation had begun (Fig. 63). According to the literature, this is the most detrimental time (reproductive stage) for leaf removal to occur, but the literature is concerned primarily with annual plants; and as such the hypothesis is correct. Harris (1973a) states that he, "believes the benefits of defoliation of perennial weeds while their carbohydrate reserves are low (midseason- June); associated with budding, flowering and fruiting is sufficiently well established so that it should be a factor for selection of biological control agents." While a reduction of seed production of Canada thistle may be important to limit dispersal, the main problem lies with vegetative reproduction by the aggressive perennial root system. I tender that, in the biological control of weeds, especially for perennials, it may be more important to stress the plant earlier in the season (attack younger plants) to decrease the vegetative reproduction of the roots, rather than

delaying until later in the season, when the majority of the root buds have no doubt been initiated. Defoliation at the later time may affect the flowering, but probably has little effect on the most aggressive mode, the regenerating roots. The author agrees with Harris (1973b) that the attack should occur when the plant is most physiologically vulnerable, but this time is in dispute.

Defoliation by Cassida rubiginosa larvae caged on individual plants caused fairly detrimental effects on the plants (Table 27), especially to parameters associated with the roots, but the defoliation levels were low. The effects, however, considering the low levels of defoliation were proportionately higher than the lower levels of simulated defoliation (25% and 50%) indicating that some factor is present with the insect damage which causes an enhanced effect. Capinera and Roltsch (1980) observed this in studies with grasshoppers versus manual clipping and suggested that it may be due to some insect salivary component.

Simulation of defoliation indicated, in general, that the higher the percent defoliation, the greater the adverse affect on the thistle. Many yield and growth parameters could be affected including height, root parameters (root bud number, root dry weight) and dry weights. Interrelated parameters such as flower and sideshoot production decreased together. Flower production normally releases the sideshoots to elongate whereas when the defoliation treatment eliminates or reduces flowering, it also reduces sideshoot production. The greatest effects occurred at the higher levels (75 and 100%). The differences between the two repeats of the one-time defoliation experiment were most likely due to the light regime. Experiment 5 had lower light intensity and the plants were shorter. Also in some cases, the results of Experiments 5 and 6 appeared reversed (height) especially in tables 32 and 33 (Ages averaged over

defoliation levels). This is probably because in experiment 6 with the better light regime, the younger plants were able to compensate better for the loss of leaves. In most other cases, similar trends were registered for the two experiments for both one-time and repeated defoliation.

For repeated defoliation, similar trends of decreased height, root bud number, dry weights et cetera were noted, except the effects were more detrimental than for the one-time treatments. The general conclusion is that repeated defoliation pressure was much more effective in depressing plant growth and vigour than one-time defoliation (Table 45). Repeated defoliation also caused mortality at young ages at high defoliation levels (Table 44). McCarty and Price (1942) found that although frequent defoliation was important, it was less so than the timing or level of defoliation and Jameson (1963) concluded that detrimental effects were increased by increased frequency of defoliation. Many perennial plants can withstand a single annual defoliation which under favourable conditions may even be stimulatory (Alcock 1964, Harris 1972, 1973b). Simmonds (1951) found that for the use of a defoliator for biological control of Cordia macrostachya (Jacq.) R. and S. single defoliations increased production, whereas multiple, even though partial, defoliation, reduced growth and reproduction.

From one-time and repeated defoliation simulation results, the stress appears greater on younger plants than on older plants, supporting the hypothesis that it may be more detrimental to attack younger plants. This indicates that some careful rethinking of biological control agents which are defoliators may be required and that careful phenological studies should be done before much emphasis is placed on the insect as a biological control candidate.

In some cases, at low levels of defoliation, there appeared to be a stimulation of growth, indicating that at least some tissue is expendable and that compensatory growth is occurring (See appendix I). Leaf area reduction need not be detrimental since many levels do not photosynthesize at their maximum potential (Hewett 1977). High levels of defoliation are required, especially if defoliation is performed at one-time only, to cause any detrimental effects. As with many crop plants, (see references in introduction), Canada thistle can withstand a high level of defoliation, especially at the older ages, without adverse effects.

Experiments to determine whether removal of the upper or lower leaves was more detrimental, tentatively indicated that removal of the upper leaves had greater adverse effects (although not significant- Tables 46,47). This is a common effect (see references in the introduction). Cassida rubiginosa larvae in the field rarely field on the young leaves, but tend to feed on the middle and lower leaves. This fact may also lower the efficiency of C. rubiginosa attack. Most insects have a preference for one leaf area or another. Harris (1973a) says that contrary to popular opinion, loss of mature leaves is most damaging. This did not seem to be the case here, although, the two repeats gave dissimilar results, one of which indicates that this may be true.

The purpose of performing the field experiment was to determine if the limited pot volume and therefore possibly limited root growth was enhancing the effects as compared to plants from the field which could potentially have a much more extensive root system. The results indicate that for the length of the experiments (approximately 3 months for both the pot and field experiments), that the effect of the pot did not seem to affect the results

(Tables 48,49).

Care was taken throughout all the simulation experiments to remove only the leaf blade and not to damage the midrib, since Cassida rubiginosa does not eat the midrib. The experiments seemed to approximate insect defoliation in the majority of cases and as Jones et al. (1982) stated, until more information is made available about defoliation, simulated defoliations are still useful. It has been shown that procedures which cut the midrib do not approximate insect blade defoliation, whereas removal of the blade is a good approximation (Poston et al. 1976, Hammond and Pedigo 1981, Fick 1982).

In summary, the greater the defoliation level, the more detrimental the effects on plant growth and vigour. Also, in general, in support of the hypothesis made concerning the age of defoliation, the results indicated that defoliation had greater adverse effects if it occurred at younger plant stages. Van den Bosch (1979) states that the appropriate timing of attack may be as important as massive defoliation. Also, defoliation of the upper leaves may have greater detrimental effects than defoliation of lower leaves. Continuous (or repeated) defoliation was more effective in reducing vigour, growth rates and yields than one-time defoliation and also resulted in mortality in treatments of high defoliation levels on young plants.

The collection of facts from this study explains why Cassida rubiginosa is not an effective biological control organism. (a) The level of insect defoliation is low and the results in this study indicated that high levels of defoliation are required to adversely affect Canada thistle. (b) The insects

attack Canada thistle fairly late in the season, long after the young stages, which this study has indicated are the most susceptible to damage. (c) The insects feed on the leaves at the middle of the plant, not on the upper leaves. (d) The insects show some preference for larger plants (Section A), which of course means they cause very little stress. The greatest problem with the insect is that it is poorly synchronized with the phenology of the plant to cause a large detrimental effect.

F. SYSTEMIC RUST PUCCINIA PUNCTIFORMIS

F.1 Introduction

> "Two years ago about an acre of our farm was overrun with Canada thistle, but by the time they were in full bloom a rust struck them and hardly a seed of the plant matured. We plowed the land in fall, and last year scarcely a thistle appeared. If this rust could be widely disseminated through the country, Canada thistle would receive a substantial check."

letter of a farmer to New Jersey Experimental Station. 1893.

F.1.1 Phytopathogenic Weed Control

The concept of utilizing plant pathogens to decrease weed populations is not new as indicated by the by-line quote. Cockayne (1916) also observed the same rust to be an effective control for Canada thistle. Other older literature examined the potential of using other rusts and pathogens (references in Hasan 1980). The concept was reawakened in the 1970's (Wilson 1969, Zettler and Freeman 1972) and resulted in a rapid increase in research (Freeman and Charudattan 1980).

In 1976, Freeman et al. listed 27 plant species being investigated for control by plant pathogens. Of these, four have reached advanced development

stages; Cercospora rodmani Conway on water hyacinth (Eichhornia crassipes (Mart.) Solms), Colletotrichum gloeosporioides (Penz.) Succ. f.sp. aeschynomene on northern jointvetch (Aeschynomene virginica (L.) B.S.P.), Phytophora citrophthora (Sm. and S..) on milkweed vine (Morrenia odorata Lindl.) and Puccinia chondrillina Bubak and Syd. on skeleton weed (Chondrilla juncea L.) (Freeman and Charudattan 1980).

The rust, Puccina chondrillina, was the first example of the deliberate introduction and successful use of an exotic pathogen as a classical biological control agent (Andres et al. 1976. Turner et al. 1981 a,b, Cullen et al. 1973, Burdon et al. 1981). Other rusts which have been investigated include Puccinia on Xanthium spp., Uromyces rumicus on Rumex crispus, Phragmidium violaceum on Rubus spp. and Puccinia punctiformis on Cirsium arvense.

Empirically, the use of an endemic pathogen would seem ineffective, since the pathogen and the host co-exist, but bioherbicide techniques are, in fact, showing great promise. This is accomplished by applying elevated spore levels at a stage when the plant is the most susceptible (Daniel et al. 1973). This type of biotechnological application and subsequent commercialization is becoming a reality (Kenney et al. 1979). Collego™, a registered mycoherbicide Colletotrichum gloeospoeioides f.sp. aeschynomene, is one example of a successful development campaign (Daniel et al. 1973, TeBeest et al. 1978. Boyette et al. 1979. McClintic 1983). The Colletotrichum genus seems to be a promising one, as several species are under investigation; C. gloeosporioides f. sp. jussiaeae against winged waterprimrose (Jussiaea decurrens (Walt) FC. (Boyette et al. 1979), C. malvarum (Braun and Casp.) South. against prickly sida (Sida spinosa L.) (Kirkpatrick et al. 1982).

F.1.2. Physiology of Pathogen Effect on the Host

It is well known that plant pathogens are capable of great destruction, with such examples as chestnut blight (Endothia parasitica (Man). And. and And.), Dutch elm disease (Ceratocystis ulmi (Buis) Moreau), and white pine blister rust (Cronartium ribicola Fisher) and numerous crop pathogens, which have shaped human lives for centuries and form an important emphasis in crop breeding programs.

Some of the general effects that pathogens have on plants are: increased respiration, interference with translocation, reduced photosynthesis, increased transpiration and so forth. Rusts have the following general effects: increased water loss, removal of nutrients, increased respiration, reduced photosynthesis and growth hormone imbalance (Agrios 1978). The potential of using the stress caused by pathogens for biological control of weeds is a promising field. The rust considered in this section has some known effects on Canada thistle (increased gibberellin levels) but the exact effects on the physiology of Canada thistle are not known and need further examination. The understanding of the nature and movement of the pathogen in the host is limited and from observation in nature, some artificial augmentation would no doubt be necessary to increase the damage, since, it is generally limited to a few shoots and tends to remain at a low level over the years (Cockayne 1915).

F.1.3 Case Study Organism : Puccinia punctiformis

Puccinia punctiformis is a microcyclic autoecious rust of the brachy form (lacking the aecial stage) (Buller 1950, Menzies 1953). The life cycle has been well described, however, some researchers record an aecial stage (Cummins 1978, Arthur 1934), whereas others recognized the lack of the aecial stage (Hotson

1934, Cunningham 1931, Savile 1970, Buller 1950, Menzies 1953). P. Punctiformis is host specific to Canada thistle and the distribution of P. punctiformis corresponds to that of its host, occurring throughout Europe, Asia, North America and New Zealand (probably moving by human agency as urediniospores attached to Canada thistle plants in packing straw) (Cunningham 1931, Arthur 1934).

The currently accepted life history is shown in figure 92. There are two types of infection (a) localized infection of isolated pustules (sori) and (b) systemic infection in which whole shoots bear either pycnia or uredinia. Plants which are systemically infected emerge in the spring usually with pycnia (haploid) and occasionally with uredinia (dicaryotic) which are primarily hypothallous. The pycnia become uredinia (heterothallism) and release urediniospores, the so-called repeating stage, which cause the secondary or localized infections (Buller and Brown 1941). The double-celled teliospores occur later in the season. The relationship between the types of infection has been a difficult problem to solve and is still difficult to understand. Cockayne (1915) suggested that new systemic infections resulted from only basidiospores originating from overwintered teliospores. Cunningham (1931) mentioned that there were differing opinions for the method of overwintering; either by systemic mycelia in the root or teliospores in the soil. Buller and Brown (1941), Buller (1950) and Menzies (1953) were able to produce systemically infected shoots by inoculation with urediniospores and found that teliospores were very difficult to germinate and suggested it was unlikely that systemic infections arise from teliospores. Turner (1981) mentions that she performed successful inoculations with teliospores, but these may have been contaminated with urediniospores.

Figure 92. Life history diagram of Puccinia punctiformis

Systemically rusted shoots which emerge in the spring are generally infected by pycnia (sometimes uredinia). In the late spring, the pycnia become uredinia and release urediniospores (the so-called repeating stage) which can infect previously non-infected plants. This results in the production of local lesions (secondary rust), which in the fall kills the lower leaves. The mycelia grow out of the leaves, into the stem and into the roots. The uredinia become telia before the leaves die. The rust can overwinter either as teliospores or as mycelia in the roots and root buds.

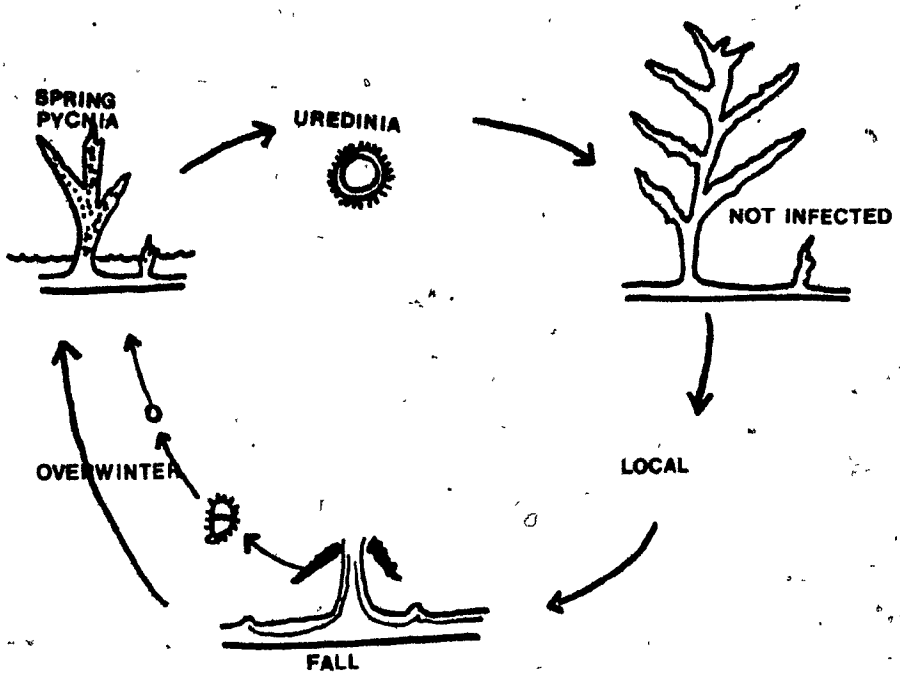

SPRING
PYCNIA
UREDINIA
NOT INFECTED
LOCAL
OVERWINTER
FALL

FIGURE 92

After secondary infection occurs, mycelia grow out of the inoculated leaves, down the stem and enter the roots. From there the mycelia can enter branch roots and root buds for overwintering.

The symptoms of systemic infection include a sickly yellow colour, thick, succulent, hollow stems, leaves which point upwards and reduced development and limited flowering. Cunningham 1931 believed no flowers were produced and Buller 1950 found mycelium in the florets of Canada thistle. The plants also tend to have longer internodes and the leaves are usually entire and narrow and with incurled margins and are less prickly (Buller 1950, Bailiss and Wilson 1967). Rusted plants also respire more than healthy plants (Keogh and Watson 1982) and also have lower osmotic pressure and several anatomical anomalies (Buller 1950). Bailiss and Wilson (1967) found elevated levels of gibberellic acid in rusted plants early in development. Cockayne (1916) mentioned that in order for the rust to become an adequate means of control, the infection must be increased above that occurring naturally.

The purpose of this study was to continue the evaluation of Puccina punctiformis as a possible biological control organism. Field levels of the systemic phase of the disease are low and seem to remain in equilibrium. Systemic infections are believed to be initiated by the previous season's secondary infections. The relationship needs to be better understood to determine procedures to enhance the infection rate. Attempts were made to understand this relationship by inoculation of plants at various ages and with single and repeated (multiple) inoculations. Analysis of present field

population dynamics was performed, as well as further studies into the physiological stress caused by P. punctiformis to Canada thistle. Microscopal examination was also done. Preliminary experiments with the production of rusted callus by tissue culture were done with the ultimate goal of the development of procedures for the mass production of spores for augmentation of field levels of the rust spores.

F.2 Materials and Methods

F.2.1 Field Observations

Survivorship was determined for systemically rusted versus nonrusted plants for the field sites examined in section A. Other additional data included the number of ramets (systemically rusted) produced at the end of the season (late August).

F.2.2. Inoculation Experiments

In most cases, spores for inoculation were collected from heavily infected plants from the field and stored at 5°C in glass vials sealed with Parafilm. Spores were collected each year (1980-1983) and used for all inoculations except for one experiment (repeat 2 of multiple inoculations) in which fresh spores were collected from plants from another inoculation experiment.

Seeds were surface sterilized in a 2% sodium hypochlorite solution by drawing a vacuum. The seeds were germinated at ambient temperature on moistened filter paper in glass Petri dishes. The small seedlings were transplanted into flats of Promix and placed in a controlled environment chamber with conditions as described in section B. Once established, vigourous seedlings were potted into 100 mm (diameter) pots in Promix. Plants were inoculated by lightly misting the plants with distilled water and applying the spores with moistened fingers to the abaxial leaf surface. The inoculated plants were placed in misted plastic bags, the bags were sealed and placed in the dark at ambient temperature for approximately 24 hours. After the dew period, the plants were removed from the bags and returned to the growth chamber or bench.

(a) Single Inoculations. This experiment was repeated twice. Plants were inoculated with Puccinia punctiformis spores at the cotyledon, 2-, 4- and 6-leaf stages. The controls were misted with water only. Data taken included height, leaf number, number of leaves infected and number of ramets (rusted and nonrusted). Regrowth was determined after top growth was removed. All treatments were replicated four times in each experiment.

(b) Multiple Inoculations. To determine the effect of successive inoculations, thistle plants were inoculated at the 2-; 4-; 8-; 2,4-; 2,4,8-; and 4,8-; and in one repeat also at the 6-; 2,4,6,8-; 4,6-; 4,6,8-; and 6,8-leaf stages. Similar data was recorded as for single inoculations. Roots were examined extensively in one repeat of the experiment to determine relative locations of rusted and nonrusted shoots to the original inoculated plant. At the 20 week stage, the plants were removed from the pots, the roots cleaned and pictures taken. A regrowth experiment with the same roots with all the top growth removed was also performed.

The effects of naturally occurring multiple inoculations were examined by collecting secondarily infected shoots and roots from the field in late August/early September and potting the roots at different intervals in a soil mixture according to Section B. Numbers of systemically rusted and nonrusted ramets were recorded.

F.2.3 Physiological Experiments

Chlorophyll Content. Chlorophyll was extracted (with cold 80% acetone for 5 minutes in 20 mL followed by an additional 2 minutes with 10 mL of added acetone in a Virtis homogenizer) from leaves of rusted and nonrusted plants

collected from the field. The samples were then centrifuged at 10,000 rpm in an IEC B-20A refrigerated centrifuge for 10 minutes. Readings of the optical density were made at 645 nm and 663 nm (Arnon 1949) with a spectrophotometer. If the density was too great, dilutions were made with 80% acetone. This experiment was repeated twice, once with four plants of the two classes and again with 6 plants per class.

Gibberellin Effects. Young plants grown from clean root pieces were potted into 100 mm (diameter) pots in Promix and grown in a controlled environment under conditions noted in Section B. Four plants were each sprayed with either distilled water (control), 1 ppm, 10 ppm or 100ppm aqueous solutions of gibberellic acid (GA3). This experiment was repeated twice. Height and leaf number were recorded weekly. After six weeks, data taken included height, leaf number, number of ramets, number of sideshoots. In repeat 2, the number of root buds, dry weight of the plant, root and ramets were also determined.

F.2.4 Microtechnique

Connections between mainstem or root and a rusted ramet collected either from the field or one of experiments in section E.2.2, were sectioned by hand and cleared by the procedure outlined in section D.2. Hand sections were cut of fresh systemically rusted material and also of callus (Section F.2.5) and stained with aniline blue in lactophenol.

Samples of rusted and nonrusted plants were collected from the field and processed according to section B for plastic sectioning and also for wax embedding. Wax sections were stained in safranin and fast green (O'Brien and McCully 1981).

Samples of leaf tissue were also prepared for Scanning Electron Microscopy by standard techniques (O'Brien and McCully 1981) and pictures taken by technician Louis Thauvette.

F.2.5 Tissue Culture of Rusted Material

Infected explants of leaves and stems from rusted plants were surface sterilized in 70% ethanol (30 seconds), 2-5% sodium hypochlorite (1-2 minutes) and rinsed in sterile distilled water and placed on tissue culture media in plastic Petri plates (Appendix K) containing varying levels of auxins and cytokinins. These operations were performed in a laminar flow apparatus.

The Petri plates were placed in an incubator in the dark to allow callus formation and examined periodically. After callus had formed, it was transferred onto fresh media every 3-4 weeks.

F.3 Results

F.3.1 Field Results

Systemically rusted plants are easily distinguished in the field from nonrusted plants by their pale green colour, the more acute angle of the leaves to the stem, longer internodes, smaller and less broad leaves and in the pycnia stage of the rust by a strong sweet odour (Fig 93). Rusted plants tend to grow more quickly at the beginning of the season than healthy plants (Table 50) but gradually their development ceases and they die. In the field, pycnia are present soon after emergence (mid May) and uredinia at least two weeks later (early June). In some cases, the systemically infected shoots emerge bearing uredinia. Secondary infections were found as early as mid June (1982) and early July (1981). Systemically infected plants rarely survive the season (Fig. 94- combined field data in form of survivorship curve) and rarely flower. If they do flower, the buds rarely open and do not seem to produce seeds.

F.3.2 Results from Artificial Inoculations

Application of rust spores results in most cases in the development of secondary rust pustules about 10 days after inoculation (Fig. 95).

F.3.2.1 One-Time Inoculations

The more leaves infected by inoculation, the greater the probability of a systemic infection and the greater the number of systemic shoots produced (Tables 51, 52). In some cases, accidental additional secondary infections also occurred (Table 52), since more leaves than were originally inoculated became infected.

Table 50. Early Season Height Comparison of Systemically Rusted and Nonrusted Thistles (Field Data 1982).

Category	Date		
	26/5	9/6	24/6 #
	Height (cm) *		
Systemic Rust	24.2±6.9	36.5±9.5	44.7±11.8
Nonrusted	19.3±6.2	32.6±10.5	45.3±15.4

* Height + standard deviation

\# 43.7% of measured thistles dead at this point.

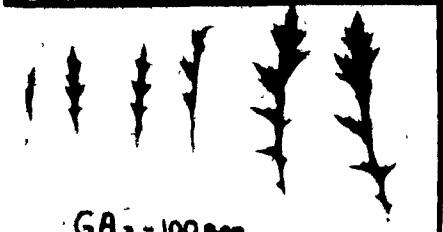
Figure 93. Comparison of nonrusted (NR) and systemically rusted (R) thistles from the field. Note the differences in leaf size and shape, internode length and stem thickness.

Figure 95. Infection of an inoculated leaf from artificial inoculation experiments (arrowheads).

Figure 96. Regrowth from inoculation experiment after removal of the top growth. Note that the rusted shoots (▲) are close to the originally inoculated plant (●), (Nonrusted shoots- ■), but in some cases, rusted shoots were found up to 25 cm along the root system from the origin.

Figure 98. Application of exogenous gibberellin. The higher levels of gibberellin(10 and 100 ppm) resulted in a plants that were much taller than the control and 1 ppm treatment.

Figure 99. The difference between 100 ppm treatment leaves and control leaves. Note the more narrow appearance of the treated leaves.



GA_3 - 100 ppm

CONTROL

99

Figure 94. Survivorship curve (%) of rusted (red) and nonrusted plants (green) averaged over field sites.

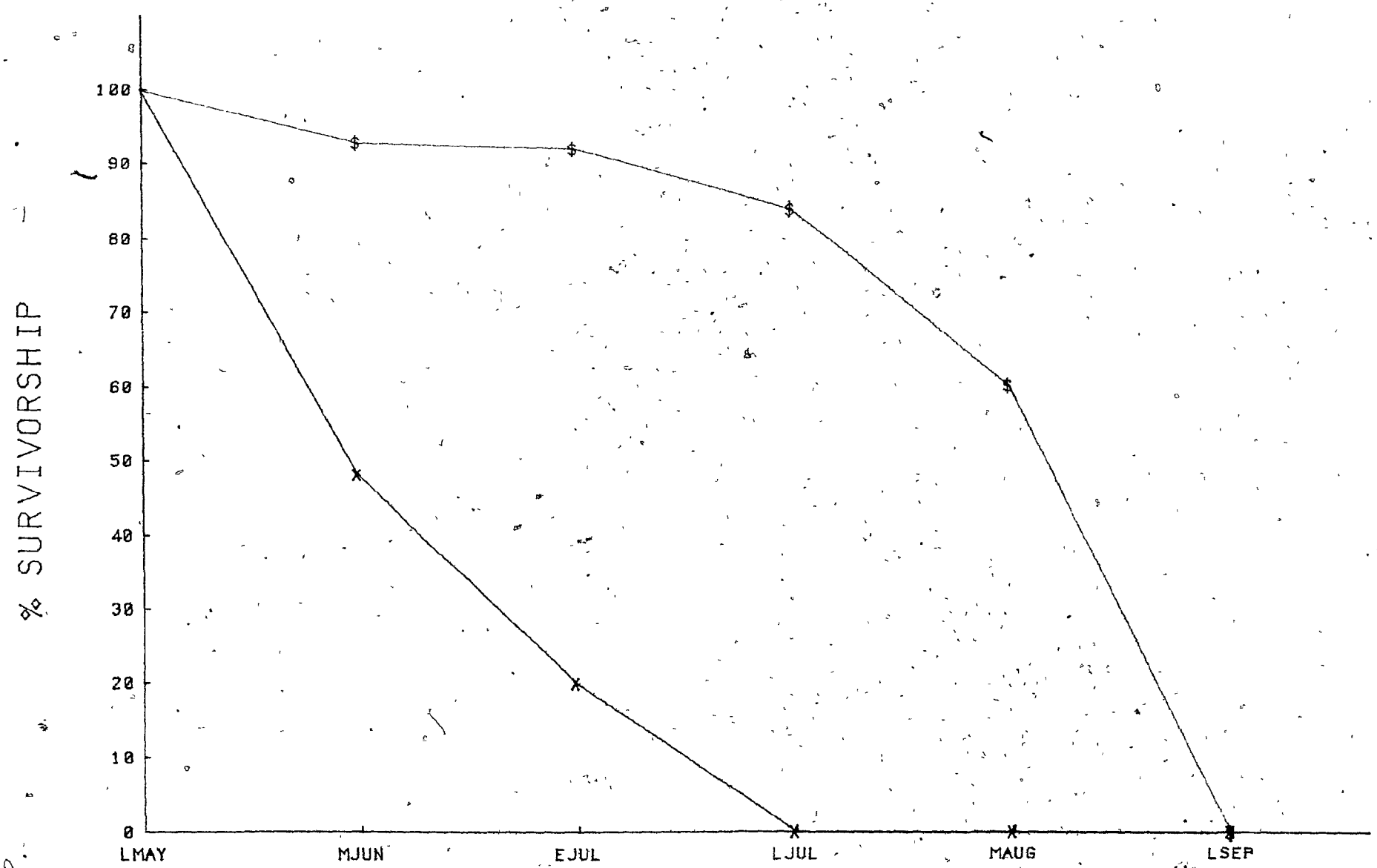


FIGURE 94. DATE

Table 51. The Effect of Single Inoculations with Urediniospores of Puccinia punctiformis on Canada Thistle Ramet Production (Repeat 1)

Inoculation	Max. No. leaves infected #	Ramet Production * 12 weeks R	12 weeks N	12 weeks %	18 weeks R	18 weeks N	18 weeks %
Cotyledon	2C	0.3	2.8	25	0.5	5.3	25
2 leaf	2C,4L	0	2.0	0	0	5.0	0
4 leaf	2C,4L	0	3.0	0	0	6.3	0
6 leaf	2C,6L	0.8	1.3	0	0.5	7.3	25
control	none	0	2.5	0	0	5.8	0

* R number of rusted ramets averaged over 4 plants
N number of nonrusted ramets averaged over 4 plants
% frequency of plants with rusted ramets

\# C=cotyledon; L=leaves

Table 52. Effect of Single Inoculations of *Puccinia punctiformis* urediniospores on Canada Thistle Ramet Production. (Repeat 2).

Inoculation	Max. No. Leaves infected #	Ramet Production * 12 week R	12 week N	12 week %	15 week R	15 week N	15 week %	18 week R	18 week N	18 week %
Cotyledon	4L	0	2.3	0	0	2.3	0	1.0	2.0	0
2 leaf	6L	0.3	3.3	25	0.8	4.8	50	1.3	1.8	75
4 leaf	5L	0	1.0	0	0.3	1.5	25	0.8	2.0	25
6 leaf	7L	0.3	1.0	25	0.3	2.3	25	2.0	1.8	100

* Ramet production in weeks following inoculation
R- average number of rusted ramets (4 plants)
N- " " " nonrusted ramets (4 plants)
%- frequency of plants with rusted ramets

L=leaves

F.3.2.2 Multiple (Repeated) Inoculations

The two repeats of this experiment are different because during repeat one, re-infection occurred other than by intended inoculation resulting in a higher number of infected leaves in most cases. In repeat 2, re-infection did not occur, so that the maximum number of leaves infected corresponded to actual inoculations. In both cases, multiple inoculations resulted in earlier and greater numbers and frequency of systemically rusted shoots (Tables 53, 54). Regrowth data indicated that the roots remained infected throughout their lifetime and were capable of producing rusted ramets for several months (Table 55). One group of plants that was accidentally infected was observed for approximately 18 months to still be producing rusted ramets. The majority of the rusted ramets were close to the original plant (Fig. 96), however, in some cases the rusted ramets were found over 25 cm along the root system from the originally inoculated plant. These experiments also indicated that the rust was capable of moving in the root system and of producing rusted ramets within 10 to 12 weeks after inoculation.

F.3.2.3 Naturally Occurring Multiple Inoculations:

In late August/early September infected plants sometimes produced ramets from the base of the stem which were systemically infected. Samples of secondarily rusted plant roots from the field grown out in the greenhouse indicated that as early as two months after local infections occurred, there were systemically infected ramets albeit, very close to the mother plant (Table 56).

Table 53. Effect of Multiple Inoculations on the Production of Systemically Rusted and Nonrusted Ramets. Repeat 1.

Inoculation	Max. no. Leav. #	4 weeks* R	N	%	6 weeks R	N	%	8 weeks R	N	%	12 weeks R	N	%	20 weeks R	N	%
Control	2/7	0	1.0	0	0	1.8	0	0.3	1.5	25	0.3	1.5	25	2.0	4.5	75
2	2/7	0	0.5	0	0	1.3	0	0	1.0	0	0.3	1.5	25	5.3	3.0	100
4	2/5	0	0	0	0	1.3	0	0	1.5	0	0.8	1.8	75	2.8	5.0	75
6	2/8	0	1.5	0	0	3.3	0	0	1.5	0	0.5	1.8	50	5.5	4.8	50
8	2/8	0	0.5	0	0	3.3	0	0	3.0	0	1.0	3.0	75	4.0	5.8	100
2,4	2/8	0.3	0.5	25	0.5	1.5	25	0.3	3.5	25	0.3	2.3	25	1.3	6.3	50
2,4,6	2/7	0	0.5	0	0.3	0.5	25	0.8	1.3	25	1.0	1.3	75	1.5	1.3	50
2,4,6,8	2/8	0.3	0	25	0.3	0.8	25	0.3	1.3	25	0.3	1.0	25	3.3	2.5	100
4,6	2/8	0	0.5	0	0	0.5	0	0.3	1.3	25	2.0	0.8	50	3.3	4.5	75
4,6,8	2/8	0.3	0.8	25	0	2.0	0	1.8	0.5	50	0	1.8	0	all dead		
6,8	2/6	0	0.5	0	0	1.8	0	0	2.5	0	0.3	1.5	50	1.3	3.8	75

evaluated 8 weeks after inoculations complete
a/b= no. of cotyledons infected/no. leaves infected

* R- rusted ramets; average for 4 plants
N- nonrusted ramets; average for 4 plants
%- frequency of plants bearing rusted ramets

Table 54. Effect of Multiple Inoculations on Production of Systemically Infected and Nonrusted Shoots (Repeat 2).

Inoculation	# Max. no. leaves	* Ramet Production 8 weeks R	N	%	10 weeks R	N	%	12 weeks R	N	%	14 weeks R	N	%
Con	2/3	0	1.2	0	0	2.6	0	0	6.0	0	0.4	7.2	20
2	2/3	0	1.2	0	0	2.0	0	0	4.0	0	0.2	5.2	20
4	2/5	0	0.2	0	0	2.0	0	0	3.8	0	0.6	5.4	40
8	2/7	0	1.4	0	0	3.0	0	0	4.8	0	0	4.4	0
2,4	2/4	0	1.2	0	0	2.2	0	0.6	3.4	40	1.0	4.4	40
2,4,8	2/7	0	1.2	0	0.2	4.0	20	0.6	6.2	60	1.2	8.0	80
4,8	2/7	0	1.2	0	0	3.0	0	0	5.2	0	0	6.0	0

* Ramet Production in weeks after inoculations
R- Rusted ramets- average/5 plants
N- Nonrusted ramets- average/5 plants
%- Frequency of plants bearing rusted ramets

\# Maximum number of leaves infected a/b=no. of cotyledons/leaves

Table 55. Regrowths of Multiple Inoculation Experiment (Repeat 2) after Removal of Top Growth

Weeks after replanting	Rust-Pycnia		Rust-Uredinia		Healthy	
	Ramet Production					
	Ht #	% *	Ht.	%	Ht.	%
3 weeks	6.8±4.5	24	10.8±5.3	6	4.0±2.1	70
5 weeks	6.0±4.5	35	4.5±0.7	3	4.4±2.8	62
6 weeks	7.4±6.9	43	5.4±2.4	9	6.7±5.6	46
7 weeks	8.6±8.7	33	7.5±4.5	11	6.2±3.5	56
8 weeks	10.4±10.1	29	10.4±6.8	15	8.0±5.5	56
9 weeks	9.3±10.0	30	13.3±9.7	18	8.4±5.5	51

#- Height(cm) ±S.D.
*- Percentage of ramets in this category

F.3.3 Physiological and Morphological Effects

F.3.3.1 Chlorophyll Content:

The amount of chlorophyll in leaves of systemically infected plants was depressed in comparison to nonrusted plants (Table 57, Fig. 97) and decreased in rusted plants over the season (based on milligrams of chlorophyll per gram fresh weight).

F.3.3.2 Gibberellin Effects:

Spraying with varying levels of gibberellic acid caused an increase in the height at higher concentrations and an increase in internode length (Figs. 98,100,101). Ramet number, root bud production and root dry weight decreased with increasing gibberellic acid content, whereas parent dry weight increased (Tables 58,59). Leaf morphology also changed, resulting in a similar leaf shape as rusted plants, (smaller leaves with indentations and narrower blades (Fig. 99).

F.3.3.3 Other Parameters:

Respiration (Appendix L) of systemically rusted plants was higher than that of nonrusted plants. Rusted plants also transpired faster than nonrusted plants (Appendix M), and also they had slightly elevated water soluble protein contents for roots, leaves and stem and elevated water soluable sugar content for the root and reduced sugar content for the stem and leaf in comparison to nonrusted plants (Appendix N).

F.3.4 Callus Formation

Table 56. Regrowth of Shoots from Secondarily Infected Field Collected Plants.

Group	Total of each Dates							
	25/9	15/10	13/11	13/12	15/1	23/2	4/3	31/5
Group1 *								
Pycnia	2	1	1	0	0	0	0	2
Uredinia	1	2	0	1	2	0	0	1
Healthy	3	5	2	2	0	2	2	0
None	6	1	5	5	5	5	5	5
% Systemic	50	33	33	0	33	0	0	100
Group2 $								
Pyncia	0	2	0	0	1	2	2	4
Uredinia	0	0	2	2	2	1	1	1
Healthy	0	9	10	9	9	7	9	12
None	14	8	8	8	8	8	8	8
% Systemic	0	18	17	18	25	30	25	29
Group 3 #								
None				0	0	0	0	0
Group 4 &								
Pycnia				0	1	1	1	0
Uredinia				0	0	0	0	0
Healthy				3	6	6	6	9
None				3	2	2	2	2
% Systemic				0	14	14	14	0

* group1 out of 8 plants potted 25/8
$ group2 out of 14 plants potted 21/9
\# group3 out of 5 plants potted 13/11
& group4 out of 6 plants potted 7/12

Figure 97. Spectra of chlorophyll (in visible range) extracted from leaves of nonrusted (A) and rusted plants (B). Note that there is more chlorophyll in the nonrusted leaves.

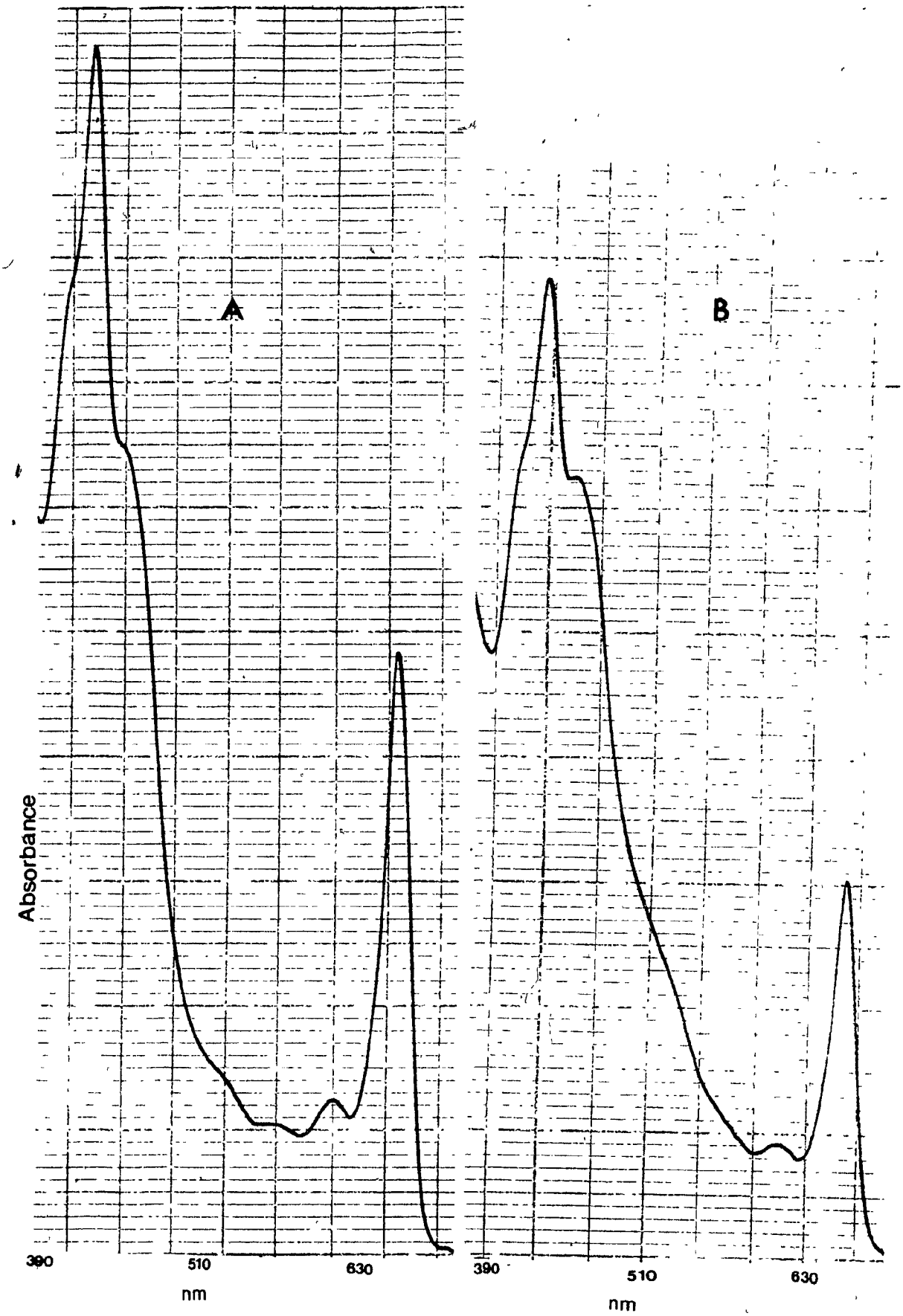


Figure 97

Table 57. Chlorophyll Content of Leaves of Systemically Rusted and Nonrusted Plants (Field Samples)

	mg/g fresh weight		mg/leaf area(cm^2)	
	Sample 1*	Sample 2	Sample 1	Sample 2
Systemically Rusted (Pycnia)	32.0±1.1	-	1.5±0.09	-
Systemically Rusted (Uredinia)	39.8±10.2	27.5±6.3	1.4±0.08	1.7±0.2
Nonrusted	38.0±5.5	47.4±10.7	1.3±0.3	1.94±0.2

* Sample 1 collected on June 1- mean± S.D.
Sample 2 collected on June 17- "

Figure 100. Effect of gibberellin on height (cm)

X= control
$= 1 ppm
K= 10 ppm
+= 100 ppm

Figure 101. Effect of gibberellin on internode length (cm)

X= control
$= 1 ppm
K= 10 ppm
+= 100 ppm

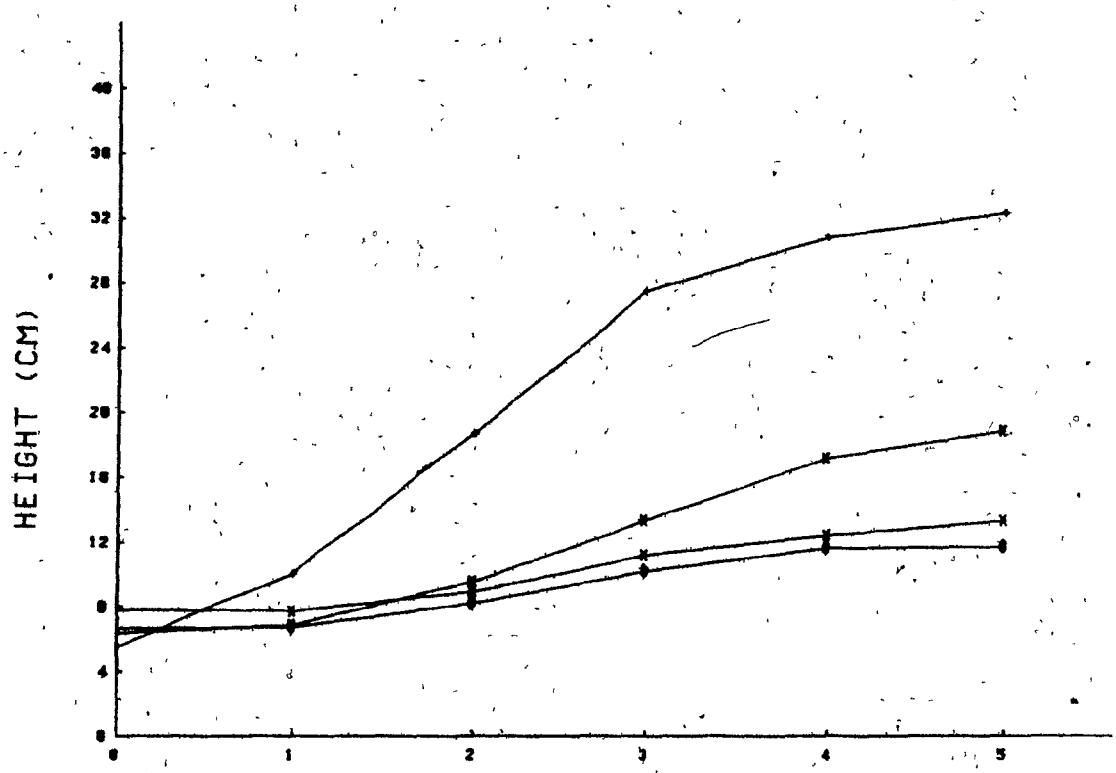


FIG. 100 WEEKS

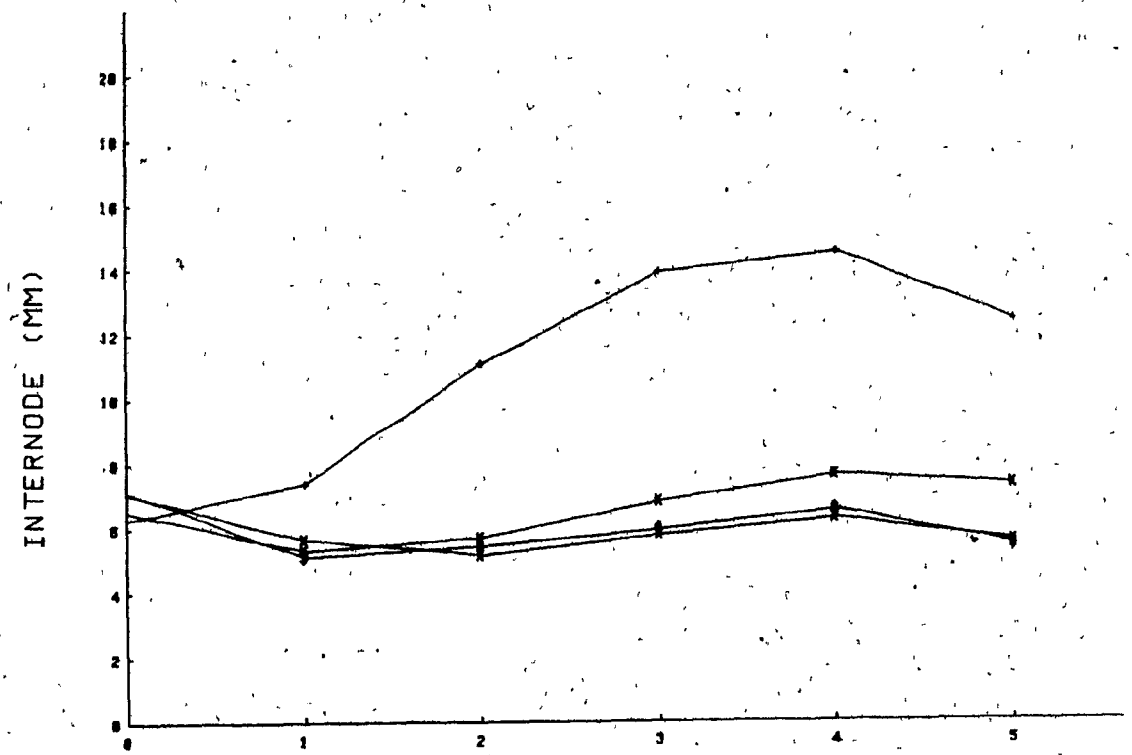


FIG. 101 WEEKS

Table 58. Effect of Exogenously Applied Gibberellin on Canada Thistle Height and Shoot Dry Weight

Gibberellin Conc.	---Plant height (Internode length) in cm------ 0 weeks	1 week	2 weeks	3 weeks	Shoot Dry Weight (g)
Control	8.3(1.3)	12.0(1.8)	18.3(1.4)	21.4(1.7)	0.5
1 ppm	12.2(1.3)	17.6(1.5)	22.8(1.6)	26.0(1.7)	0.3
10 ppm	2.4(1.4)	22.8(2.0)	33.8(2.2)	37.8(2.4)	1.4
100 ppm	17.0(1.7)	23.1(1.9)	35.8(2.3)	39.6(2.1)	1.1

Table 59. Effect of Exogenously Applied Gibberellic Acid on Yield Parameters of Canada Thistle (Repeat 2).

Parameter	--Gibberellic Acid Conc. (ppm)--- 0	1	10	100	LSD (0.05)
Height(cm)	13.4	11.9	19.1	32.6	3.0
Internode Length(cm)	0.6	0.6	0.7	1.3	0.9
Ramet number	2.8	1.8	2.0	0.3	1.4
Ramet height (cm)	2.7	1.7	3.6	2.7	1.2
Root bud number	15.0	22.3	8.3	4.0	6.1
Leaf number	24.0	22.0	26.0	26.0	2.7
Dry weight(g)					
Plant	2.2	2.3	2.6	3.2	0.4
Root	1.2	1.4	1.0	1.0	1.4
Ramet	0.3	0.1	0.5	0.3	0.4

After testing a range of hormone types and combinations, the best combination for callus formation was 1 mg L^{-1} 2,4-D (2,4-dichlorophenoxy acetic acid) and 1 mg L^{-1} zeatin. Several other combinations were examined and good callus was found on several combinations and shoot formation was also initiated.

The callus formed on good media was fluffy, biege and oftne had rust coloured spots on the surface (Figs. 102-104). Hand sections through callus tissue showed that the tissue was very heavily infected with the rust (Figs. 105-108). Haustoria morphology was similar (with encapsualation) to natural infections but more variable. The mycelia occurred intercellularly and also aerially in the outer portion of the callus.

F.3.5 Microtechnique and Pustule Formation

After inoculation, it takes approxiamtely 10 days before the symptoms of rust infection occur, by the ofrmation of round chlorotic areas on the leaves. Later after the pustule (sori) develops, the area around is first chlorotic (Figs. 109-110) and then at a later date, when the majority of the leaf is sying, the areas around the sori have greened up again, an example of the so-called "green-islands". Sometimes satellite pustule formation occurred (Fig. 109) which indicates that re-infection from the centre sori has occurred.

Leaves: Sections through the leaves of infected plants show the stages of development of the pycnia through to the uredinia from the light microscope (Figs. 111-118) and the scanning electron microscope (Figs. 119-122). The results indicate that the pycnia have a flat hymenium in the intraepidermal postion. The uredinia have ruptured the epidermis and the urediniospores are spherical with many spiny echinulations.

Callus formation.

Figure 102. Callus formed from infected leaf tissue.

Figure 103. A closeup of the callus. Note the firm but not hard texture of a good callus.

Figure 104. Surface of callus showing pustule-like processes (arrowheads)

Figure 105. Hand section of callus. This outer portion of the callus is heavily infected intercellularly and the mycelia also extend aerially. There are also several haustoria (X125).

Figure 106. Closeup of the outer portion of callus. Note the large amount of mycelia both intercelluarly and aerially (X 410).

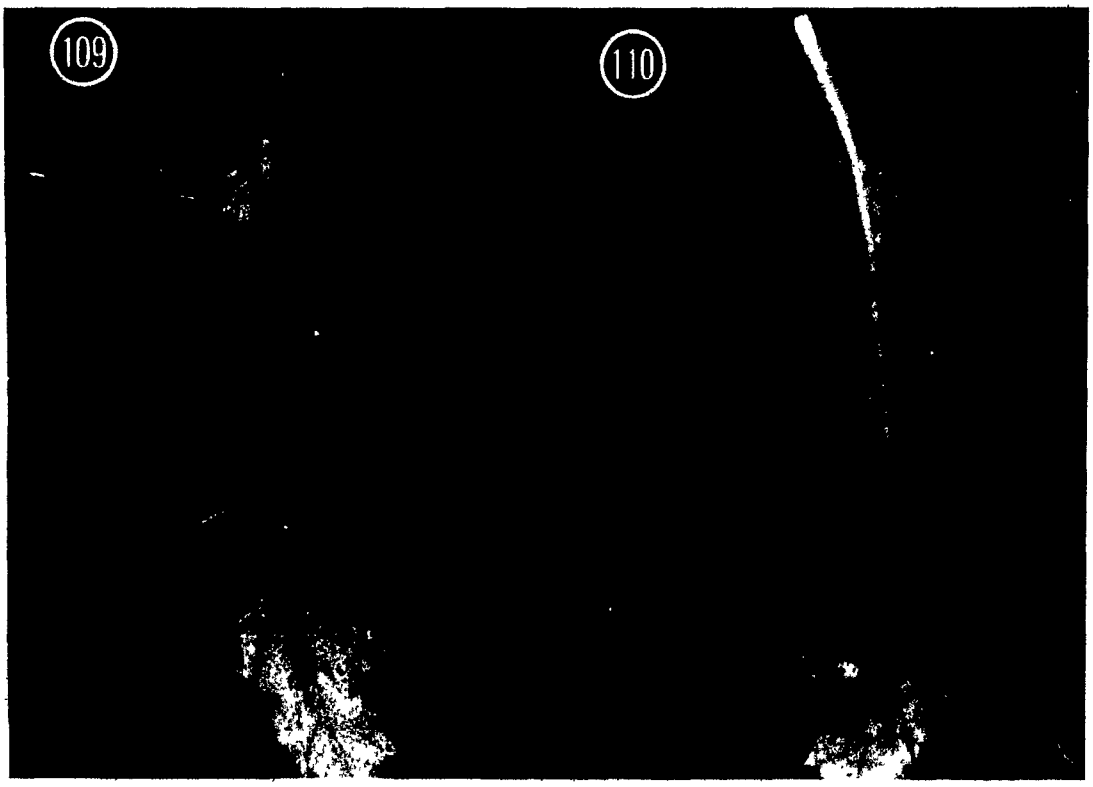
Figure 107. An enlargement of a portion of figure 106 (upper middle) showing details of mycelia (X 1000).

Figure 108. An haustoria and associated intercellular mycelia (X200).

Figures 109 and 110. An artificially infected leaf with secondary infection viewed from the adaxial and abaxial surfaces.

Figure 109. The leaf from the abaxial surface. Note the satellite pustules (large arrowhead).

Figure 110. When the leaf was turned and viewed from the upper surface, the chlorotic regions correspond to the location of the sori on the underside (match arrowhead sizes for comparison).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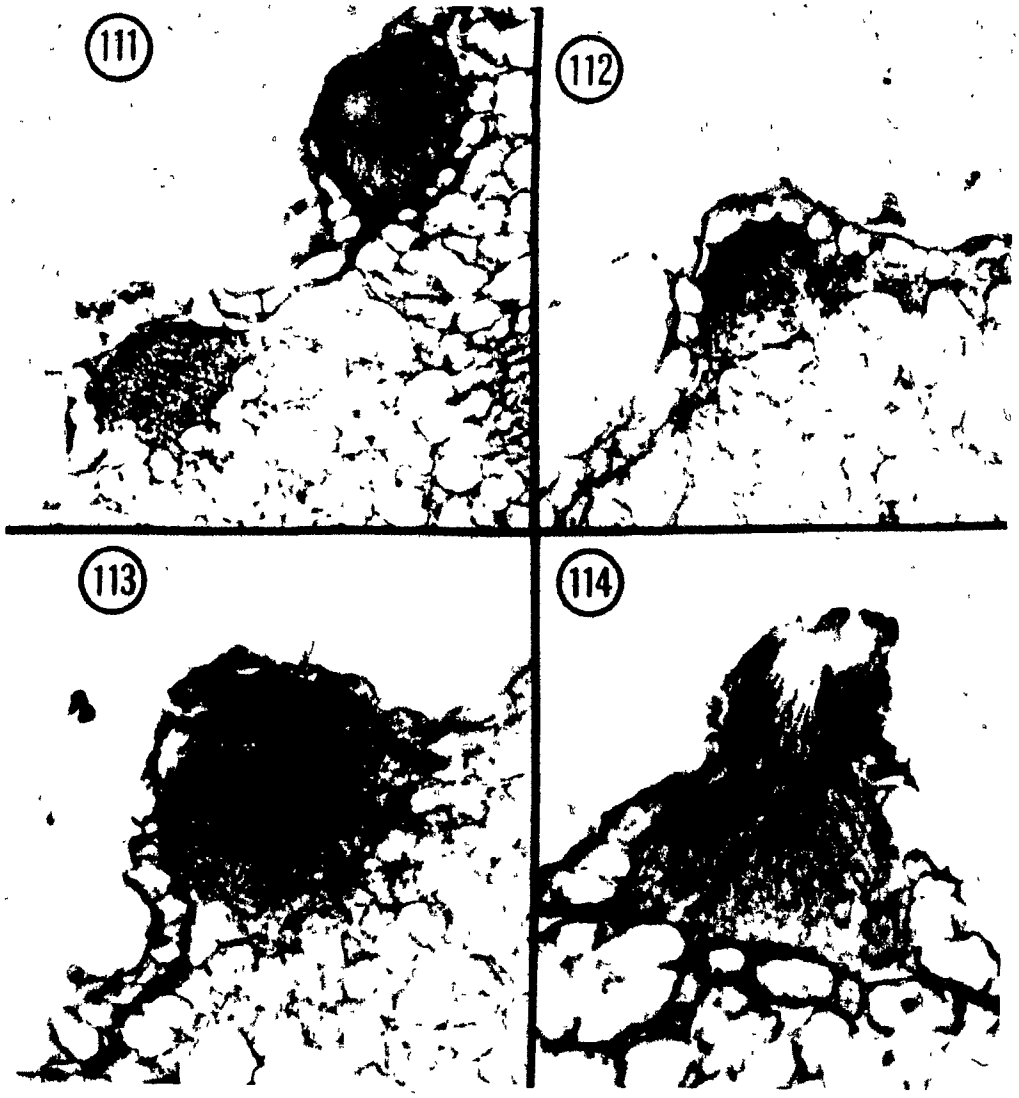
Figures 111 to 114. Pycnia. Note that the pycnia are intraepidermal (Wax sections stained with safranin-fast green)

Figure 111. Two pycnia close together on the leaf (X 150).

Figure 112. A typical pycnia totally covered by the epidermal layer (X 150).

Figure 113. A pycnia showing different staining characteristics of the structure. The darker area was stained green and the outer layer red (X 150).

Figure 114. A mature pycnia (or possibly uredinia) which appears to be on the verge of rupture. The epidermis has already begun to break open (X 150).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113
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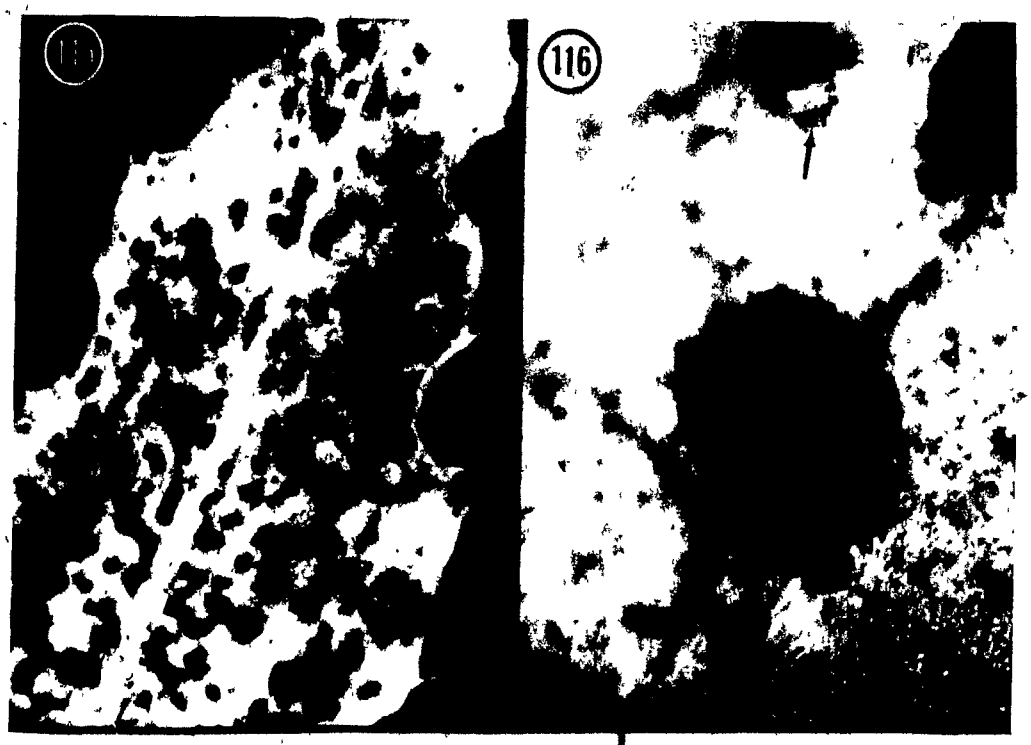
Figures 115 to 118. Uredinia and urediniospores.

Figure 115. The abaxial surface of a leaf showing the heavy systemic infection at the uredinia stage.

Figure 116. Uredinia on a cleared leaf. Note there are some teliospores present (arrowhead).

Figure 117. A uredinia in cross section showing the urediniospores and the ruptured epidermis. (Plastic section stained with toluidine blue) (X 170).

Figure 118. A young urediniospore still in the two cell stage. The lower cell will become the pedicel and the upper the spore (Plastic section-toluidine blue) (X 1750).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117


118

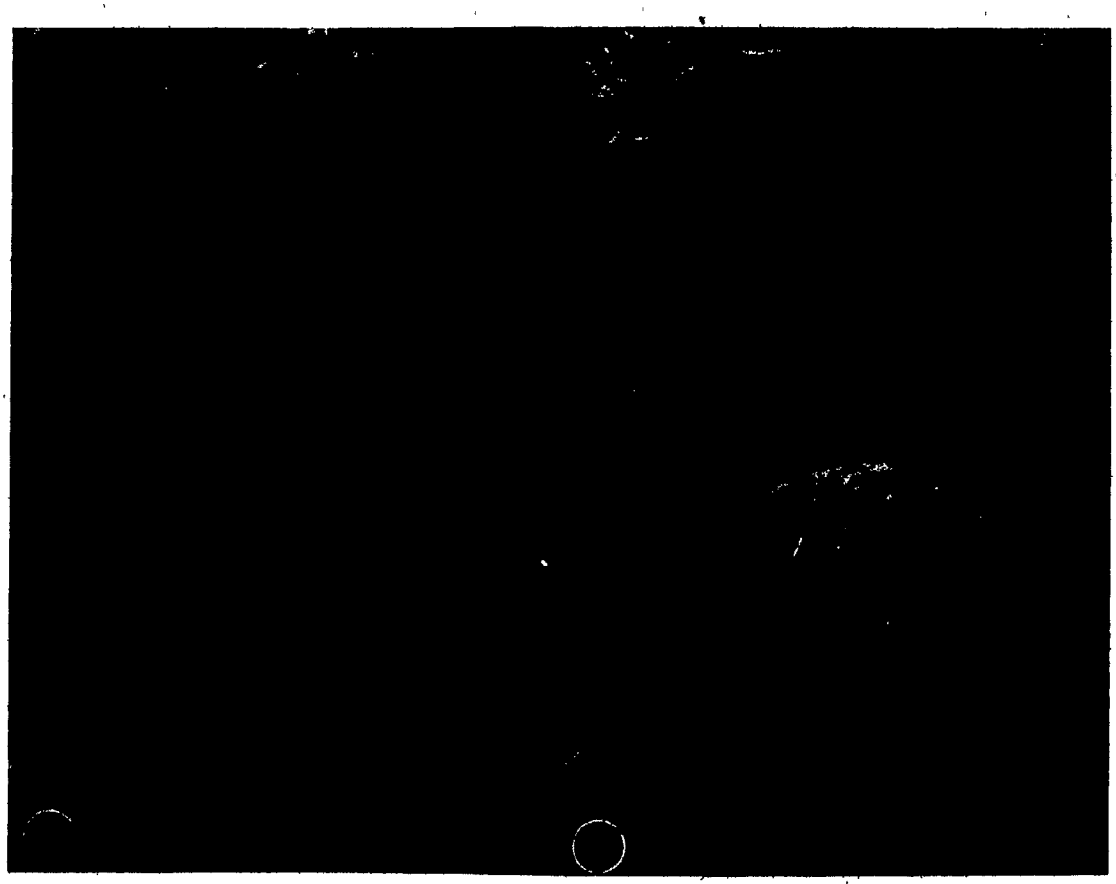
Scanning electron microscopy.

Figure 119. Pycnia. Note the complete covering of the epidermis.

Figure 120. Uredinia showing the ruptured epidermis and the large number of urediniospores

Figure 121. Closeup of the urediniospores in the uredinia.

Figure 122. The surface details of the urediniospores showing the minute echinulations.



Stem and Roots: The stems of rusted plants are usually hollow, fleshy and more succulent than those of nonrusted plants. Mycelial connections between secondarily rusted plants and systemically rusted ramets or sideshoots moved directly through the the cortex primarily from infected roots or stems (Figs. 123-126). The mycelia occurred most commonly just centrifugal from the vascular tissue and also sometimes in the phloem regions. Several haustoria were observed. The haustoria were of different shapes and sizes (Figs. 127-130). Mycelia were primarily intercellular and seemed to grow as a strand of related mycelia (Figs. 131-132).

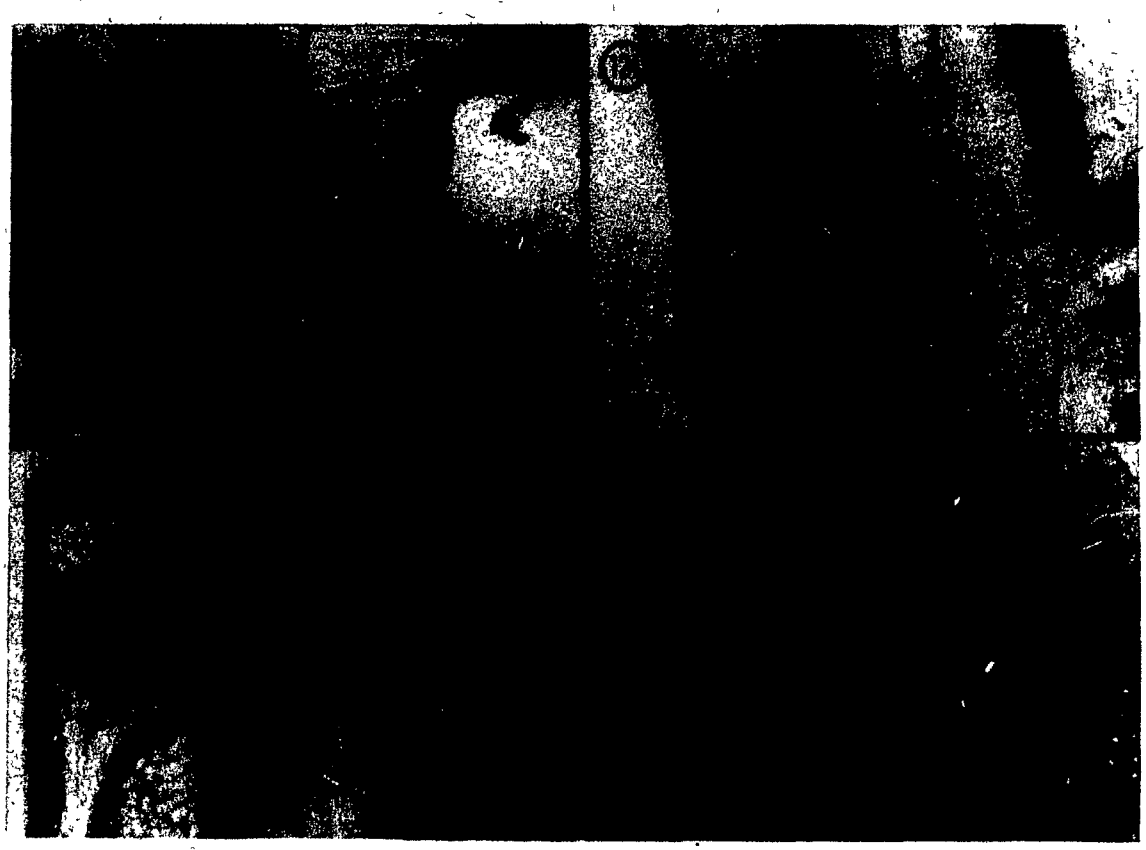
Mycelial growth in tissue after infection. All are cleared hand sections unstained.

Figure 123. Mycelia growing through the outer cortex and branching into sideshoots (X 50).

Figure 124. Section through the outer cortex showing a number of mycelial strands growing through the cortex (X 50).

Figure 125. A mycelial strand growing in the cortex near the vascular tissue (X 100).

Figurea 126. A closeup of a mycelia strand showing how the mycelia grow intercellularly and remain connected in a strand (X 300).



Hand sections of systemically rusted shoots.

Figures 127 to 129. Different shapes and sizes of haustoria.

Figure 127. A branched haustoria (X 600).

Figure 128. An unbranched haustoria (arrowhead)(X 280).

Figure 129. A haustoria with toes (arrowhead) (X 260).

Figure 130. A simple haustoria clearly showing the encapsulation (arrowhead) (X 600).

Figures 131 to 132. Intercellular growth of mycelia (arrowheads).

Figure 131. (X 220).

Figure 132. (X 600).


120
150

F.4. Discussion

Utilization of plant pathogens for biological weed control is a new and promising field and much more emphasis and hope is directed towards this technique. Field data on Puccinia punctiformis indicate that the naturally occurring field level is low, rarely eliminates a Canada thistle population and also oscillates and is difficult to predict over time (Appendix O). This suggests that some population management, probably by augmentation, would be required to cause greater damage. As early as 1916, Cockayne realized that, "to become an adequate means of control,... must increase the infection beyond that occurring naturally."

Field data showed that shoots which emerge systemically infected in the spring rarely survive the season and flower infrequently. These plants are easily distinguished from nonrusted plants by their unusual morphology and by their strong odour. The rapid height increase which occurred early in the season by rusted plants (Table 50) was previously observed by Buller (1950). The majority of the systemically infected plants have died by late June /early July and secondary infections begin to become apparent in late June/ early July. From these secondary infections, must come next year's systemically infected shoots. According to the most widely accepted theory of systemic infection production, the rust must grow out of these inoculated leaves, into the stem and finally down into the roots to infect the root buds (Buller and Brown 1941, Buller 1950, Menzies 1953). Buller and Brown (1941), Buller (1950) and Menzies (1953) accomplished the production of systemically infected ramets by inoculation by urediniospores, but Menzies (1953) says that the mycelia can be very slow growing. In this study, rusted ramets were also produced by

urediniospore inoculation.

In single artificial inoculation experiments, the greater the number of leaves infected, the greater the probability of systemic infection. With multiple inoculations, more frequent inoculations resulted in earlier, greater numbers and frequency of systemically infected shoots. Such multiple inoculations occur commonly in the field and may be one factor required for systemic infection. For the artificial inoculations, systemic shoots were found as early as 4 weeks.

In response to this rapidity, in nature, by mid September, some secondarily infected plants produce small ramets at the base of the stem (within 2 months of the first inoculation period). Buller (1950) also found this phenomenon. One problem that has been observed with the inoculations is that not all root buds emanating from inoculated plants become infected. The reason for this is unknown, but could be due to a phenomenon where only the portion of the root closely connected to the mycelia which grows down from the leaves would become infected. This could indicate a possible problem to the attempts to augment the infection rate, but may be solved by increased infection rates resulting in better mycelia infection.

The stress caused by Puccinia punctiformis on Canada thistle is sufficient to cause plant death, therefore making it the most effective of all the natural enemies covered in this study. It causes the stress by altering the physiology of the weed; there is increased respiration, and transpiration and a decrease in chlorophyll content. These combined would result in a decreased photosynthetic rate and the elevated respiration, indicates that the plant may be using more energy than it is manufacturing. Increased transpiration means increased water loss and indeed rusted thistles wilt more quickly than

nonrusted thistles under similar water stress (personal observation). The mechanism most probably includes the effects of elevated gibberellin levels. Bailiss and Wilson(1967) postulated that the symptoms of chlorosis and internode elongation are a result of increased gibberellin levels. This is emphasized by the production of the same symptoms in healthy thistles by exogenous application of GA_3. Bailiss and Wilson (1967) reported an increase in leaf area and width with gibberellin application, whereas in this study, the opposite was found. These symptoms of decreased leaf area and width correspond better to the actual disease symptoms. An increase in shoot weight was also found by Bailiss and Wilson (1967), but they owe this to the increase leaf size, whereas, it was more probably due to the increase in the stem volume. Other effects noted previously in the literature included, changes in development and differentiation of the vascular tissue and mechanical support tissues and lowered osmotic pressure (Menzies 1953).

Microscopal examinations confirm Menzies' (1953) observations that the mycelium travels down the outer cortex in the stem from inoculated leaves and that further down it migrates into the inner cortex and the phloem. From these mycelia, root buds can become infected. Microscopal studies of the pycnia and uredinia on leaves indicated that the pycnia are the type with flat hymenium and also are intraepidermal. Upon uredinia formation, the epidermis is ruptured and remains of the ruptured epidermis are visible around the edges of the sori. The urediniospores are spherical and decorated with spikey echinulations, a common pattern for the Puccinia rusts.

Inoculation with spores produces a pustule within approximately 10 days. Prior to pustule formation, the area around where the pustule will form (2mm+ diameter) becomes chlorotic. Sometimes the pustule, once it forms can reinfect

the area around it resulting in the star pattern. Later, as the leaf becomes chlorotic, the areas that were previously chlorotic tend to remain or even become greener than the surrounding tissue. This phenomenon is referred to as "green-island formation" and it is speculated that it may be due to changes in cytokinin levels.

The approach for the use of P. punctiformis for control of Canada thistle would require the augmentation of the field levels of spores, perhaps at younger growth stages and laboratory production of spores would therefore be required. Rusts are considered obligate parasites, which means that they normally require the presence of the host to grow. The establishment of artificial cultures of rust fungi (with and without the presence of tissue culture of the host material) have been reported (Hotson 1953, Harvey and Grasham 1969 and references therein, Harvey and Woo 1971). Some rusts have been cultured on artificial media; Gymnosporium juniperi-virginiana and Puccinia graminis f. sp. tritici. This may be possible at some point for P. punctiformis. Preliminary studies reported here indicated that at least P. puntiformis will grow in tissue culture. The tissue culture callus produced was well infected by mycelia and under some conditions plants could be produced by the callus. Further research into this aspect should be performed, as it may be important in the future of the biological control of Canada thistle. The greater variability of haustoria morphology and pustule like structures on the callus surface have been observed for other host-parasite tissue culture relationships (Harvey and Grasham 1969, Harvey and Woo 1971).

In summary, Puccinia punctiformis can be a very effective control when it occurs as a systemic infection, unfortunately, the rust seems to stabilize

itself at a relatively low level in the population. The reason for this remains unknown; does it kill its host too young, are the older leaves not as receptive to the infection and is the transmission of the rust limited by leaf death before migration of the rust from the leaves, is there a climatic factor suppressing the increase in the infection? Turner et al. (1981) postulate that variability among and within ecotypes of Canada thistle may be one factor limiting rust infection. If there is some way of augmenting the infection rate, P. punctiformis could be a very effective biological control organism against Canada thistle.

G. SUMMARY OF THE EFFECTS OF THE EXAMINED NATURAL ENEMIES

The aim of this study was to extensively examine the stress physiology of Canada thistle under the attack of various natural enemies. The natural enemies were chosen to include several modes of attack (gall formation, defoliation, stem/root boring, et cetera) to provide an opportunity to evaluate the effectiveness of each attack mode. In addition, the organisms were also chosen to embrace attack on all parts of the plants (seed head, stem, leaves root crown and an all-inclusive disease(systemic)).

The effect of each organism is briefly summarized in order of effectiveness:

(a)Orellia ruficauda-seed head fly

The effect of O. ruficauda on the plant as a whole is minimal. Only about 21% of the heads per plant were damaged and only about 20% of the seeds per head were attacked. While this may be important for decreasing dispersal into unpopulated regions, seed production is not very important in pasture situations (the main Canadian habitat of Canada thistle), where seeds rarely germinate or seedlings survive. Seeds damaged by O. ruficauda are not viable owing to extensive internal tissue damage.

(b)Cleonus piger- root crown weevil

C. piger can attack a major portion of the Canada thistle population. In

this study, a maximum of 27% of sampled plants contained C. piger larvae. The main field symptom of C. piger attack is wilting. Occasionally attacked plants die, but this occurredmainly on areas of poor soil (on gravel- Site 1 in this study). Microscopal examination indicated that the reason C. piger can cause wilt is due to damage to the xylem tissue. The larvae remove a large portion of the vascular tissue in the root crown region. However, the tissue can regenerate and in the simulation experiment severed vascular tissue reconnected. The necrotic zone bordering on the larval cavity consisted of many dead cells and dark deposits between the cells. Due to the apparent resistance of Canada thistle to vascular tissue damage, C. piger causes only a minor stress by itself. C. piger, being a large insect, also seems to be found in the larger plants of the population, decreasing, by this habit, its capability of causing plant death.

(c) Cassida rubiginosa- Defoliator

In the field, C. rubiginosa causes minimal damage to Canada thistle plants, and as Cleonus piger seems attracted to large vigourous plants. Simulation experiments indicated that maximum stress occurs on young plants and at high defoliation levels (50% leaf removal or more) and that repeated defoliation is more detrimental than a single defoliation. The limitation that causes C. rubiginosa to be a poor natural enemy is its lack of synchrony with the phenology of Canada thistle. The majority of C. rubiginosa feeding occurs at a point where Canada thistle plants are nearing mature height and have initiated flowering rather than at a younger, more susceptible stage.

(d) Urophora cardui- stem gall fly

U. cardui gall formation caused more stress if the galls occurred on the

mainshoot of the plant and if it occurred on young plants. Radioactive tracer studies (^{14}C-fructose) and elevated sugar content indicate that the galls were weak physiological sinks when young, but as they aged, they accumulated very little radioactive precursor. Microscopal studies showed that the nutritive tissue was composed of large, noncytoplasmic cells, probably not an extensive physiological stress. Results also confirm that the greater the number of larvae (more nutritive tissue), the greater the stress of the plants. In the field, U. cardui shares the same problem as Cassida rubiginosa; it is poorly synchronized with its host. The main ovipositional period occurs in mid to late June when the plant height is about 80% of mature height and about 50% of the plants have initiated flowering. Since the flies do not lay their eggs in flower buds, they lay them instead in the sideshoots. Sideshoot galls cause much less stress than mainshoot galls. If the flies were synchronized with younger Canada thistle stages, stress effects could most probably be elevated.

(e) Puccinia punctiformis- systemic rust

Of the five natural enemies, P. punctiformis is the most effective. Plants which are systemically infected die prematurely, rarely flower and generally the roots will die after a time. In the field, however, P. punctiformis attack is maintained at a low level in the population. In order to use P. punctiformis as a control agent, some technique to augment the disease (cause an epidemic) must be employed. Examination of artificial inoculations indicated that repeated (multiple) inoculations resulted in earlier and higher numbers of rusted shoots than single inoculations. Preliminary tissue culture indicated that there may be some future in attempting to grow P. punctiformis in callus culture plants for the production of spores.

In nature, these organisms sometimes occur together on a plant. In combination, they no doubt cause more stress and each one of them fills a niche (no overlap) and causes some stress to the plant population. Without them, Canada thistle may be a greater problem. Indeed, in the western provinces, where Cassida rubiginosa and Cleonus piger are uncommon and Puccinia punctiformis is rare, (Peschken unpub.) Canada thistle is a greater problem. This may indicate that the combined stress (although minor individually) of these organisms is probably affecting Canada thistle population dynamics.

III. MODELLING THE EFFECTS OF NATURAL ENEMIES ON THE POPULATION DYNAMICS OF CANADA THISTLE.

1. Introduction

The use of mathematical models, especially the Leslie matrix model (Leslie 1945) has proven useful for demographic studies. Leslie matrices simulate the growth of populations from rates of survival and fecundity. Applications of such models have been used often by zoologists for examination of animal population dynamics (Jeffers 1978 and references therein). Usher (1966, 1976) extended the applications of the Leslie matrix to tree stand management. Subsequent plant applications followed (Sarukhan and Gadgil 1974, Mortimer et al. 1979, 1980, McMahon and Mortimer 1980, Maillette 1982, Mortimer 1983). Life cycle diagrams (a type of pre-matrix model which translate easily into matrix form) were first used for plant applications by Sarukhan and Gadgil (1974) and Sagar and Mortimer (1976). The life cycle diagrams presented by Sagar and Mortimer (1976) were the first used to describe weed populations. The natural extensions of these into matrix form are found in Mortimer et al.(1978, 1980) and McMahon and Mortimer (1980). They simulated population dynamics of two weed species, Avena fatua L. and Poa annua and used them for the prediction of the use of a herbicide as a control measure (Mortimer 1978, Mortimer et al. 1980). In addition, they simulated the population dynamics of a perennial weed (Agropyron repens).

and used the model to evaluate management practices, cropping procedures and the cost effectiveness of eradication versus containment of weed populations using herbicides (Mortimer et al. 1980, McMahon and Mortimer 1980).

2. The Model

This model and approach were initially developed independently and later found to be similar to that of Mortimer and his colleagues from which ideas and some modifications were drawn. A prototype of the model was presented as a paper in 1981 (Forsyth and Watson 1982). The life cycle of Cirsium arvense is represented in diagrammatic form (Fig. 133) (cum Sagar and Mortimer). Each arrowhead represents a transition from one stage to another. An expanded diagram (Fig. 134) results if this life cycle diagram is extrapolated over the different seasons of the year (after Sagar and Mortimer 1976). The transition arrows can then be pinpointed to the time of year in which they occur.

Each of the transition periods can be represented as a Transition Matrix (Fig. 135), where the arrows on Figure 134 become transition proportions and fecundity values. An initial population matrix can be used to generate each successive generation which in turn is used to generate the following one by matrix multiplication. The population matrices are column matrices with 5 rows and the transition matrices are square (5X5). The rows in the population matrices and both the rows and the columns in the transition matrices (from top and to the right represent the various life stages in this order: seed, seedling, vegetative shoot, flowering shoot and root bud.

Notation used below (m_{ij}) is standard matrix notation where m= name of the matrix in figure 135, i=row and j=column. The values are all based per m^2. The initial population vector A' values will be referred to as a'_{ij}.

Figure 133. Diagrammatic representation of Canada thistle life cycle.

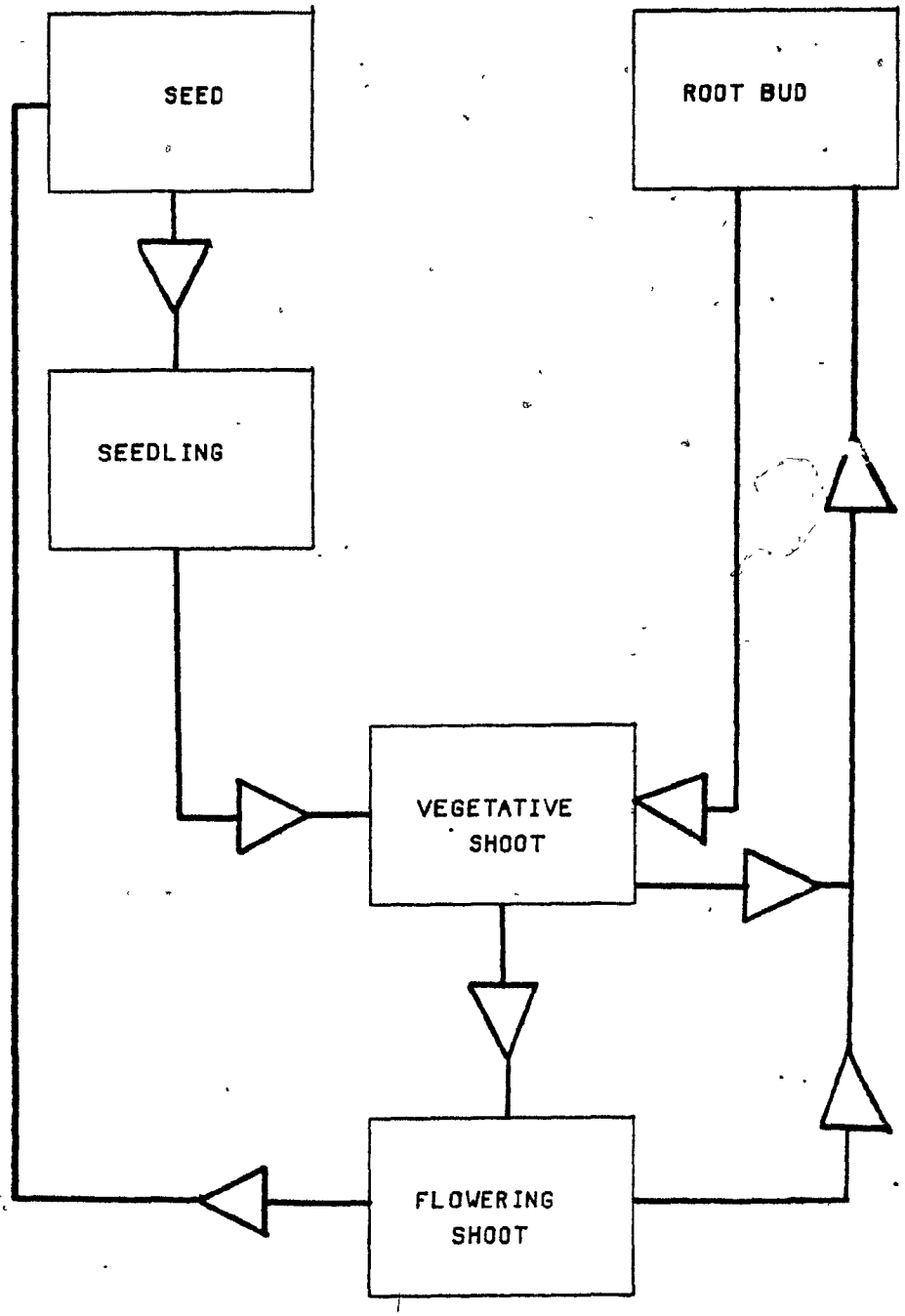


Figure 133

Figure 134. Expanded diagrammatic representation of the Canada thistle life cycle to include the seasons. Each of the transition regions can be represented by a transition matrix (Fig. 135) in which case each arrow becomes either a probability or a fecundity.

vs=vegetative shoot, fs=flowering shoot, ES= early spring. LS= late spring, S=summer, LSM= late summer, EA=early autumn, OW=overwinter.

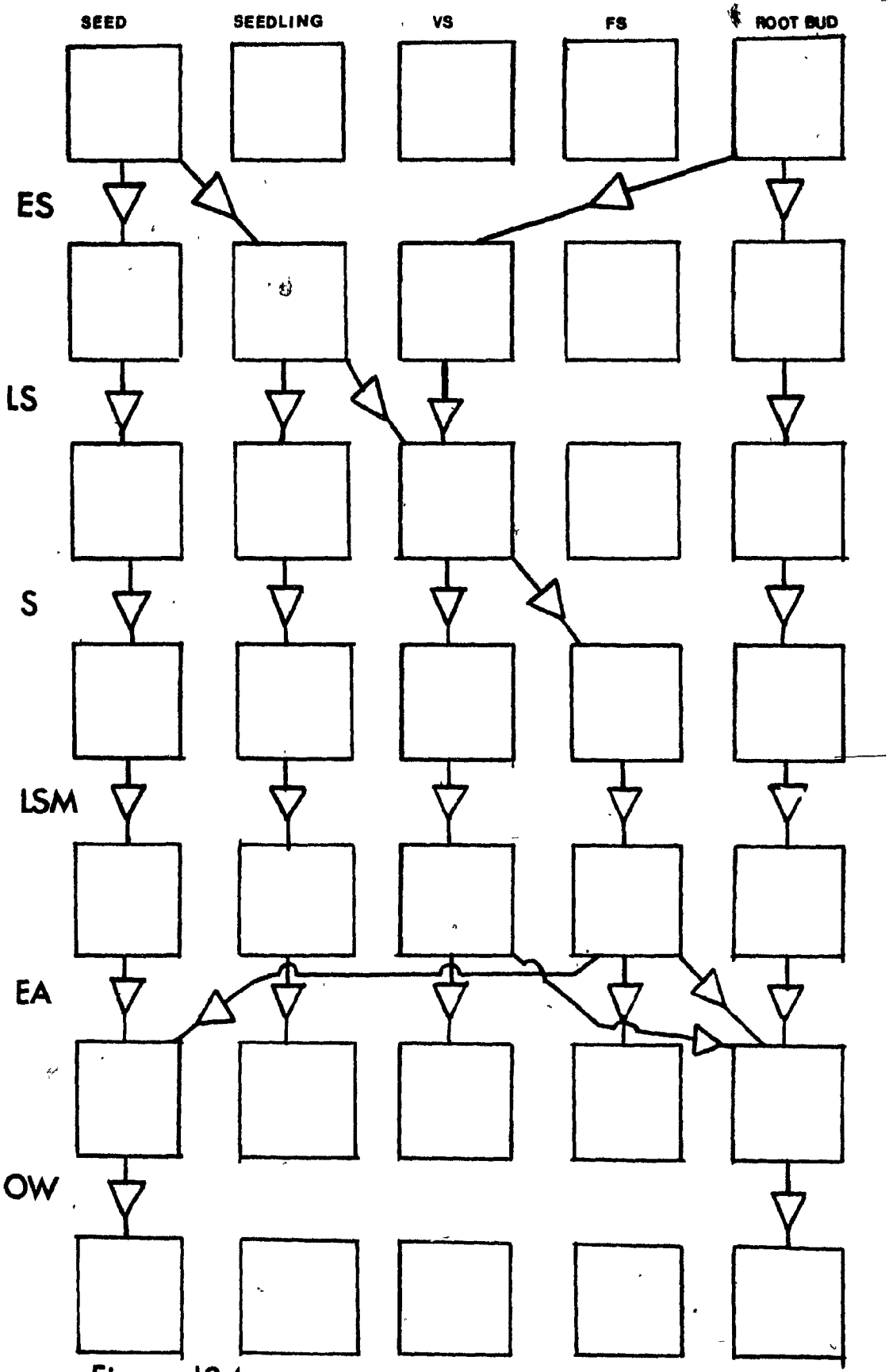


Figure 134

Transition Matrices

A

	sd	sl	vs	fs	rb
sd	0.9	0	0	0	0
sl	0.03	0	0	0	0
vs	0	0	0	0	0.15
fs	0	0	0	0	0
rb	0	0	0	0	0.8

Initial Population Vector A'

Early spring

38000
0
0
0
250

B

1	0	0	0	0
0	0.001	0	0	0
0	0.0005	1	0	0
0	0	0	0	0
0	0	0	0	1

Late spring

C

1	0	0	0	0
0	1	0	0	0
0	0	0.25	0	0
0	0	0.75	0	0
0	0	0	0	1

Summer

D

1	0	0	0	0
0	1	0	0	0
0	0	1	0	0
0	0	0	0.9	0
0	0	0	0	1

Late summer

E

1	0	0	760	0
0	1	0	0	0
0	0	1	0	0
0	0	0	1	0
0	0	5	10	1

Early Fall

F

0.9	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0.9

Overwintering

sd=seed, sl=seedling, vs= vegetative shoot,
fs=flowering shoot, rb= root bud

Figure 135. Transition Matrices for Matrix Model

The initial seed population, a'_{11}, is set at 38,000 (from a value extrapolated from Amor and Harris 1974). The number of root buds, a'_{12}, was set at a value of 250 m^{-2} (extrapolated from field studies- Appendix P and Bakker 1960).

The transition matrice values were chosen either from the literature or to correlate with field data collected, with the aim of production of a final winter population vector similar to the initial vector(over one season), only slightly augmented. Note that the model is simulating a pasture situation.

Transition Values:

a_{11} seed to seed in early spring- 90% of seed remains viable

a_{21} seed to seedling in early spring. Only 3% (a high estimate) of seeds germinate to produce seedlings

a_{35} root bud to shoot; about 15% emerge giving an estimate of about 38 shoots m^{-2}.

a_{55} root bud to root bud, unemerged and still surviving- 80%

b_{11} seed to seed- 100%

b_{22} seedling to seedling- a token figure of 0.001 probability meaning that seedlings rarely survive

b_{32} seedling to vegetative shoot- another token value of 0.0005 probability representing very few seedlings that become vegetative shoots

b_{33} vegetative shoots to vegetative shoots- number of shoots formed from seedlings that survive - 100%

b_{55} root bud to root bud- still remaining in soil 100%

c_{11}, c_{22} and c_{55} maintenance of seed, seedling and root bud number

c_{33} vegetative to vegetative- 25% remain vegetative i.e. do not flower

c_{43} 75% become flowering shoots

d_{11}, d_{22}, d_{33} and d_{55} - maintenance of previous levels of seeds, seedlings, vegetative shoots and root buds

d_{44} 90% of flowering shoots survive

e_{11}, e_{22}, e_{33}, e_{44} and e_{55} - maintenance of previous levels of seeds, seedlings, vegetative and flowering shoots, and root buds.

e_{14} flowering shoot fecundity of seed 760 seeds/shoot (a value reported by Hay 1934 of 1530 seeds/plant divided by 2 to account for possibility that one half of the population is male)

e_{53} root bud fecundity from vegetative shoots- 5 per shoot

e_{54} root bud fecundity from flowering shoots- 10 per shoot.

e_{53} and e_{54} are estimates and further work on these numbers should be performed. Some pot experiments indicated that flowering plants may in fact produce fewer root buds because of energy and resource reallocation to flower production, but until further knowledge is

collected the values will be left intact.

f_{11} overwintering of seeds- 90% survive

f_{55} overwintering of root buds- 90% survive

To run the data, a computer program (written by fellow graduate student D. Cloutier in MBASIC and translated to SBASIC by the author (Appendix Q)) was used to determine the matrix values over 15 years. The program allowed limits to be set and the following limits were set: 100,000 for seeds, 300 for seedlings, 500 for vegetative shoots, 300 for flowering shoots and 1000 for root buds.

Once the model was established, it was used to simulate and predict the effects of the insects and the pathogen examined on the population dynamics of Canada thistle.

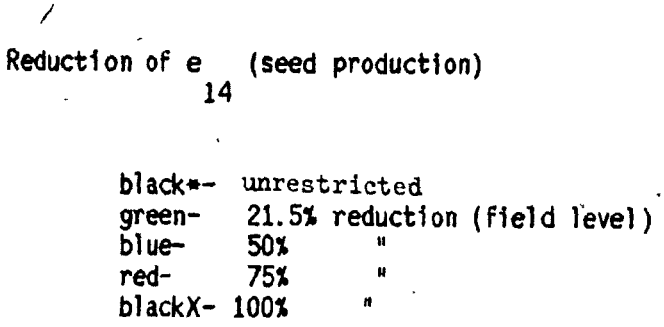
3. Applications of the Model

The results from the model are represented in graphical form (Figs. 136-146) for seed and root bud production, the overwintering forms of Canada thistle. Effects of each of the insects were simulated separately at different levels to see the effect on the population dynamics of the weed and then combined . Each plot also contains a curve which represents the case where there are no restrictions (black- symbol X).

(a) Orellia ruficauda:

The effects of seed production only were examined for this insect (Fig. 136). The green line represents the field level of attack (21.5) where e_{14} was changed to 570. This level has little depressing effect on

Figure 136. Modelling of the effect of Orellia ruficauda damage on seed production.



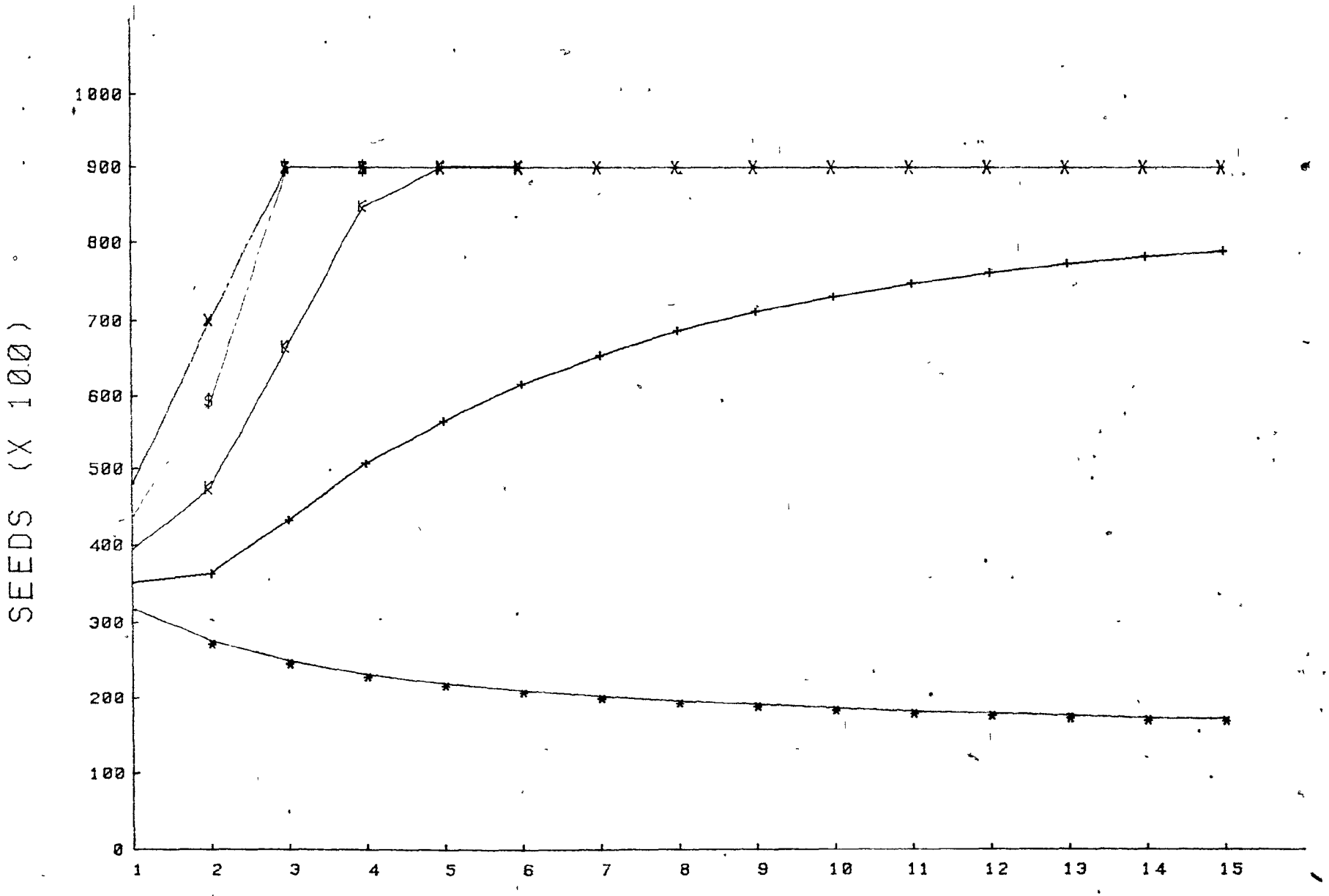


FIGURE 136. YEARS

the population; it reaches the carrying capacity within the same time period as the unrestricted case. The blue curve represents a 50% predation (e_{14} =380), which results in a delay in the population reaching the carrying capacity of 2 years, whereas 75% (e_{14} =160), red curve, does not reach the carrying capacity but is increasing. Only 95% reduction of seed production (e_{14} =38, black curve, * symbol) results in a gradual decrease in seed production.

(b) Cleonus piger:

C. piger effects are most appropriately simulated by altering the transition value from vegetative shoots to flowering shoots (c_{43}). The effects on seed production are given in figure 137. The field value (about 27%) of C. piger attack is represented by the green curve (assumption that they kill all they inhabit; high but ideal)(c_{43} =0.56). Further reductions (c_{43} =0.40, red curve and c_{43} =0.3, blue curve) delayed the tendency to approach the carrying capacity, but did not eliminate it. Only a reduction to 10% transition (c_{43} =0.1, black curve, * symbol) results in a gradual reduction of the seed population. The effects on root bud production follow similar trends i.e. reduction of c_{43} to 0.56, 0.4 amd 0.3 do not eliminate the attainment of the carrying capacity, whereas c_{43} =0.1 results in a very slow linear climb which has reached only one third (300) of the carrying capacity after 15 years (Fig.138).

(c) Cassida rubiginosa:

C. rubiginosa can potentially reduce the transition from vegetative to flowering shoots and survival of the vegetative shoots -c_{33} and c_{43}

Figure 137. Modelling of the effect of Cleonus piger damage on seed production.

Modification of c_{43} (vegetative to flowering shoots)

Reduction:		
Black*-	0%	
green-	27%	field level
red-	50%	
blue-	60%	
blackX-	87%	

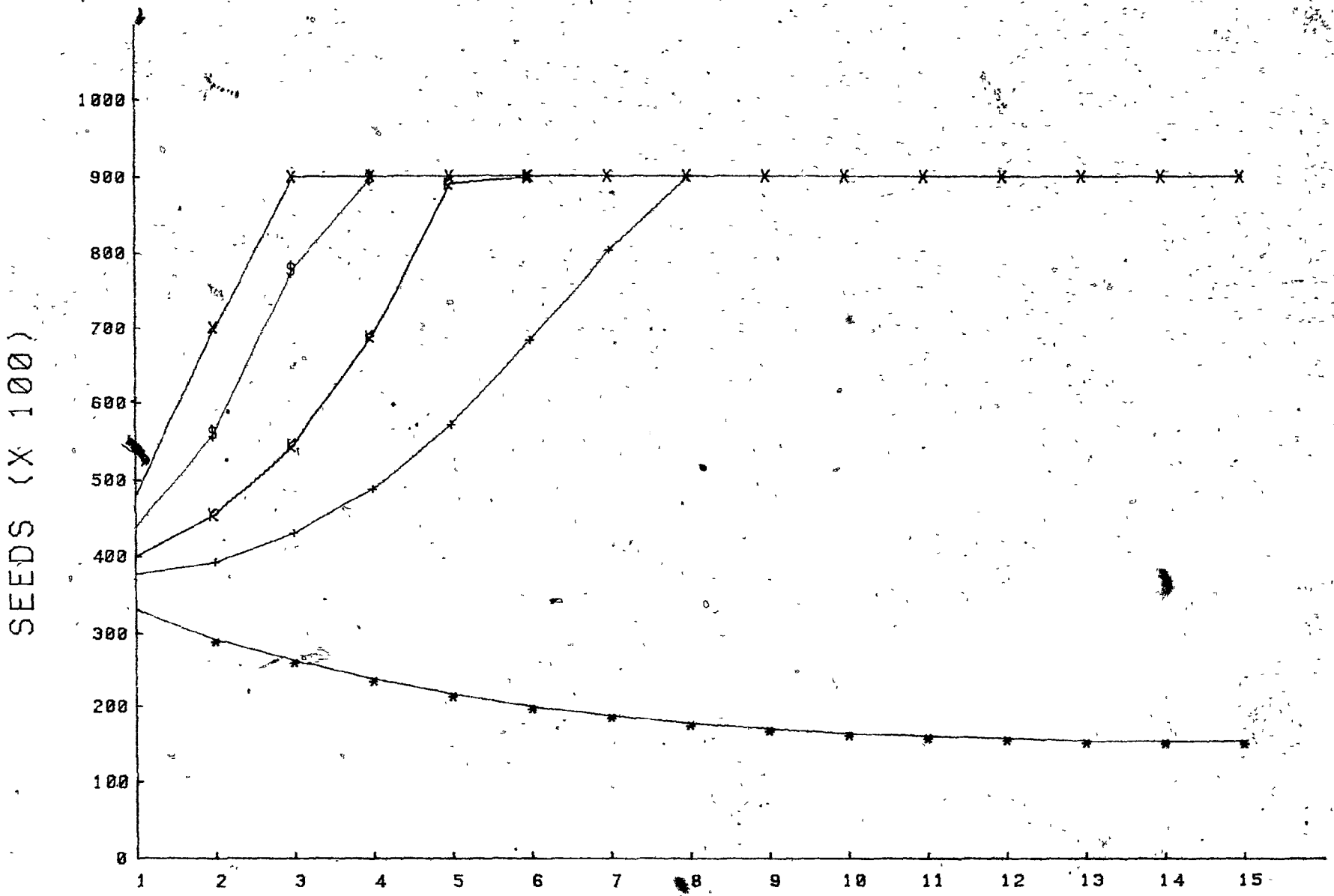


FIGURE 137. YEARS

Figure 138. Modelling of effect of Cleonus piger damage on root bud production.

Modification of vegetative to flowering shoots.

Reductions:

black*-	none
green-	27% (field level)
red-	50%
blue-	60%
blackX-	87%

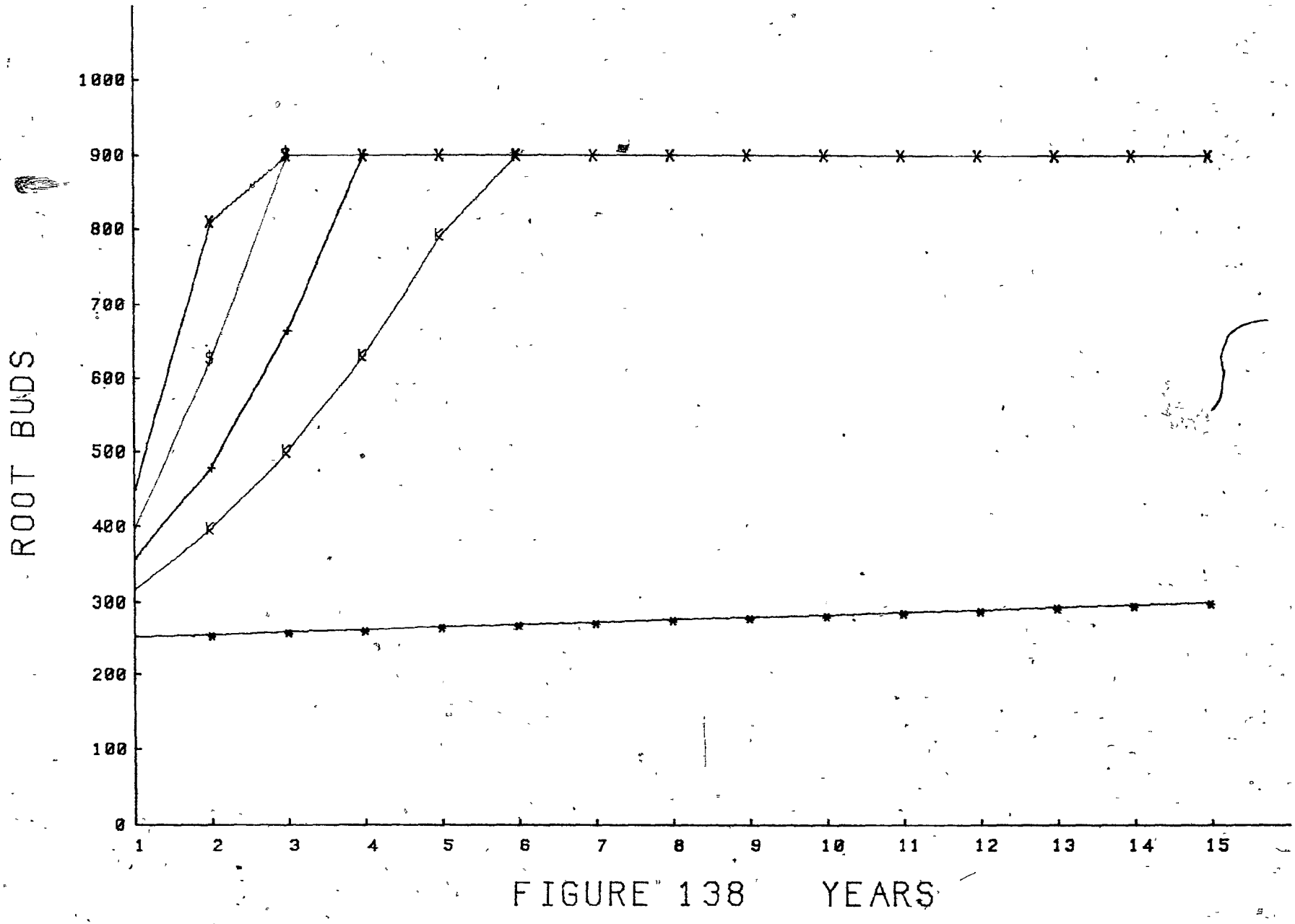

ROOT BUDS
1000
900
800
700
600
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
YEARS

FIGURE 138

and the production of seeds and root buds- e_{14}, e_{53} and e_{54}.

The effect on seed production of modified values is given in figure 139. If the seed and root reduction only are implemented (e_{14}=570, e_{53}=4 and e_{54}=8) the blue curve results. If reduction to the vegetative survival and transition to flowering shoots are included (c_{33}=0.1 and c_{43}=0.4 based on a hypothetical and high defoliation level), the green curve results. Although it does not result in a reduction in the population, it does delay the increase. If the root bud and seed production are further reduced (e_{14}=190, e_{53}=3 and e_{54}=6) the red curve results (with c_{33} and c_{43} returned to normal values) and the population has taken on a sigmoid shape and takes a long time to reach the carrying capacity. If c_{33} is again reduced to 0.1 and c_{43} to 0.4 and combined with the latter seed and root bud fecundities, the black curve (* symbol) results, an effective reduction. The effects of the same reductions (corresponding reduction to curve colours) for root bud production are illustrated in figure 140. Only the final combination (black- * symbols) has any real effect on root bud production.

(d) Urophora cardui:

U. cardui can potentially affect seed fecundity (e_{14}) and root bud fecundity (e_{53} and e_{54}). The results for seed production are given in figure 141. The green curve represents a reduction in e_{14} to 250 (33%), e_{53} to 4 and e_{54} to 8. The rest of the curves all have the seed fecundity set at 95% (e_{14}=38). All of these curves result in seed production reductions. Figure 142 represents the simulations for root bud production. Reductions in fecundity of e_{53}=4 and e_{54}=8, green curve;

Figure 139. Modelling of the effect of Cassida rubiginosa damage on seed production.

Modification of vegetative to vegetative and flowering and seed root bud fecundity.

Reductions:

	c 33	c 43	e 14	e 53	e 54
blackX-	-	-	-	-	-
blue	-	-	21.5%	20%	20%
green	60%	45%	"	"	"
red	-	-	75%	40%	40%
black*	60%	45%	"	"	"

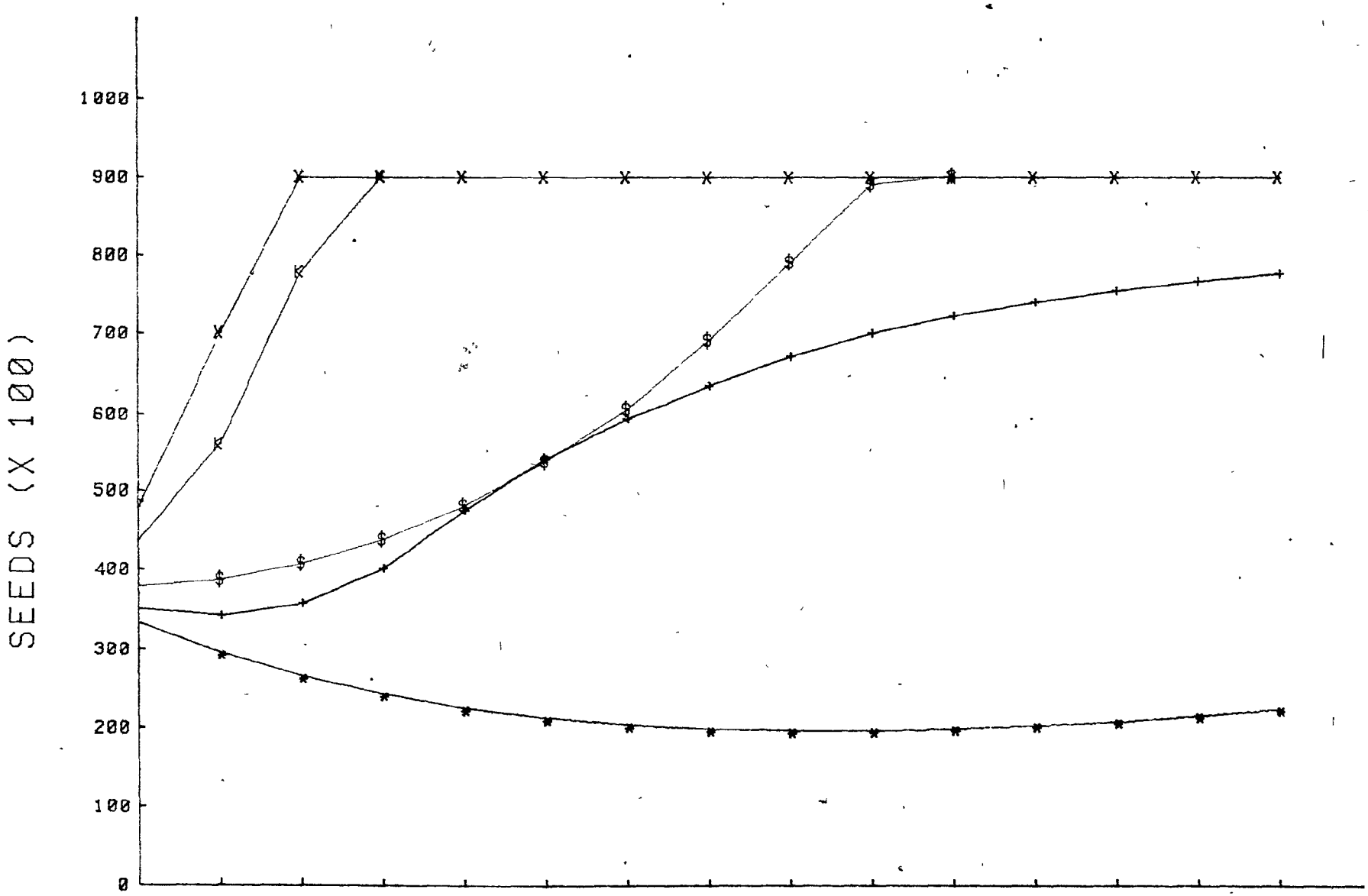


FIGURE 139. - YEARS

Figure 140. Modelling of the effect of Cassida rubiginosa on root bud production.
Modification of vegetative to vegetative and flowering shoots and of seed and root bud fecundity.

Reductions:

	c_{33}	c_{43}	e_{14}	e_{53}	e_{54}
blackX	-	-	-	-	-
blue	-	-	21.5%	20%	20%
green	60%	45%	"	"	"
red	-	-	75%	40%	40%
black*	60%	45%	"	"	"

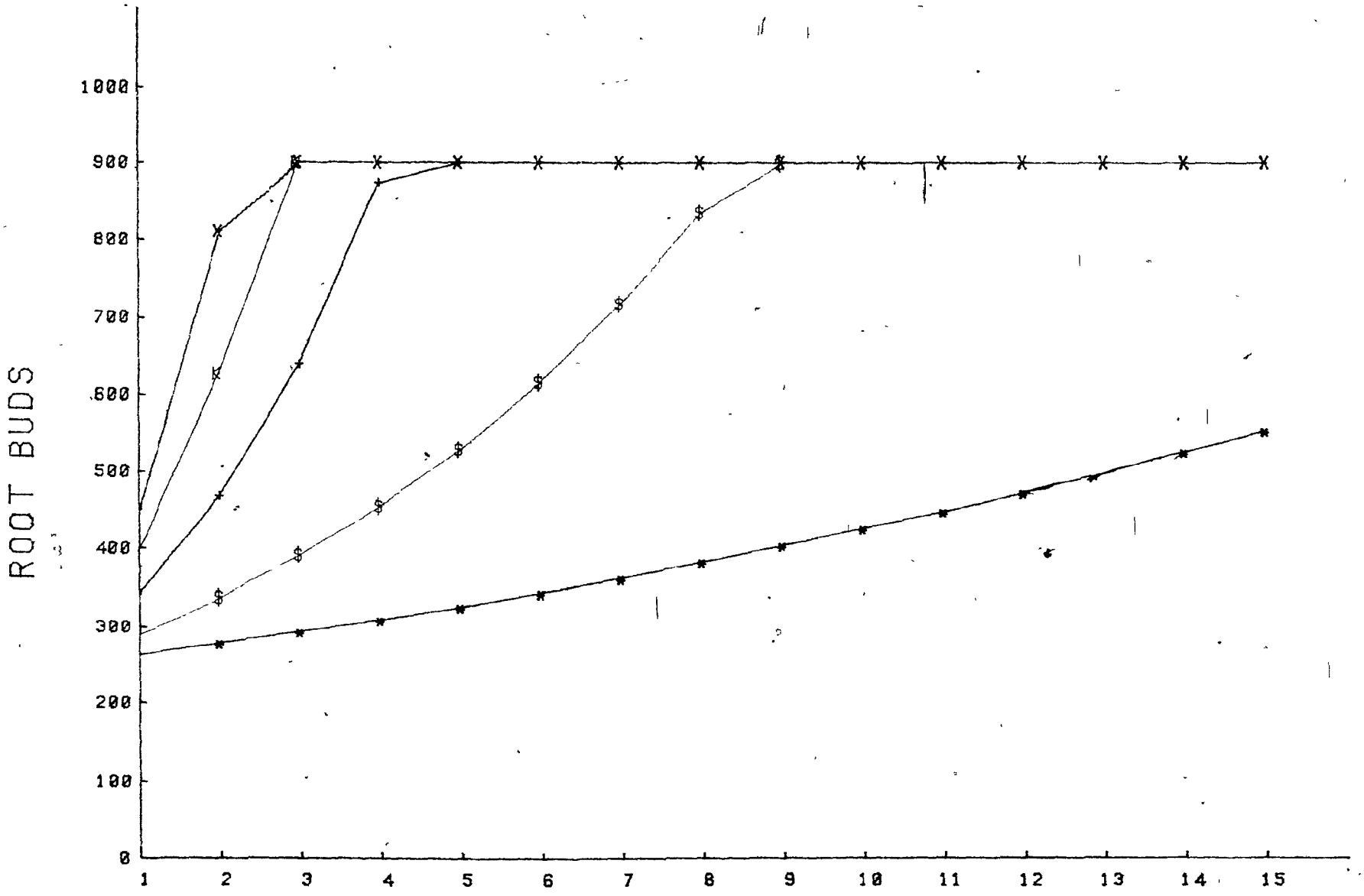

ROOT BUDS
1000
900
800
700
600
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
YEARS

FIGURE 140.

Figure 141. Modelling of the effect of Urophora cardui damage on seed production.

Modification of seed and root bud fecundities.

Reductions:

	e 14	e 53	e 54
blackX	-	-	-
green	33%	20%	20%
blue	95%	40%	40%
red	"	60%	60%
black*	"	80%	80%

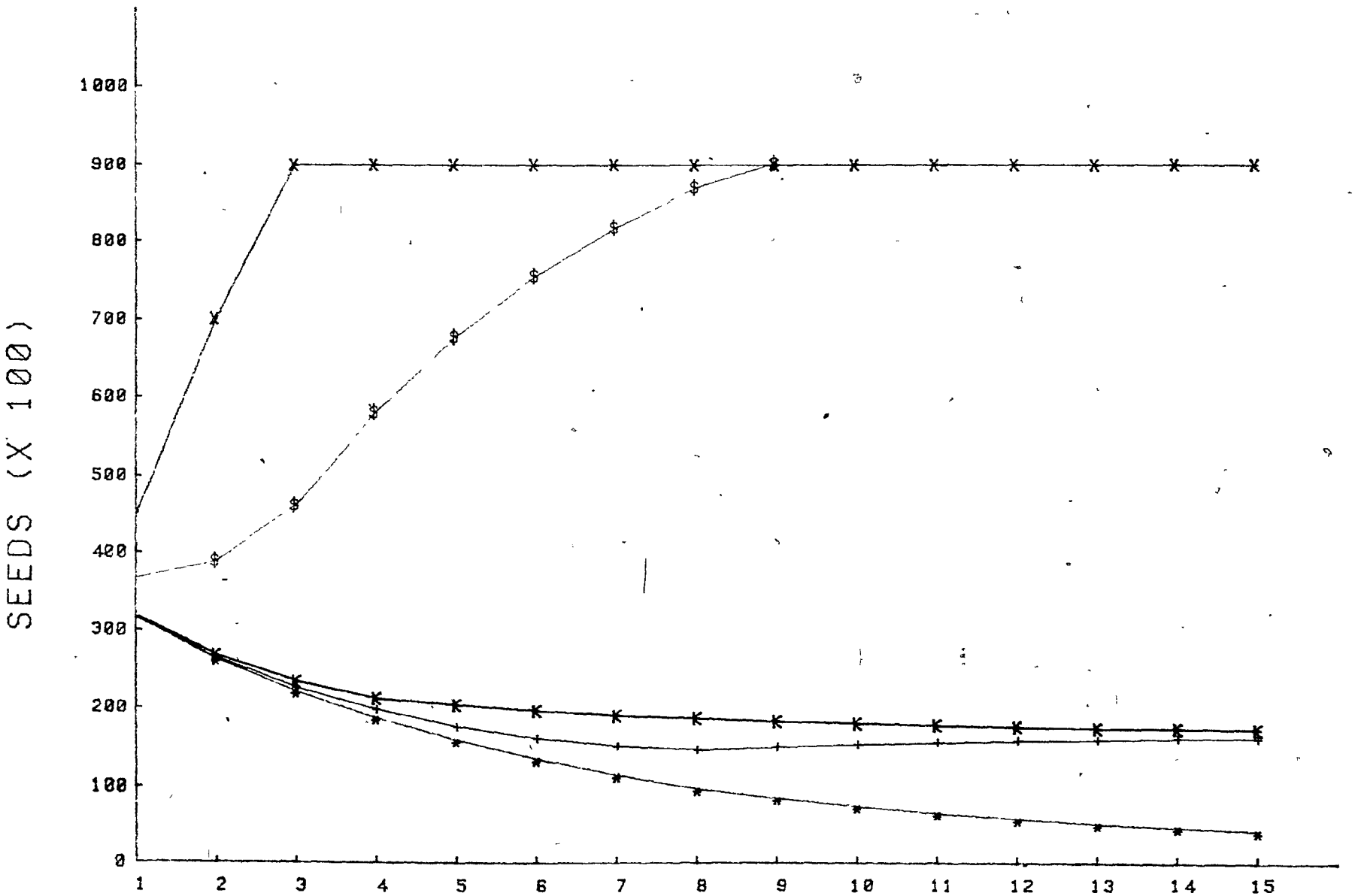


FIGURE 141. YEARS

Figure 142. Modelling of the effect of Urophora cardui damage on root bud production.

Modification of seed and root bud fecundities.

Reductions:

	e_{14}	e_{53}	e_{54}
blackX	-	-	-
green	33%	20%	20%
blue	95%	40%	40%
red	"	60%	60%
black*	"	80%	80%

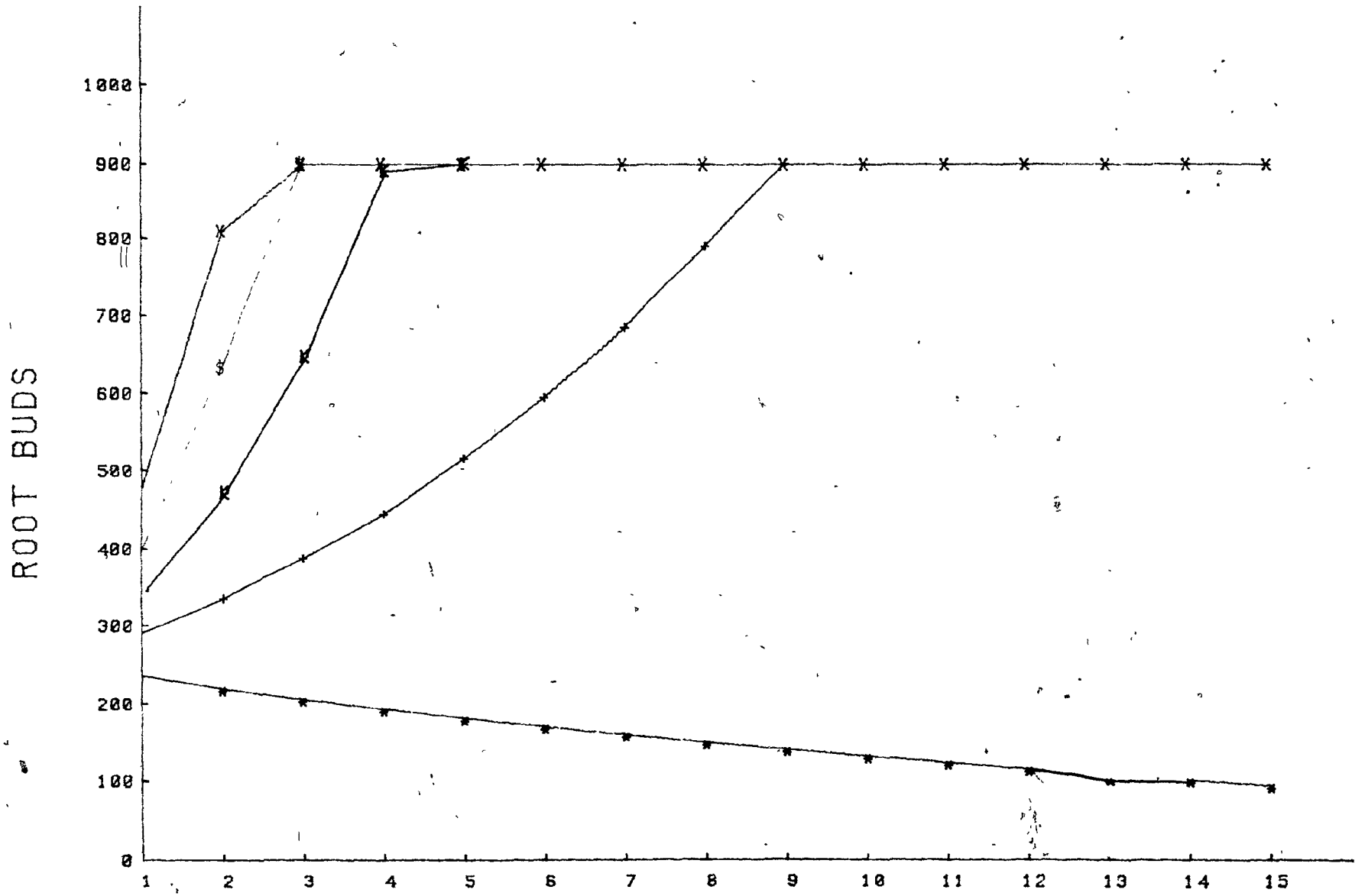

ROOT BUDS
1000
900
800
700
600
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
YEARS

FIGURE 142.

e_{53} =3 and e_{54} =6, blue curve; and e_{53} =2 and e_{54} =4, red curve result in delays of reaching the carrying capacity but do not decrease the ultimate value. Reductions of e_{53} to 1 and e_{54} to 2 (black curve, * symbol) are required before root bud production is checked.

(e) Puccinia punctiformis:

P. punctiformis affects primarily the survival of the vegetative shoots (c_{33}) and the transition to the flowering stage (c_{43}). Simulation of the effect on seed production is given in figure 143. The field level of P. punctiformis is simulated by the green curve (c_{33} =0.2, c_{43} =0.6). Further reductions of c_{33} =0.15, c_{43} =0.45 (blue curve, K symbol) and c_{33} =0.1, c_{43} =0.3 (red curve) result in delays in reaching the carrying capacity but no reduction. Changes of values to c_{33} =0.05, c_{43} =0.15 (black-* symbol) and c_{33} =0.01, c_{43} =0.04 (blue , X symbol) result in reduction. The root bud production (figure 144) has very similar responses as does the seed production.

(f) Combination

Combinations of all the most stringent field reductions in values of all the natural enemies were inputted to obtain the green curves of figures 145 and 146 (e_{53} =4, e_{54} =8, e_{14} =250, e_{33} =0.1, e_{43} =0.4). Seed production rate was reduced but still increasing and root bud production is also reduced but still managed to reach the carrying capacity. If the root bud production and transition to flowering are further reduced (e_{53} =3, e_{54} =6 and c_{43} =0.3) the blue curve results and root bud and seed production are both reduced. If the values are further decreased (assuming very high levels of the rust- c_{33} =0.01, c_{43} =0.04), seed

Figure 143. Modelling of the effect of Puccinia punctiformis damage on seed production.

Modification of vegetative to vegetative and flowering shoots.

Reduction:

	C 33	C 43
black*	-	-
green	60%	40%
blueK	70%	55%
red	80%	70%
black*	90%	85%
blueX	98%	96%

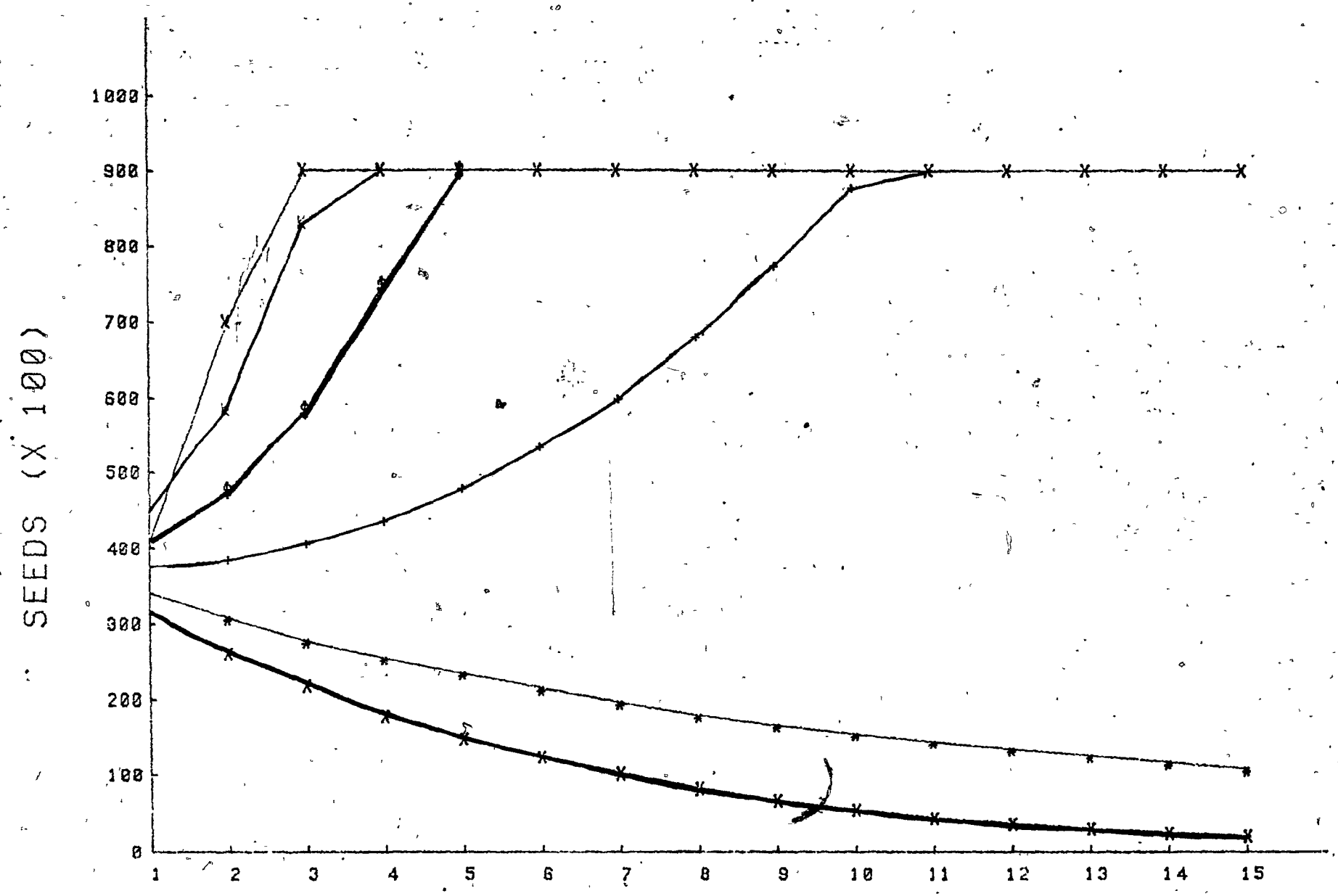

SEEDS (X 100)
1000
900
800
700
600
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
YEARS

FIGURE 143.

Figure 144. Modelling of the effect of Puccinia punctiformis damage on root bud production. Modification of vegetative to vegetative and flowering shoots.

Reductions:

	C 33	C 43
blackX	-	-
green	60%	40%
blueK	70%	55%
red	80%	70%
black*	90%	85%
blueX	98%	96%

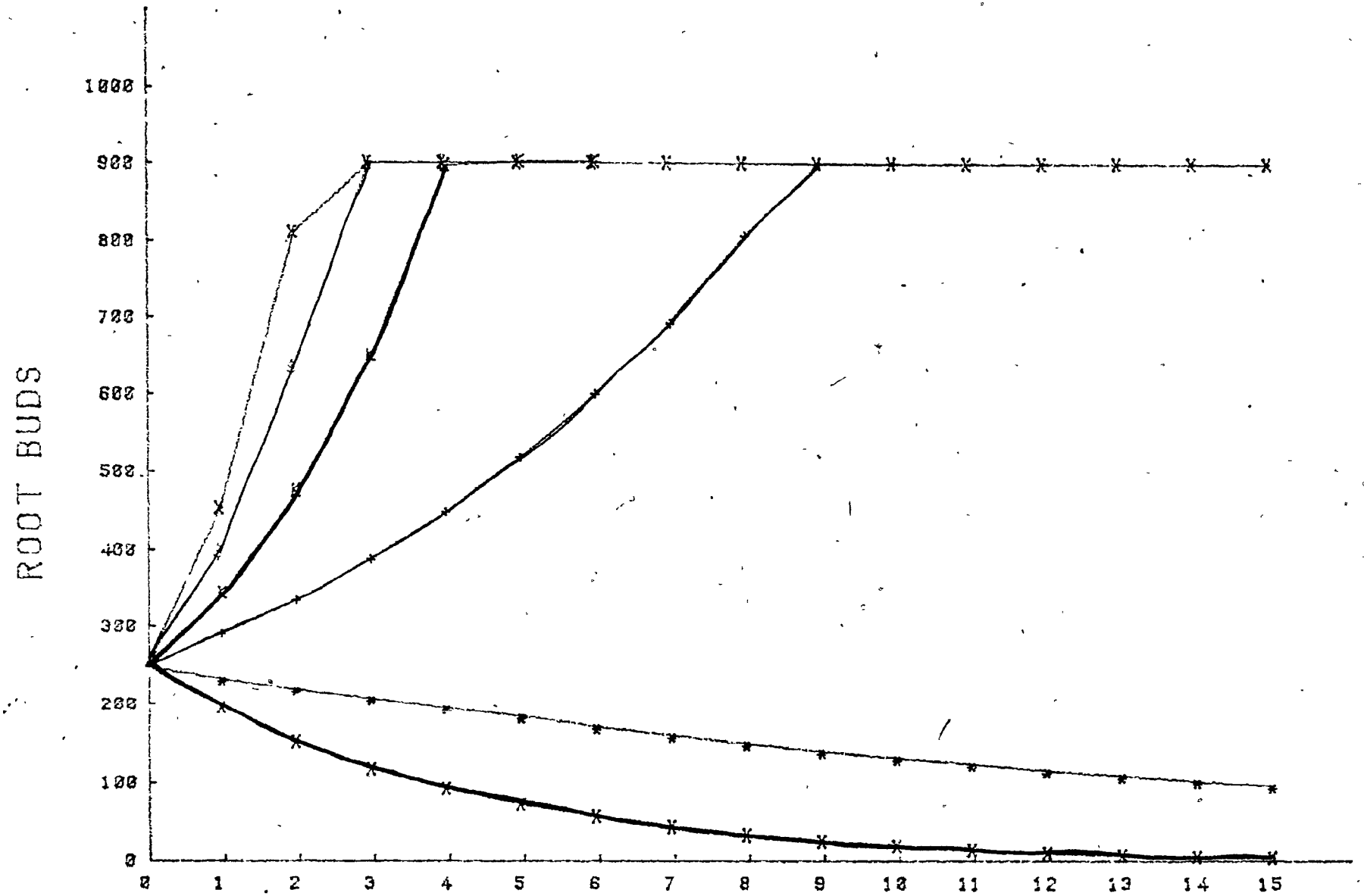


FIGURE 144. YEARS

Figure 145. Modelling of the effect of a combination of damages from all natural enemies on seed production.

Reductions:

	e_{53}	e_{54}	e_{14}	c_{33}	c_{43}
blackX	-	-	-	-	-
green	20%	20%	70%	60%	50%
blue	40%	40%	70%	60%	60%
red	80%	80%	95%	98%	96%

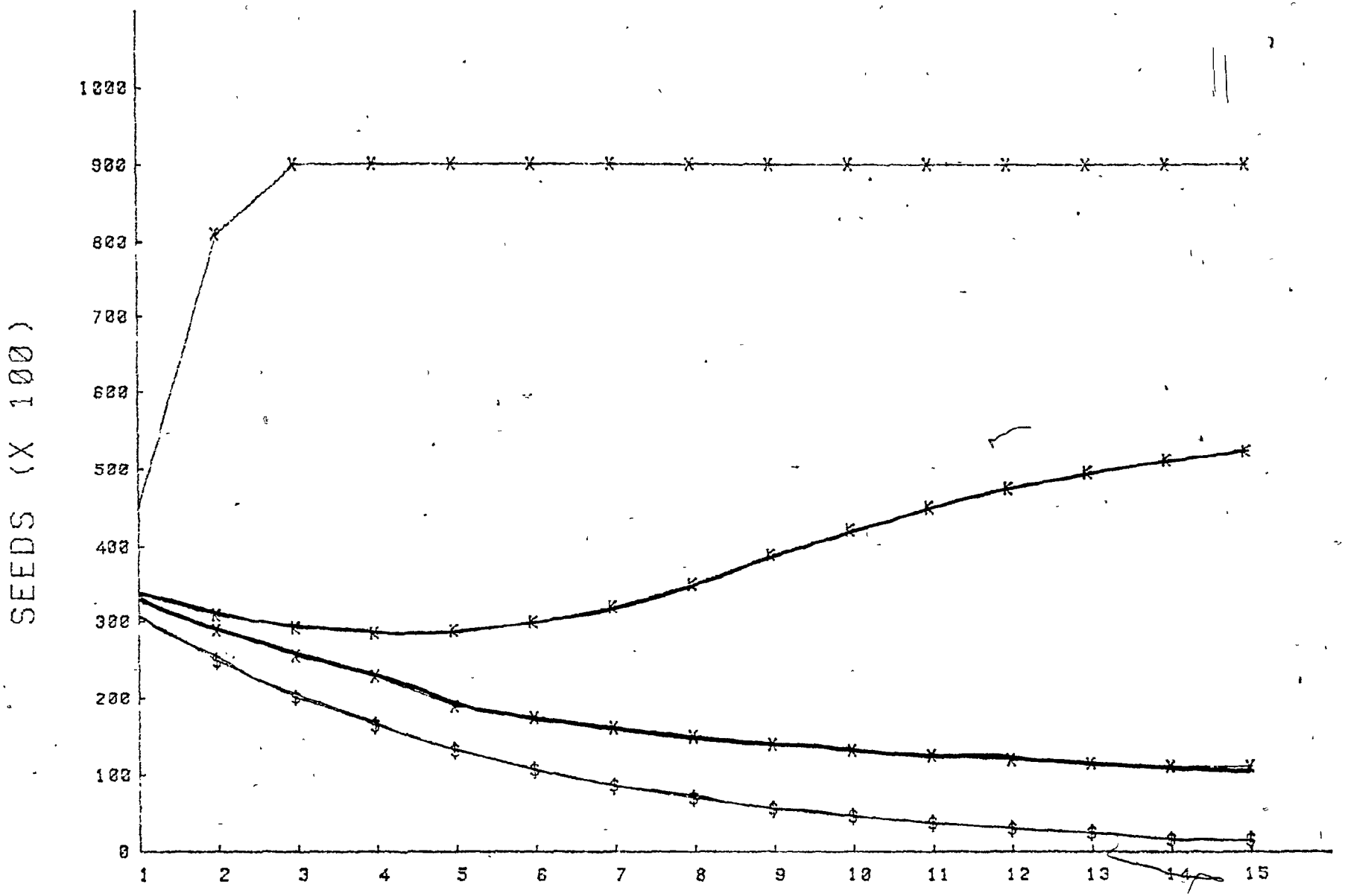


FIGURE 145. YEARS

Figure 146. Modelling of the effect of a combination of damages from all natural enemies on root bud production.

Reductions:

	e_{53}	e_{54}	e_{14}	c_{33}	c_{43}
blackX	-	-	-	-	-
green	20%	20%	70%	60%	50%
blue	40%	40%	70%	60%	60%
red	80%	80%	95%	98%	96%

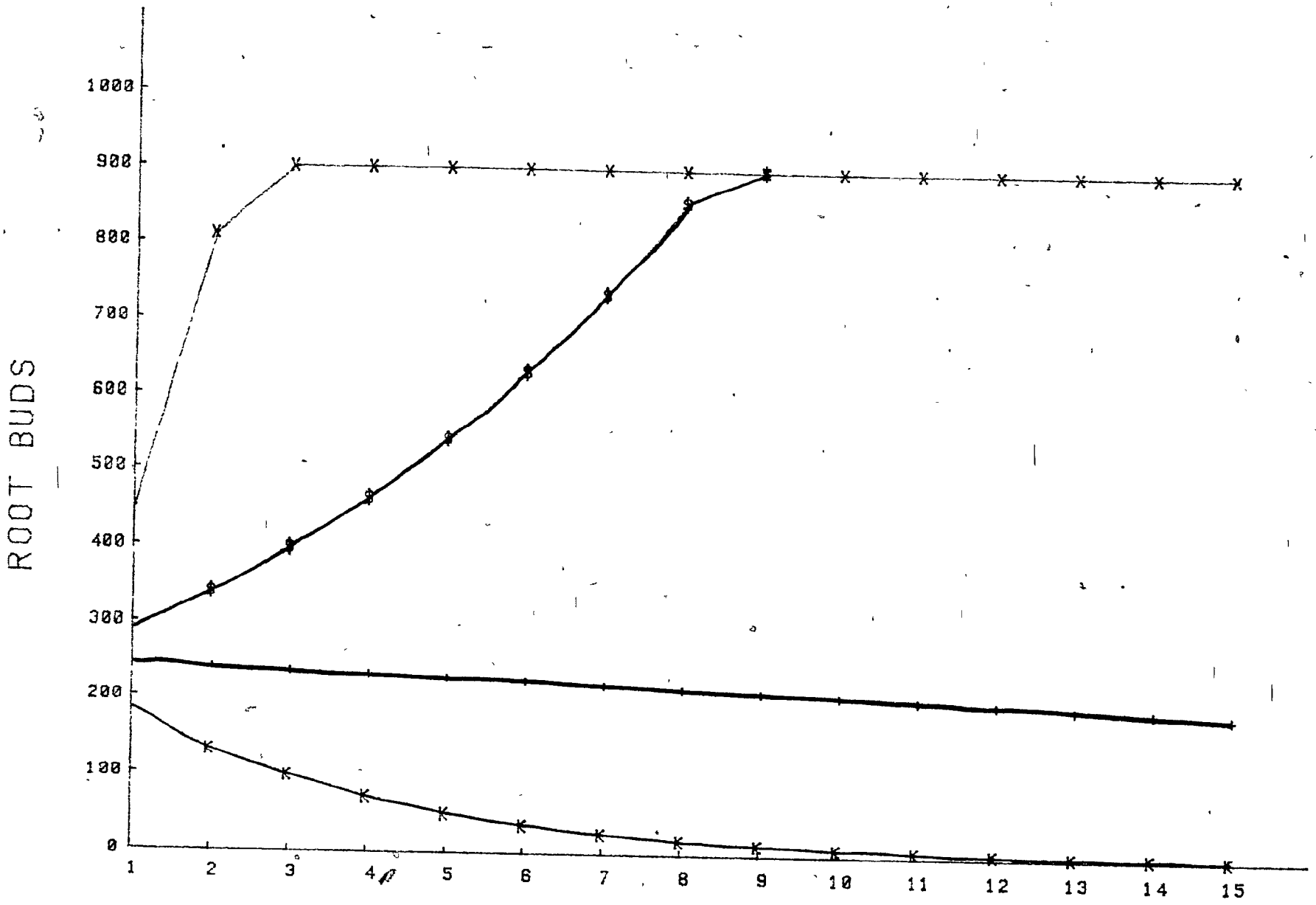


FIGURE 146. YEARS

fecundity dramatically reduced (e_{14}=38) and the root bud fecundity reduced (e_{53}=1, e_{54}=2),(a very heavy infestation of all insects) the red curves result. If this level of insect damage and rust infection were maintained, Canada thistle would probably be eventually eliminated.

The model has allowed an insight into the effects of the natural enemies over time. As has been seen throughout this research, the five natural enemies are only capable of maintaining the population at a certain level, but not of reducing the population. The damage of the natural enemies would have to be increased to result in regulation of Canada thistle populations.

IV. GENERAL SUMMARY AND CONCLUSIONS

The research herein and the application of the matrix model both indicated that on the whole, the group of natural enemies examined at their present field levels and damage are probably maintaining the thistle population at its present level, but are not resulting in a population reduction.

In order for the seed head predator, Orellia ruficauda to be more effective, it would have to attack a greater percentage of the heads and the seeds.

Cleonus piger, the root crown inhabitant, is limited by its large size to large plants and thereby limits its effectiveness. Augmentation of the population may be effective, but this is not recommended since the insect can potentially attack artichoke.

The effectivenesses of Cassida rubiginosa, the defoliator and Urophora cardui, stem gall fly are both reduced because of the lack of synchrony of the insect life cycle with the phenology of the plant. This study has indicated that both of these insects have more detrimental effect on plants much younger than those they attack in the field. C. rubiginosa would also require an increase in population to cause greater damage. The U. cardui population is gradually increasing, but the effects are minimized by the synchrony problem which forces the flies to lay their eggs into the sideshoots resulting in the formation of the less stressful sideshoot galls. If there were a way to delay

Canada thistle maturity (i.e. competitive pasture grass growth, sheep grazing), the insects may be able to attack the plants at a younger and more susceptible age.

Puccinia punctiformis is perhaps the most promising of all the natural enemies. A recent small body of literature is dedicated to the possible use of the rust as a mycoherbicide. It is not known at present what limits the population level in the field (why the infestation does not reach epidemic proportions). The theory is that there is an insufficient number of spores and that is why preliminary work into artificial spore augmentation was initiated.

Insights into the biological control of weeds have been gained in this study. Probably the most appropriate conclusion is that studies of this type should become a part of the biological control program to eliminate further study on insects which are insufficiently synchronized, do not cause a high level of damage and cause damage to areas of the plant which are highly resistant to damage. Studies such as this could also be used to indicate that damage to desirable native plants related to the target weed by a potential candidate are not severe enough to be serious. A number of insects and pathogens are at this stage at present and studies on this problem of conflict of interest may be important in the future.

V. CLAIMS OF ORIGINAL WORK

This study is the first concerted and organized effort to answer the problems of understanding the stress caused by natural enemies for a program of biological weed control in answer to the challenges of Harris (1980b) and Andres (1980b). The original discoveries are listed below under the organism name.

1. Orellia ruficauda. Seed head predator

The first microscopy of seed damage caused by the predator and the seed head containing the larvae. Field work has been previously reported and was extended by this work.

2. Cleonus piger. Root crown inhabitant

(a) The first microscopy of the damage caused by the larval feeding in the root crown area.

(b) The first simulation experiment (notching) of the severing of the stele and observations of subsequent vascular regeneration.

3. Cassida rubiginosa. Defoliator

(a) The simulation experiments are the first done on Canada thistle. The results indicated that higher levels of defoliation on young plants are most effective, which is opposed to the general concept that it is more detrimental to the plant for defoliation to occur when it is flowering. Also, repeated

defoliation pressure is more detrimental than one-time defoliation.

(b) The first microscopy of damage caused by the insect.

4. Urophora cardui. Stem gall fly

(a) The typical evaluation protocol was modified to distinguish between those plants emerged before and after fly emergence, which allowed for better understanding of the stress of gall formation.

(b) Similar controlled environment experiments as performed by Peschken and Harris (1974) were performed with more detailed examination of the stress of the gall. It was reaffirmed that gall formation was more stressful if the gall occurred on the mainshoot and was more detrimental to young plants.

(c)The gall was determined to be a weak physiological sink by radioactive precursor (^{14}C-fructose) studies and by sugar content analysis.

(d) Analysis of soluble and total protein content was also performed for the first time of gall and other thistle tissues.

(e) Microscopal studies indicated the gall to be a typical type of gall containing the "inner gall" surrounded by thick-walled parenchyma.

(f) Correlations made between gall size (amount of nutritive tissue) and parameters of yield and growth indicated that larger galls were more damaging that small ones.

(g) The realization that the most probable reason U. cardui does not result in a large stress is its lack of phenological synchronization with the most susceptible stage (younger plants).

5. Puccinia punctiformis. Systemic rust

(a) Extension of the knowledge on artificial inoculations indicating that multiple inoculations result in a greater and earlier yield of systemically rusted ramets.

(b) Microscopy of the pycnia and uredinia (SEM) and of the rust growth in the stem, some original and some repeats of other work.

(c) First successful attempt at producing infected callus and the microscopy thereof.

6. Modelling.

The first application of the Leslie matrix model to the simulation of the effect of natural enemies on the population dynamics of a weed.

VI. RECOMMENDATIONS FOR FUTURE WORK

1. It is recommended that further studies such as this become a common emphasis in the biological weed control protocol.

2. A continuation of the field studies on these organism should be performed.

3. Cleonus piger. The simulation work should be done on older plants.

4. Cassida rubiginosa. The simulation experiments should be extended to include insect defoliation under controlled environment conditions on different ages of plants and additional work on the parasite problem should be done.

5. Urophora cardui. Further studies should be made into the determination of the most susceptible age of the plant and that a study should be done to implement some management practice to provide better synchronization of U. cardui and Cassida rubiginosa with Canada thistle phenology.

6. Puccinia punctiformis. Research into the infection process should be continued and also into the artificial culture of the rust.

7. The model should be given added flexibility to allow for the addition of a time factor i.e. so that it could simulate the difference between damage in the spring versus damage in the late summer.

VII. APPENDICES

Appendix A. Calculations and Calibration Curve for Anthrone's Reagent (Soluble Sugar Content).

Three replicates of each sample were analyzed by the Anthrone's method. The absorbance for each was read and the value for the three was averaged. The glucose concentration (mg) was read from the calibration curve using the regression line (Fig A1). Since this value was for 0.1 mL of the sample, the calculated value was then multipled by the appropriate factor to determine the total amount of glucose for the total volume of extracted solution. This value was then divided by the total sample weight to determine mg glucose/g fresh weight. If the solution was too dense a dilution was performed (usually 1:3), and the absorbance was multipled by 4 before calculations proceeded. Once these values were determined, they could be used in analysis of variance, in which case averages would be taken of all different replicates for the same tissue type.

Sample Calculation: Sample of gall tissue

Abs. (0.1 ml) .33 .36 .37 (diluted 1:3) averaged at 0.35

$$\text{mg glucose} = \frac{\text{Abs.} - 0.70}{5.75}$$

$$\frac{0.35 - 0.70}{5.75} = 0.049$$

Multiply by the dilution factor (4)=0.195
Total volume of extracted liquid was 35 mL.

multiply 0.195 X 350=68.17 mg/35 mL

Total weight of the sample was 1.1g. Therefore
mg glucose/mg=68.17/1.1=61.97 mg/g fresh weight

Figure A1. Calibration curve for anthrone's reagent used to determine glucose concentration.

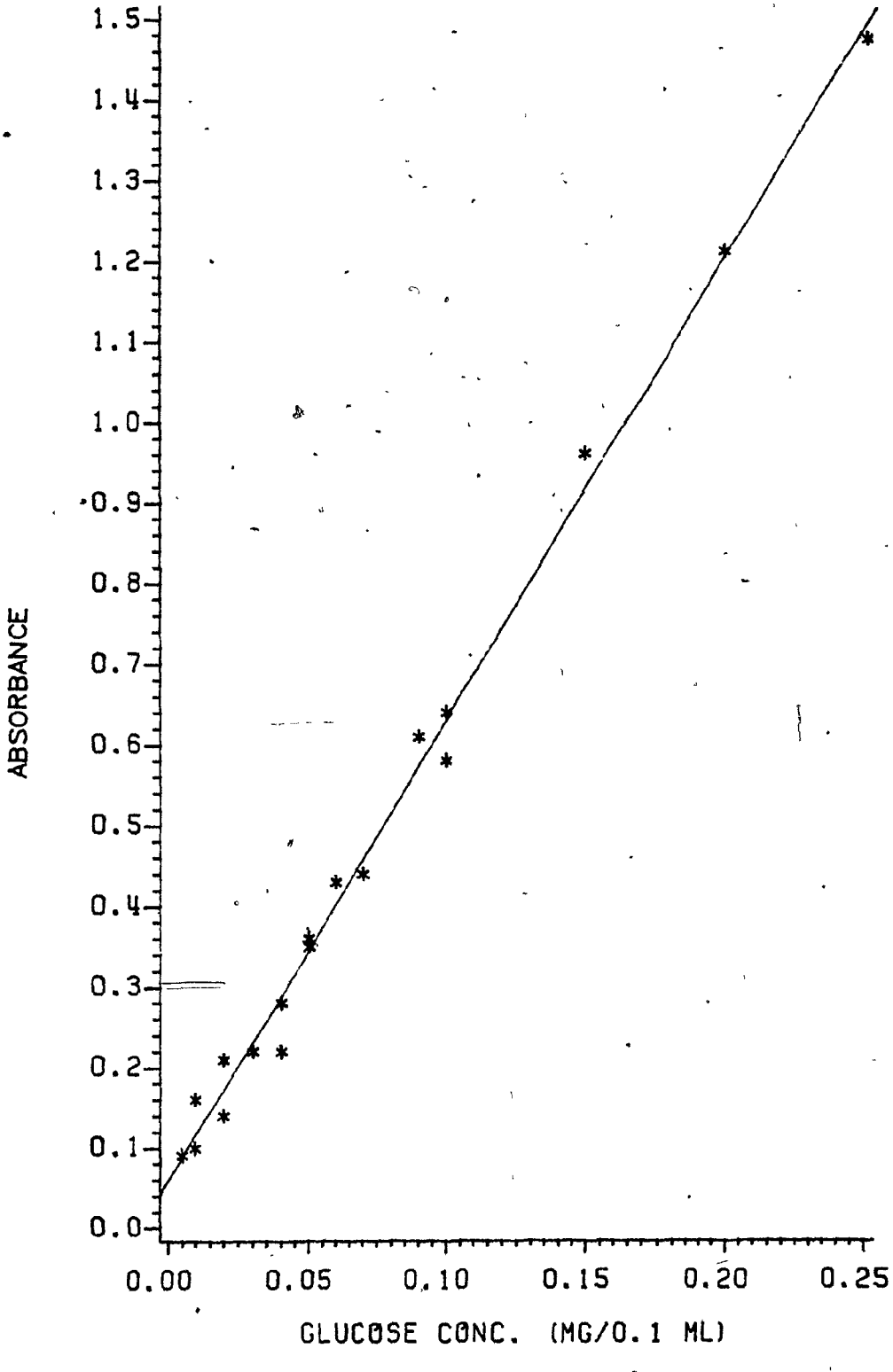

ABSORBANCE
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.00
0.05
0.10
0.15
0.20
0.25
GLUCOSE CONC. (MG/0.1 ML)

FIGURE A1

Appendix B. Calculations and Calibration Curve for Lowry's Reagent (Water Soluble Protein Content)

Three replicates of each sample were analyzed by the Lowry's method. The absorbance for each was read and an average value was calculated. The mg protein were calculated from the regression equation of the calibration curve (Fig A2). Since this value was for 0.25 mL of the sample was multiplication by the appropriate factor to determine the total amount of protein for the total volume of extraction solution was performed. This value was then divided by the fresh weight of the sample to determine mg protein/g fresh weight of tissue. These values were then used for analysis of variance and average per tissue were determined.

Sample calculation on same sample as used in Appendix A.

Abs. of three replicates 0.21 0.21 0.21 average 0.21

Calculate from the calibration curve

mg protein= 0.562 Abs. - 0.0386

= 0.562(0.21) -0.0386

=0.079 mg in 0.25 mL.

mg protein in total volume (35 mL)=

0.079 X 35 X 4=11.06mg

amount of protein per gram. Total fresh weight was 1.1 g.

11.06/1.1=10.1 mg protein/g fresh weight

Figure A2. Calibration curve for Lowry's reagent used to determine protein concentration.

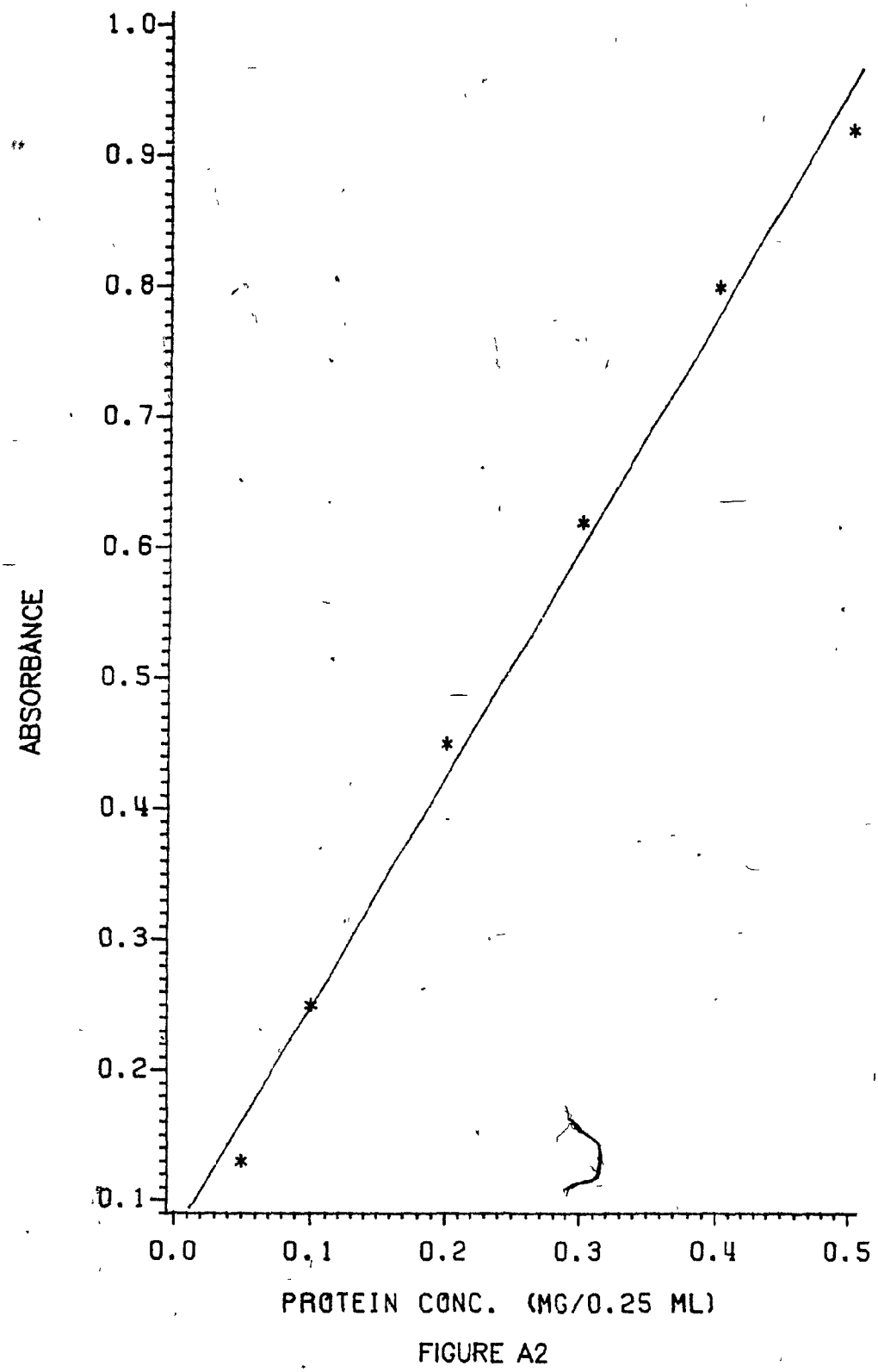

1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
ABSORBANCE
0.0
0.1
0.2
0.3
0.4
0.5
PROTEIN CONC. (MG/0.25 ML)

FIGURE A2

Appendix C. Calculations Associated with the MicroKjedahl Procedure.

Calculations of microKjedahl results are based on a titration of the sample with a standardized acid. The volume of the titration acid is corrected by the blank volume and then the corrected value is multipled by the normality (N) of the acid to determine the milliequivalents. The milliequivalents are multipled by the factor of 14.007 to determine mg of nitrogen in the sample. This value is divided by the mg of the sample and multiplied by 100 to determine the % nitrogen. Percent protein is calculated by multiplying by the factor 6.25.

Sample calculation: for a sample of a gall

Volume of titration acid=0.75 mL
- Blank volume = 0.218mL

Corrected volume = 0.532 mL

Normality of the acid= 0.0145 N

Milliequivalents= corrected volume X N of acid

= 0.532 X 0.0145

= 0.0077 milliequivalents

mg N= milliequivalents X 14.007

= 0.0077 X 14.007
=0.0108

$$\% N = \frac{mg\ N}{mg\ sample} \times 100 \qquad mg\ sample = 11.62$$

$$\% N = \frac{0.108}{11.62} \times 100 = 0.93\%$$

% Protein =% N X 6.25= 5.8%

Appendix D. B/A Channel Ratio Method for Calculating Disintegrations per Minute for Liquid Scintillation Counting

Each sample used for liquid scintillation experiments was sampled three times and each of these replicates was counted on the liquid scintillation counter 4 to 7 times to check precision and increase accuracy. ^{14}C quench curves for the machine used for counting were prepared by Chemistry department graduate students Ramble Ankumah and William O'Niel and passed on to those students who used the machine.

The technique used to calculate disintegrations per minute is referred to as the Channel Ratio method (Wang et al. 1975). For each sample, the averages of the counts per minute, the channel A and channel B counts were calculated. The value of the channel B/channel A ratio was determined. Two regression equations were used to calculate efficiencies one for low B/A ratios (1-1.3) (high efficiency) and onefor high B/A ratios (1.8-2.5) (low efficiencies). The efficiencies were then calculated:

(A) for low efficiencies (high B/A ratios)

$$Y = -0.409Z + 1.130$$

(B) for high efficiencies (low B/A ratios)

$$Y = -0.749Z + 1.644$$

where Y= efficiencies and Z= B/A ratio

The disintegrations per minute were then calculated by dividing the average counts per minute by the efficiency calculated by the preceeding method.

Appendix E. Preliminary Experiment of Gall Formation and Defoliation Stress Combined

Purpose: To determine if the combination of defoliation and gall formation resulted in greater stress than gall formation alone

Materials and Methods:

Plants grown from root pieces were exposed to flies when between 5 to 10 cm in height. The plants were planted in a soil:peat moss:sand (3:1:1) mixture in 155 mm diameter plastic pots. After gall formation three plants were defoliated weekly at the 75% defoliation level until week 10 and three other plants were not defoliated, but gall formation proceeded normally.

Results and Discussion:

Plants with galls plus defoliation tended to be shorter, had lower dry weights and also reduced root bud and ramet production when compared to plants with galls only (Table A1). This indicated that a combination of the two stresses is more effective that just one alone.

Table A1. Effect of Gall and Gall plus Defoliation on Plant Height, Dry Weight and Root Bud Production.

Time	Gall only	Gall+ 75% defoliation
	----------Height (cm) ±S.D.----------	
0 weeks	9.8±1.8	12.5±1.4
3 weeks	12.8±1.5	12.2±1.2
7 weeks	16.8±1.4	13.8±1.1*
11 weeks	21.8±2.8	16.0±3.5
19 weeks	25.7±4.5	23.0±11.3
HARVEST DAY		
Parameters		
Root bud number	19.0±2.0	14.5±7.8
Ramet number	16.8±7.0	5.8±4.9
Dry weight (g)		
Plant	3.1±1.0	3.5±1.8
Root	7.6±0.4	5.2±0.7
Ramets	7.4±2.0	5.2±0.7
Gall	0.7±0.3	1.3±1.1

* one of three plants was dead at this point

Appendix F. Calculations Associated with Cassida rubiginosa Feeding Experiments under Controlled Environment Conditions

Data for insect defoliation under controlled environment conditions was handled by summing all the visual ratings (recorded as %) for all the leaves on the plants and dividing by the total number of leaves. The plants were then classified into four groups according to this calculated value; I=0 control, II=0.1-5%, III=5.1-10% and IV=10.1+%. This was based on % leaf removal rather than the number of larvae because depending on the age of the larva, leaf intake was variable and due to the nature of Cassida rubiginosa phenology, collection of identical larvae was not possible, however correlation between the number of larvae and calculated average % of leaf damage was 0.88.

Sample calculations:

% leaf removal	Total %	No. Leaves	Average % damage	Rating
10	10	44	0.2	II
20,5,5,5,20,15	70	37	1.9	III
10,5,10,20,5,20, 10,30,30,40,10, 10,10,20,15,5,10, 5,10,5,10,5,5,5, 25,5,5,30,55,20, 10,10,10,5	490	42	11.7	IV

Appendix G. Parasitism of Cassida rubiginosa

Pupae were collected from the field in late summer and placed in vials with cork caps and observed later in the fall. Out of 31 pupae examined 10 were found to be parasitized (32%) by a wasp parasite (probably Tetrastichus rhosaces Walker reported by Ward and Peinkowski 1978b). Harris and Zwolfer (1971) indicated that this parasitism may be an important factor which limits population buildup of this insect. This is the first report of the parasite in Quebec as far as can be found.

Appendix H. Analysis of Variance Tables (Section E)

Table A2. One time defoliation Repeat 1

		Height	Ramets	Root buds	Sideshoot	DWP
Mean Square		62.85	7.6	175.67	16.36	1.57
Sources	DF	----------	----------	PR>F	----------	----------
R	3	0.97	0.72	0.29	0.52	0.82
AGE	3	0.0001**	0.09	0.07	0.28*	0.002*
DEFOL	4	0.0085**	0.03*	0.05*	0.01**	0.0002**
AGE*DEFO	12	0.055	0.94	0.38	0.47	0.60
COMBO	19	0.0001**	0.28	0.09	0.03*	0.001**

	Ramet Ht.	DWR	DWO	DWT	SHRT	LEAF NO.
Mean Square	19.30	4.65	1.17	10.07	0.83	15.1
SOURCES	----------	----------	PR>F	----------	----------	----------
R	0.09	0.93	0.07	0.61	0.48	0.86
AGE	0.08	0.0001**	0.04*	0.0001**	0.004**	0.01**
DEFOL	0.04*	0.0001**	0.13	0.0001**	0.0001**	0.61
AGE*DEFOL	0.89	0.009**	0.24	0.095	0.58	0.63
COMBO	0.19	0.0001**	0.08	0.0001**	0.0001**	0.20

** 0.01 Significance
* 0.05 Significance
DWR- dry weight root, DWO- dry weight ramets,
DWT- dry weight total, DWP- dry weight parent,
SHRT- Shoot/root ratio

Table A3. One Time Defoliation Repeat 2.

		Height	Leaf no.	Ramet no.	Fl. no	Side no.	RB no.
MEAN SQUARE		183.75	44.58	32.04	24.00	35.3	30.5
SOURCES	DT	PR>F					
R	3	0.04*	0.42	0.06	0.72	0.83	0.67
AGE	3	0.02*	0.41	0.06	0.87	0.10	0.06
DEFOL	4	0.005**	0.27	0.10	0.008**	0.12	0.97
AGE*DEF	12	0.24	0.14	0.10	0.24	0.88	0.42
COMBO	19	0.009**	0.16	0.03*	0.07	0.40	0.38

	DWP	DWR	DWO	DWT	SHRT	Ramet ht.
MEAN SQUARE	6.03	1.97	5.11	13.32	0.63	56.06
SOURCES	PR>F					
R	0.36	0.009**	0.0001**	0.0001**	0.06	0.11
AGE	0.001**	0.21	0.04*	0.01**	0.68	0.01**
DEFOL	0.006**	0.002**	0.45	0.01**	0.03*	0.04*
AGE*DEFOL	0.44	0.005**	0.16	0.17	0.04*	0.25
COMBO	0.004**	0.0006**	0.10	0.009**	0.03*	0.02*

** Signficant at 0.01 level
* Significant at 0.05 level
Fl. no.=flower number, Side. no.= sideshoot number
RB no.= root bud number, DWP=dry weight parent,
DWR= dry weight root, DWO= dry weight ramets,
SHRT= shoot/root ratio, DWT= dry weight total.

Table A4. Continuous Defoliation. Repeat 1.

		Height	Ramet no.	RB no.	Side. No.	Ramet Ht.
MEAN SQUARE		36.53	15.41	144.31	8.34	20.03
SOURCES	DF	PR>F				
R	3	0.84	0.01**	0.59	0.62	0.58
AGE	3	0.004**	0.06	0.35	0.67	0.72
DEFOL	4	0.01**	0.002**	0.01**	0.31	0.57
AGE*DEFO	10	0.29	0.22	0.08	0.31	0.97
COMBO	17	0.02*	0.004**	0.01**	0.41	0.97

	DWP	DWO	DWT	DWR	SHRT
MEAN SQUARE	1.76	1.66	11.31	2.73	1.15
SOURCE	PR>F				
R	0.48	0.04*	0.36	0.50	0.33
AGE	0.47	0.46	0.07	0.07	0.45
DEFOL	0.0001**	0.74	0.0001**	0.0001**	0.08
AGE*DEFOL	0.40	0.68	0.25	0.14	0.04*
COMBO	0.0001**	0.80	0.0006**	0.0002**	0.03*

** Significant at 0.01 level
* Signficant at 0.05 level

Side. no.=sideshoot number, RB no.=Root bud number, DWP=dry weight parent, DWR= dry weight root, DWO= dry weight ramets, DWT= dry weight total, SHRT= shoot/root ratio

Table A5. Continuous Defoliation. Repeat 2.

		Height	Leaf no.	Ramet no.	Fl. no.	Ramet ht.	Side no.
MEAN SQUARE		747.18	48.23	70.23	8.75	39.32	186.57
SOURCE	DT	-PR>F-					
R	3	0.23	0.001**	0.0007**	0.002**	0.06	0.04*
AGE	3	0.0001**	0.82	0.005**	0.01**	0.89	0.0001**
DEFOL	4	0.0001**	0.0001**	0.0001**	0.0001**	0.13	0.0001**
AGE*DEF	12	0.0006**	0.05*	0.0012**	0.26	0.61	0.0001**
COMBO	19	0.0001**	0.003**	0.0001**	0.0001**	0.51	0.0001**

	RB No.	DWP	DWR	DWO	DWT	SHRT
MEAN SQUARE	116.55	16.08	8.54	7.33	73.45	8.90
SOURCE	-PR>F-					
R	0.0002**	0.011*	0.12	0.24	0.51	0.02*
AGE	0.10	0.03*	0.0004**	0.001**	0.0001**	0.0001**
DEFOL	0.0007**	0.00001**	0.0001**	0.02*	0.0001**	0.22
AGE*DEFOL	0.29	0.02*	0.0003**	0.13	0.0001**	0.29
COMBO	0.012*	0.0001**	0.0001**	0.008**	0.0001**	0.001**

** Significant at 0.01 level
* Significant at 0.05 level

Fl. no.= Flower number, Side. no.= Sideshoot number, RB no.= Root bud number, DWP= dry weight parent, DWR= dry weight root, DWO= dry weight ramet, DWT= dry weight total, SHRT= Shoot/root ratio

Appendix I. Applications of Defoliation Data:

I Regression:

Linear regressions were performed for several parameters over different levels of defoliation. The data presented (Fig. A3) is of heights from a continuous defoliation experiment for the four different leaf stages. The effect of defoliation has more detrimental effects on the 5- and 10-leaf stages as indicated by the higher negative slope over increasing defoliation levels. The effect on the 15- and 20-leaf stages is less. Similar treatment of results from one-time defoliation shows little differences between the regression for various leaf stages over the different defoliation levels.

II Tammes' Curves:

The relationship between plant yield and insect feeding (measured as numbers of insects or feeding injuries) can be described by Tammes' curve. The typical shape of a Tammes' curve approximates a reversed sigmoid (Fig. A4). At low damage levels, theoretically the plant is capable of compensating for the damage and there is no decrease in productivity, resulting in an upper plateau (Fig A4,A). In some cases, the plant may even overcompensate resulting in a rise. The furtherest edge of the plateau, before the damage begins to affect the productivity is referred to as the threshold. At slighty higher damage levels, beyond the threshold level, compensatory growth becomes less effective and the productivity decreases. The theoretical curve then straightens and a portion of a linear relationship occurs between yield loss and insect damage (Fig. A4,B). A lower plateau can also occur indicating that plants with underground storage are difficult to destroy (Fig A4,C) (Jackson 1980,

Figure A3. Linear regression and equations for continuous defoliation results for the height (cm) parameter over the different levels of defoliation. (red= 5-leaf, blue=10-leaf. green= 15-leaf and black= 20-leaf).

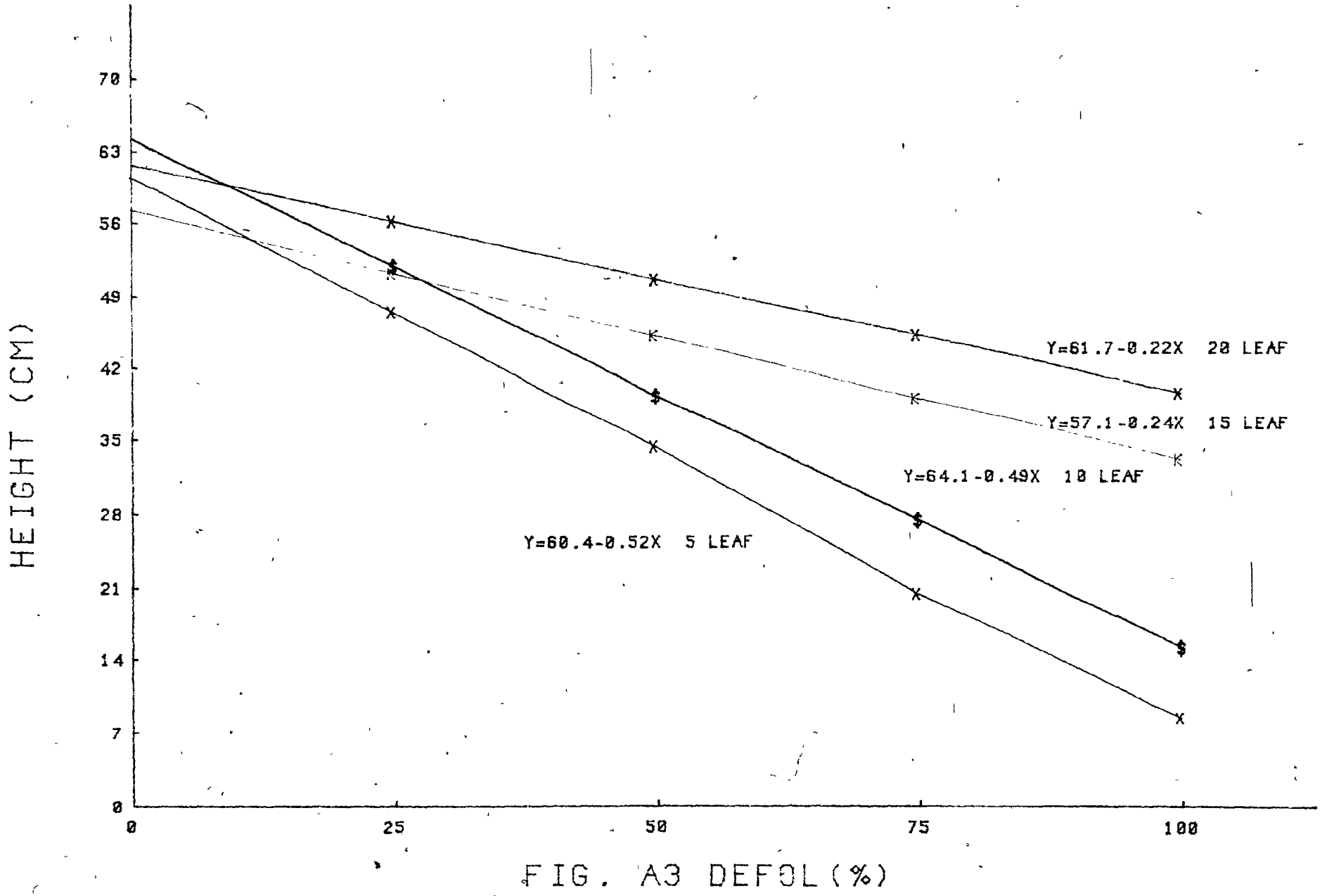

HEIGHT (CM)
70
63
56
49
42
35
28
21
14
7
0
0
25
50
75
100
Y=61.7-0.22X 20 LEAF
Y=57.1-0.24X 15 LEAF
Y=64.1-0.49X 10 LEAF
Y=60.4-0.52X 5 LEAF
FIG. A3 DEFOL(%)

Figure A4. Typical Tammes' curve.

A reversed sigmoid shape with an upper plateau (A) indicating compensation for damage, a threshold beyond which compensation for damage is no longer active, (B), the linear phase of the curve where there is a linear relationship between the decrease in productivity and insect damage, (C), the lower plateau which indicates underground storage resources which cannot be tapped by insect damage.

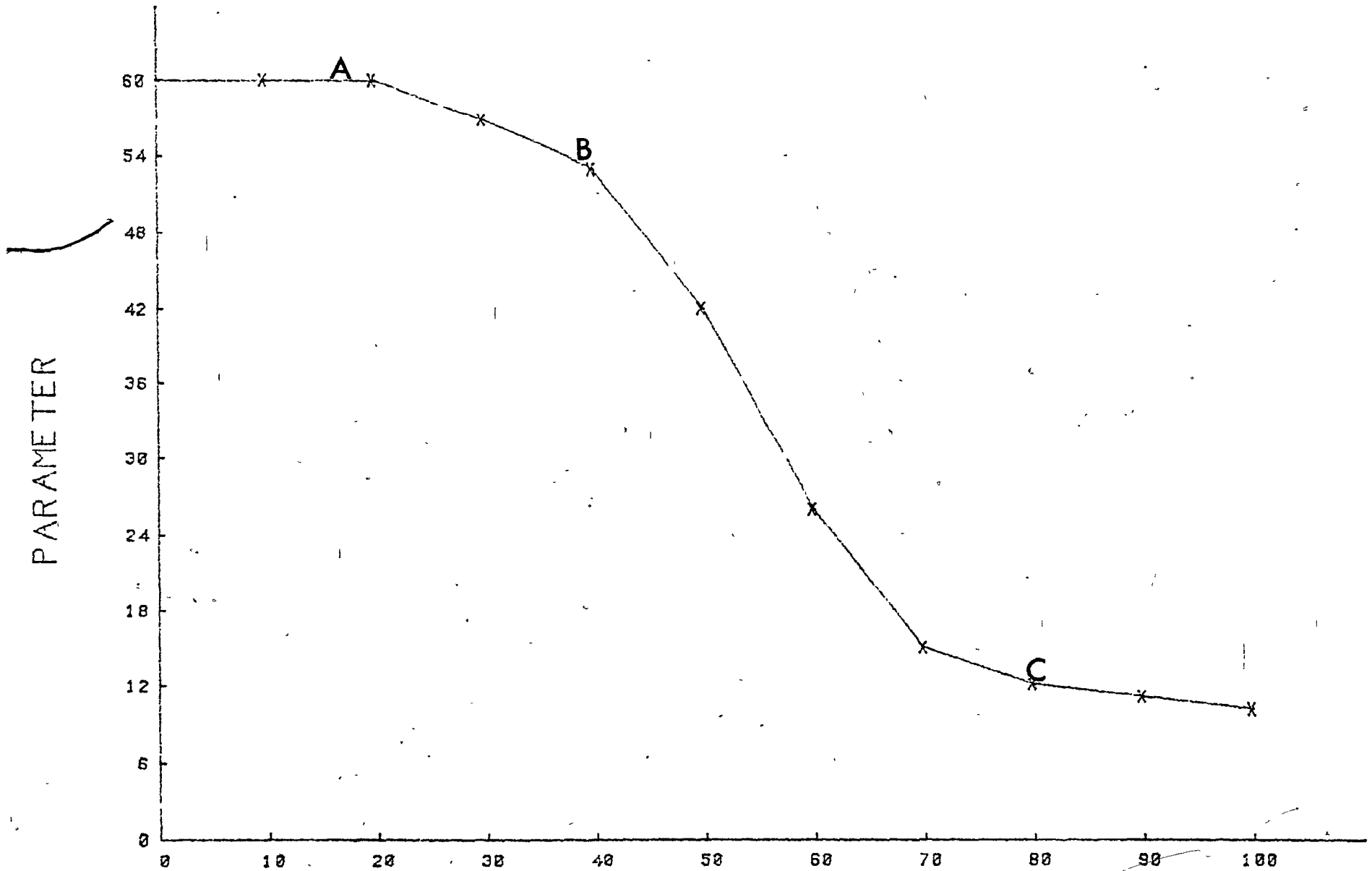


FIGURE A4. VALUE

Hodkinson and Hughes 1980).

When this type of curve was plotted for some of the one-time defoliation data (Fig. A5), it is difficult to pick out the threshold levels. Graphing of height versus defoliation does not result in a typical Tammes' curve shape. Canada thistle is capable of compensating for a high level of damage if it occurs only once.

Similar graph plots for a continuous defoliation indicated some near typical Tammes' curves (Fig A6). Thresholds for 5- and 10- leaf stages were somewhere between 25 and 50% defoliation and for the other stages at higher levels of defoliation.

III Defoliation Pressure Index (DPX):

The defoliation pressure index (DPX) is a concept originated by Nakano(1977, 1980) to integrate the different factors involved in the action of a defoliator.

Using an example given in Nakano(1977) data for one-time defoliation experiments was translated into DPX values (Table A6). To determine the DPX values, $\log_{10}$ was used rather than $\log_2$. A sample of the application of the DPX values is given in figure A7. As Nakano (1980) states, a negatively linear relationship exists between DPX and these two examples of "quantitative indices of plant growth."

Figure A5. Graphs of height (cm) and defoliation for a one-time defoliation experiment. No typical Tammes' curve shapes were found. Black- 5 leaf, Blue$- 10 leaf, green- 15 leaf, red-20 leaf, and blue*- average over all leaf stages.

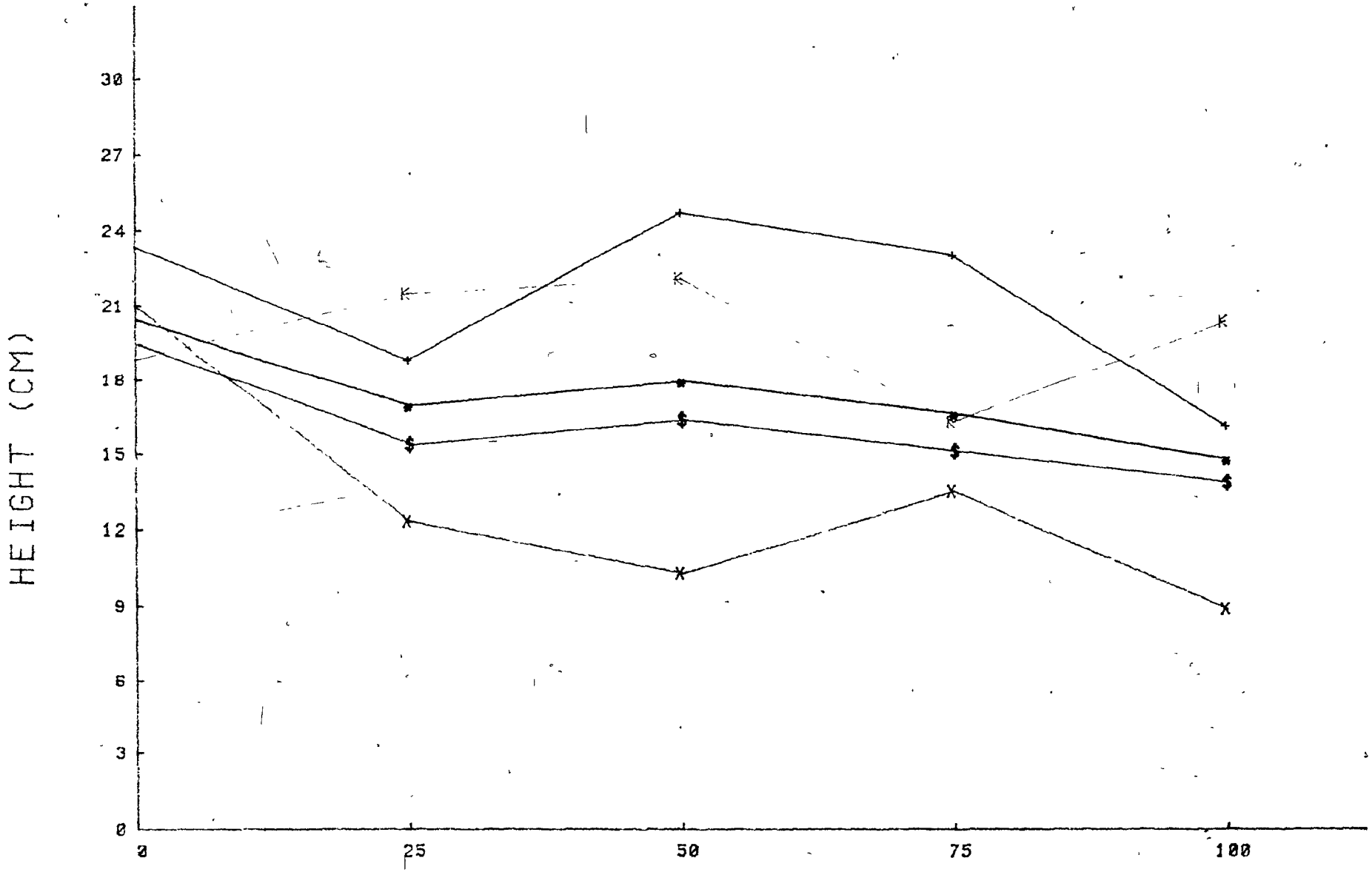


FIG. A5.

Figure A6. Graphs of height (cm) versus defoliation (%) for continuous defoliation. Some near typical Tammes' curves were found. Approximate threshold levels are indicated by dropped perpendiculars to the X-axis. Same symbols used as figure A5.

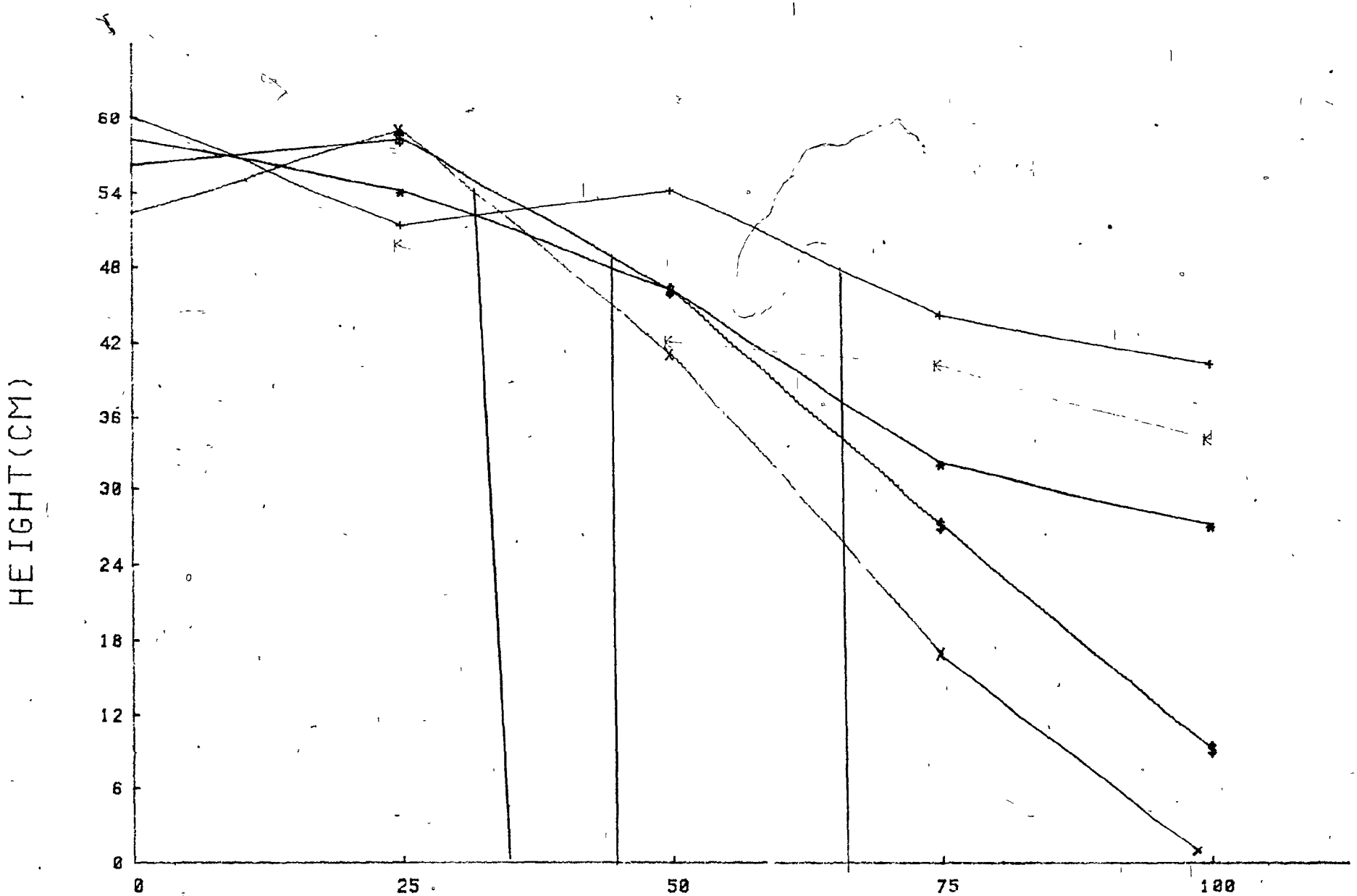


FIG A6. DEFOL (%)

Table A6. Calculated DPX Values for One-Time Defoliation Experiments.

Leaf Stage	Defoliation Level				
	0	25	50	75	100
	DPX				
5	0	0.12	0.3	0.6	-
10	0	0.11	0.26	0.5	1.0
15	0	0.10	0.20	0.36	0.6
20	0	0.04	0.08	0.11	0.18

Sample calculation:
10-leaf stage.

%Def.	No. days without defol. (A)	No. days with Defol X factor (B) $	Calcul. value (A+B)	Ratio &	DPX *
0	7	77X1	84	1	0
25	7	77X0.75	65	0.77	0.11
50	7	77X0.50	46	0.55	0.26
75	7	77X0.25	26	0.31	0.51
100	7	77X0	7	0.08	1.0

$ factor=100-level of defoliation/100
& ratio= calculated value/value for 0 defoliation
* DPX= $\log_{10}$ (ratio)

Figure A7. Graph of dry weight of total plants and dry weight of roots versus defoliation pressure index (DPX). Negatively linear relationships occur. (The lines drawn are regression lines.)

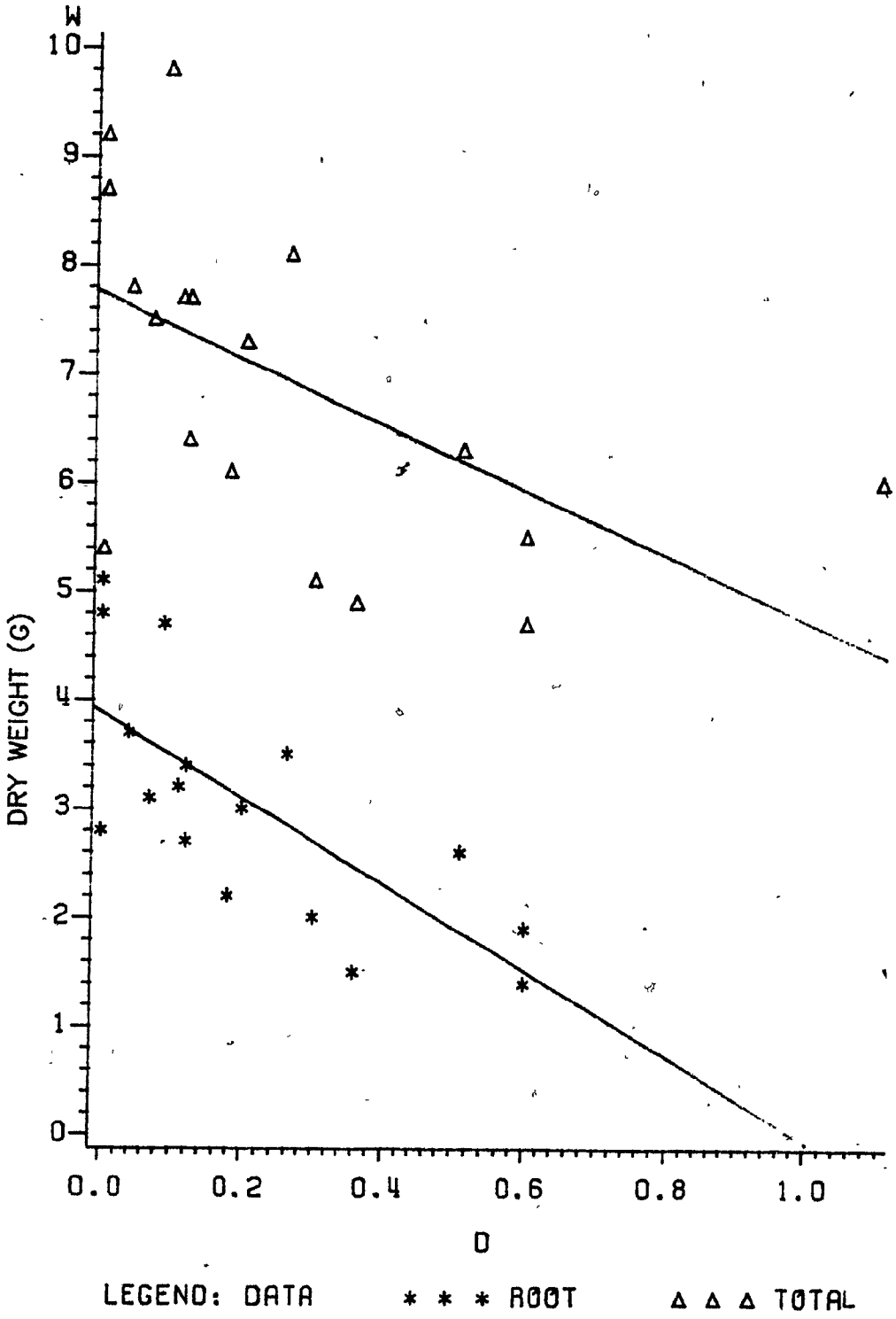

W
10
9
8
7
6
5
4
3
2
1
0
DRY WEIGHT (G)
0.0
0.2
0.4
0.6
0.8
1.0
D
LEGEND: DATA * * * ROOT Δ Δ Δ TOTAL

FIGURE A7

Appendix J. Calculations for Chlorophyll Determinations (Section F).

Absorbances of the chlorophyll extracts were read at 645 nm and 663 nm according to Arnon's (1954) procedure. The chlorophyll content was determined by the following equation:

$$\text{Chlorophyll a + b} = 8.05\ A663 + 20.20\ \frac{}{A465}$$

If the extract was diluted prior to reading (usually 1:9), the correction factor was employed at this point in the calculations. The value is then calculated per g or per cm^2 by dividing by the leaf weight and leaf area of the sample.

Example Calculation:

Absorbance at	645	0.45
	663	0.81

Chlorophyll content = 8.02(0.81) + 20.20(0.45)
= 15.59

This was diluted 1:9, therefore it was multiplied by 10 give 155.86 mg.

The weight of the sample was 4.49 g and the mg chlorophyll per g tissues was 34.72 mg/g fresh weight

The area of the leaves was 105.5 cm^2. Therefore,

1.48 mg chlorophyll/cm^2

Appendix K. Tissue Culture Media

One litre of media contained 100 mL of macronutrient solution (A), 1 mL of micronutrient solution (B), 10 mL of vitamin solution (C) and 1 mL of iron solution (D), 3 % sucrose (30 g), 1 g of casein hydrolysate, 5 mg inositol plus a source of an auxin and a cytokinin. The pH was tested and adjusted to 5.7 if required. Agar (0.8 % Difco Bacto-Agar) was then added and the media was autoclaved for 15-20 minutes at 212 °C and 15 lbs per sq. inch pressure. Plates were poured and either used after sufficient cooling or stored at 10 °C. The media was adapted from Murashige and Skoog (1962).

A Macronutrient Solution (g/L)

NH_4NO_3	16.5
KNO_3	19.0
$MgSO_4 .7H_2O$	3.7
KH_2PO_4	1.7
$CaCl_2 .2H_2O$	4.4

B Micronutrient Solution (mg/100 mL)

H_3BO_3	620
$MnSO_4 .4H_2O$	2230
$ZnSO_4 .7H_2O$	860
$Na_2MoO_4 .2H_2O$	25
$CuSO_4 .5H_2O$	2.5
$CoCl_2 .6H_2O$	2.5
KI	83

C Vitamin Solution (mg/L)

Nicotinic acid	50
Thiamine HCl	10
Pyridoxine	50
Glycine	200

D Iron solution (g/L)

FeNaEDTA	20

Many of the chemicals and technical advice for this procedure were obtained from Bruce Gray and Jessie Nelles.

Good callus was formed with a combination of 1 mg/L 2,4-D and 1 mg/L zeatin. In another experiment a series of combinations of IAA (indoleacetic acid) and BA were used as auxin and cytokinin sources. All possible combinations (20) of four levels of BA (0.1, 1.0, 2.0, 5.0 mg/L) and five levels of IAA (0, 0.1, 1.0, 2.0 and 5.0 mg/L) were made. Preliminary results indicated that higher levels of cytokinin are required for good callus.

Appendix L. Comparison of the Respiration of Systemically Rusted and Nonrusted Plants.

Materials and Methods:

Pairs of plants (one rusted, the other nonrusted) of similar height and leaf number were selected and each plant was enclosed in a plastic bag fitted with two port connections (secured with silicon sealant) at opposite ends of the bag. One port was connected to a carbon dioxide source (either a CO_2 tank (323 ppm) or the external atmosphere) and the other to an infrared gas analyzer (Model 225, Analytical Development Co. Ltd.). When the external atmosphere was used, the CO_2 concentration was predetermined by calibration against the tank gas. The plants were kept in the dark and reading of the spikes at first sampling were compared. This experiment was repeated twice, with 2 pairs of plants examined in one experiment and 4 pairs in the other.

Results:

The respiration as measured by the release of carbon dioxide by the plants was higher for rusted plants (841.2 $\pm$ 410.1 ppm) than for nonrusted plants (448.8 $\pm$ 199.8 ppm).

Discussion:

Elevated respiration is commonly observed effect of pathogen development on their host. It is one of the effects of Puccinia punctiformis which makes it an effective natural enemy.

Appendix M. Comparison of Transpiration of Rusted and Nonrusted Plants.

Throughout the author's observations, it was noticed that rusted plants tended to wilt more easily than nonrusted plants under the same conditions. This is a common effect with plants infected with rust.

Materials and Methods:

To quantify the amount of water loss, plants (rusted and nonrusted) were transplanted into Promix in styrofoam cups and the soil and the cup were covered with plastic bags and closed around the plant stem so that the only loss of water was through the plant itself. The pots were weighed periodically.

Results and Discussion:

The rusted plants did not survive transplanting well and often died too early in the experiment. The results indicated that losses of weight were higher for the nonrusted thistles than for the rusted plants. This was more than likely due to the fact that the rusted plants were dying.

Appendix N. Sugar and Protein Contents of Rusted and Nonrusted Plants.

Methods for extraction and analysis of proteins and sugar were similar to those employed in section B.

Protein content for roots was slightly higher in rusted plants (10.5 ± 1.1 g/g fresh weight) than for nonrusted plants (9.2 ± 5.7) and also for leaves (11.3 ± 1.1 as opposed to 9.0 ± 1.4) and for the stem (10.2 ± 2.1 versus 7.9 ± 3.1). Sugar content was higher in the roots of rusted plants (3.5 ± .43 versus 1.2 ± 0.6) whereas it was lower for the leaf and the stem (leaf- 1.3 ± 1.4 versus 1.9 ± 1.9); stem- 0.6 ± 0.4 versus 2.0 ± 0.3).

The increase of water soluble protein content means more proteins in a transportable form in rusted plants and the lower sugar content may mean that there are depleted carbohydrate sources in the rusted plants.

Appendix O. Rust Population Dynamics

Rusted populations on Macdonald College were examined in the course of two undergraduate projects (Keogh 1979, Nadeau 1980) prior to the work presented in this study. Results (Table A7) indicate that the population dynamics of the occurrence of the rust is variable from field to field and also from year to year. The highest % field infection found was 47%. Turner (1981) found a maximum of 52.5% in Montana. None of the levels found in natural populations were capable of control.

Table A7. Rust Population Dynamics in Field Populations

Year	Site 1*	Site 2	Site 3	Site 4
	% rusted shoots			
1979	5	-	33	47
1980	5	-	5	26
1981	4	23	27	22
1982	21	34	15	21

* sites correspond to those in section A.

Appendix P. Root Bud Production of Field Populations.

Samples of soil were dug and examined in the field. The samples were 30X30X30 cm in volume. Roots were collected and taken to the laboratory where root buds were counted, root length and root weight determined. Samples were taken in the spring (May 5/82) and the fall (Oct 20/82) (from sites 1 and 4 of section A). Ten to 12 samples were taken per field at each sampling date. Five samples from site 1 and 4 were planted in the greenhouse and allowed to grow. Shoots produced were counted.

The results indicated that in an area of 1 m^2, between 70 and 500 root buds occur in the soil (Table A8). A value of 250 buds per m^2 was chosen as the value for the model.

Table A8. Root Bud, Root Length and Root Weight from Field Samples#

SPRING

	Site 4	Site 1
Root bud number	17.3±12.9	21.9±12.2
Root weight (g)	28.1±12.5	48.1±16.6
Root length (cm)	168.7±81.8	155.8±50.4

FALL

Root bud number	53.4±36.2	44.9±21.6
Root weight(g)	17.0±16.7	8.4±1.9
Root length (cm)	166.8±81.5	180.4±73.5

ROOT BUD PRODUCTION

Spring collection	20.0±12.1	43.2±14.0
Fall collection	6.3±3.5	16.2±5.5

#- based on volume of 30X30X30 cm.

Appendix Q. Listing of Program for Matrix Model.

```
VAR T,R2,J1,J2,R1,K,I,L,LM,E,F,P,R4,R5=REAL
VAR R6,R7,FM,SM,MS,MF,J,MR,ZZ,N,A,D,B,C=REAL
VAR A$=STRING(5)
1000  MR=0
ZZ=0
INPUT "NUMBER OF DIFFERENT MATRICES";D
INPUT "MATRICES ORDER";N
DIM REAL X(D,N,N) G(N) Y(N) M(N) O(N)
DIM REAL Q(N) S(N) Z(N) FW(N) SW(N) H(N)
FOR A=1 TO D
PRINT "MATRIX NUMBER";A
FOR B=1 TO N
PRINT "ROW NUMBER";B
FOR C=1 TO N
INPUT X(A,B,C)
NEXT C
NEXT B
NEXT A
1160 A=0
1170 A=A+1
1175 J1=0
PRINT "MATRIX NUMBER";A
PRINT
FOR B=1 TO N
FOR C=1 TO N
PRINT X(A,B,C);
NEXT C
PRINT
NEXT B
PRINT
1250 PRINT "ARE THERE ANY VALUES THAT NEED TO BE CHANGED?"
GOSUB 10000
IF J=1 THEN 1280 ELSE 1330
1280 INPUT "WHICH ROW?";B
INPUT "WHICH COLUMN";C
INPUT "ENTER THE VALUE";X(A,B,C)
J1=1
GOTO 1250
1330 IF J1=1 THEN 1175 ELSE 1340
1340 IF A=D THEN 1360 ELSE 1170
1360 PRINT "DO YOU WANT TO ENTER THE POPULATION VECTOR?"
GOSUB 10000
IF J=1 THEN 1390 ELSE 1550
1390 FOR B=1 TO N
INPUT "VECTOR";G(B)
Y(B)=G(B)
NEXT B
1420  J2=0
PRINT "THE VECTOR IS"
```

```
FOR B=1 TO N
Y(B)=G(B)
PRINT G(B)
NEXT B
1460 PRINT "DO YOU WANT TO MODIFY ANY VALUES?"
GOSUB 10000
IF J=1 THEN 1490 ELSE 1540
1490 INPUT "WHICH ROW";B
INPUT "ENTER THE VALUE";G(B)
Y(B)=G(B)
J2=1
GOTO 1460
1540 IF J2=1 THEN 1420 ELSE 1590
1550 FOR B=1 TO N
G(B)=10
Y(B)=G(B)
NEXT B
1590 PRINT "DO YOU WANT TO DO STABLE-AGE DISTRIBUTION ONLY?"
GOSUB 10000
IF J=1 THEN 1630 ELSE 1650
1630 ZZ=3
GOTO 1781
1650 ZZ=0
PRINT "DO YOU WANT THE SPRING VECTOR RATIO  PRINTED FOR EACH"
PRINT "TIME UNIT?"
GOSUB 10000
IF J=1 THEN R1=1 ELSE R1=2
PRINT "DO YOU WANT THE ACTUAL SPRING VECTORS PRINTED?"
GOSUB 10000
IF J=1 THEN R2=1 ELSE R2=2
PRINT "DO YOU WANT TO LIMIT THE NUMBER OF TIME UNITS?"
GOSUB 10000
IF J=1 THEN 1750 ELSE 1780
1750 INPUT "ENTER THE NUMBER OF TIME UNITS";MR
GOTO 1781
1780 MR=0
1781 PRINT "DO YOU WANT TO LIMIT SOME VECTOR VALUES?"
GOSUB 10000
IF J=1 THEN 1784 ELSE 1787
1784 PRINT "ENTER THE VALUES"
FOR B=1 TO N
PRINT "ROW";B
INPUT H(B)
PRINT
NEXT B
PRINT #1
PRINT #1; "THE VECTOR VALUES ARE";
FOR B=1 TO N
PRINT #1; H(B)
NEXT B
PRINT #1
GOTO 1789
```

```
1787 FOR B=1 TO N
H(B)=2E+35
NEXT B
1789 IF ZZ=3 THEN 2000 ELSE 1790
1790 PRINT "DO YOU WANT THE RATE IF INCREASE PRINTED"
PRINT "FOR EACH TIME UNIT?"
GOSUB 10000
IF J=1 THEN R4=1 ELSE R4=2
R5=2
PRINT "DO YOU WANT THE FALL VECTOR RATIO PRINTED?"
GOSUB 10000
IF J=1 THEN R6=1 ELSE R6=2
PRINT "DO YOU WANT THE FALL VECTOR VALUES PRINTED?"
GOSUB 10000
IF J=1 THEN R7=1 ELSE R7=2
2000 P=0
MF=0
MS=0
FM=0
SM=0
2010 K=0
I=0
L=0
LM=0
E=0
F=0
FOR B=1 TO N
M(B)=0
O(B)=0
Q(B)=0
S(B)=0
Z(B)=0
NEXT B
P=P+1
A=0
2050 A=A+1
IF A=1 THEN 2070 ELSE 2080
2070 FOR B=1 TO N
K=K+Y(B)
NEXT B
2080 FOR B=1 TO N
FOR C=1 TO N
Z(B)=Z(B)+(X(A,B,C)*Y(C))
NEXT C
NEXT B
FOR B=1 TO N
IF Z(B)>H(B) THEN Z(B)=H(B)
NEXT B
FOR B=1 TO N
Y(B)=Z(B)
Z(B)=0
NEXT B
```

```
IF A=5 THEN 2120 ELSE 2150
2120 FOR B=1 TO N
M(B)=Y(B)
NEXT B
2150 IF A=D THEN 2160 ELSE 2200
2160 FOR B=1 TO N
O(B)=Y(B)
I=I+Y(B)
NEXT B
2200 IF A=D THEN 2210 ELSE 2050
2210 F=O(1)
E=M(1)
FOR B=1 TO N
IF E=0 THEN E=1
IF F=0 THEN F=1
IF E>M(B) THEN 2240 ELSE 2250
2240 E=M(B)
IF E=0 THEN E=1
2250 IF F>O(B) THEN 2260 ELSE 2270
2260 F=O(B)
IF F=0 THEN F=1
2270 NEXT B
FOR B=1 TO N
IF E=0 THEN E=1
IF F=0 THEN F=1
Q(B)=INT((M(B)/E)+.5)
S(B)=INT((O(B)/F)+.5)
NEXT B
IF K=0 THEN K=1
L=I/K
IF ZZ=3 THEN 2341 ELSE 2400
2341 PRINT
PRINT P;
IF D<5 THEN 2344 ELSE 2343
2343 FOR B=1 TO N
PRINT Q(B);
NEXT B
2344 FOR B=1 TO N
PRINT S(B);
NEXT B
FOR B=1 TO N
IF D<5 THEN 2348
IF FW(B)=Q(B) THEN 2347 ELSE 2348
2347 FM=FM+1
2348 IF SW(B)=S(B) THEN 2350 ELSE 2352
2350 SM=SM+1
2352 NEXT B
IF D<5 THEN 2356
IF FM=N THEN MF=MF+1 ELSE MF=0
2356 IF SM=N THEN MS=MS+1 ELSE MS=0
IF MF>10 OR MS>10 THEN 2372 ELSE 2360
2360 FOR B=1 TO N
```

```
FW(B)=Q(B)
SW(B)=S(B)
NEXT B
FM=0
SM=0
GOTO 2010
2372 R1=1
R2=1
R4=1
R5=1
R6=1
R7=1
MR=P
2400 PRINT #1;"FOR THE TIME UNIT";P
ON R1 GOTO 2420,2500
2420 PRINT #1;"THE SPRING VECTOR RATIO IS"
FOR B=1 TO N
PRINT #1;S(B);
NEXT B
PRINT #1
2500 ON R2 GOTO 2510,2600
2510 PRINT #1;"THE SPRING VECTOR VALUES ARE"
FOR B=1 TO N
PRINT #1;O(B);
NEXT B
PRINT #1
2600 ON R4 GOTO 2610,2700
2610 PRINT #1;"THE RATE OF INCREASE IS";L
2700 ON R5 GOTO 2710,2800
2710 PRINT #1;"THE SUM OF THE SPRING VECTOR IS ";LM
2800 IF D<5 THEN 3000
ON R6 GOTO 2810,2900
2810 PRINT #1;"THE FALL VECTOR RATIO IS"
FOR B=1 TO N
PRINT #1;Q(B);
NEXT B
PRINT #1
2900 ON R7 GOTO 2910,3000
2910 PRINT #1;"THE FALL VECTOR VALUES ARE"
FOR B=1 TO N
PRINT #1;M(B)
NEXT B
PRINT #1
3000 IF P=MR THEN 3050 ELSE 2010
3050 PRINT "THE RUN IS COMPLETED, MORE.....";A$
INPUT A$
3060  IF A$="M" THEN 3070 ELSE 3060
3070 PRINT "DO YOU WANT TO MODIFY THE MATRICES?"
GOSUB 10000
IF J=1 THEN 1160 ELSE 3110
3110 PRINT "DO YOU WANT TO CHANGE THE VECTOR?"
GOSUB 10000
```

```
IF J=1 THEN 1420 ELSE 3140
3140 PRINT "DO YOU WANT TO WORK WITH NEW MATRICES?"
GOSUB 10000
IF J=1 THEN 1000  ELSE 3170
3170 PRINT
10000 INPUT A$
IF A$="Y" THEN 10020 ELSE 10010
10010 IF A$="N" THEN 10030
10020 J=1
GOTO 10040
10030 J=2
10040 PRINT
10050 RETURN
```

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