STUDIES ON MASS CULTURING OF *PARANGUINA PICRIDIS* KIRJANOVA AND IVANOVA, AND ITS HOST-PARASITE RELATIONSHIP WITH *ACROPTILON REPENS* (L.) DC. (RUSSIAN KNAPWEED)

by

(c) OSAMA ANAS

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

Department of Plant Science
Macdonald Campus of
McGill University,
Montreal.

August 1983 

Short title

CULTURING OF *PARANGULINA PICRIDIS* AND ITS DAMAGE TO *ACROPTILON REPENS*

Osama Anas

Short title

CULTURING OF PARAMECIUM PICKIDIS AND ITS DAMAGE TO ACEPTILOM [illegible]

Osama Anas

DEDICATED TO MY FATHER

LATE PROF. MOHAMMAD ANAS.

ABSTRACT

M. Sc. OSAMA ANAS Agronomy

STUDIES ON MASS CULTURING OF *PARANGUINA PICRIDIS* KIRJANOVA AND IVANOVA, AND ITS HOST-PARASITE RELATIONSHIP WITH *ACROPTILON REPENS* (L.) DC. (RUSSIAN KNAPWEED)

Russian knapweed is an aggressive noxious introduced weed widespread in North America. The potential of the leaf and stem gall nematode *Paranguina picridis* Kirjanova and Ivanova as a biocontrol agent has been recognized. Tissue culture methods have been utilized to obtain large quantities of some nematode species, and could be utilized to mass produce *P. picridis* for inundative releases. Culturing of *P. picridis* has not previously been attempted. The B5 medium, with high concentration of macronutrients, vitamins, sugar, and low concentration of FeEDTA supported penetration of *P. picridis*. Nematode penetration also took place in cultures when gibberellic acid (GA_3) was added in the medium in combination with benzyladenine (BA) and α-naphthaleneacetic acid (NAA), but no nematode reproduction occurred.

Studies were conducted using radioactive tracers to determine the effect of *P. picridis* gall formation on Russian knapweed. The nematode galls act as powerful 'physiological sinks' causing considerable damage to the weed host and this study demonstrates the potential of *P. picridis* as an effective biocontrol agent.

SOMMAIRE

M. Sc. OSAMA ANAS Agronomy

ETUDES SUR LA CULTURE EN MASSE DE PARANGUINA PICRIDES KIRJANOVA AND IVANOVA ET SUR LES INTERACTIONS ENTRE L'AGENT PATHOGENE ET ACROPTILON REPENS (L.) DC. (CENTAUREE DE RUSSIE)

La centaurée de Russie est une mauvaise herbe introduite qui est répandue en Amérique du nord. Le potentiel du nématode Paranguina picridis Kirjanova and Ivanova comme agent de contrôle a déja été reconnu. Comme de larges quantités de certaine espèces de nématodes ont déja été produites grâce aux techniques de la culture de tissu, la culture de P. picridis pour distribution pourrait ainsi être réalisable quoi que cela n'ai jamais été entrepris auparavant. Le milieu B5, avec des concentrations élevées en macroéléments, vitamines, sucres et des faibles concentrations en FeEDTA a permis la pénétration de nématodes. Cette pénétration a aussi observée lorsque le milieu a été additionné de GA_3 en combinaison avec du benzyladenine (BA) et de l'acide α-naphthalèneacétique (NAA) mais les nématodes ne se sont pas reproduits.

Des études sur les effets de la formation de galles par P. picridis sur la centaurée de Russie ont été effectuées en utilisant des traceurs radioactifs. Ces galles agissent comme de puissantes zone de réception endommageant considérablement la plante hôte ce qui démontre le potentiel de P. picridis comme agent de controle biologique.

ACKNOWLEDGEMENTS

I wish to extend my appreciation and sincere gratitude to Dr. A.K. Watson for his continuous guidance, encouragement, support and constructive suggestions in the preparation of this thesis.

I thank Dr. N.N. Barthakur for the use of facilities in the W.F. Oliver Laboratory where the radiotracer work was performed.

My thanks are due to Dr. A.K. Watson, Dr. C. Chong, Dr. R.H. Estey and Dr. N.N. Barthakur for acting as members of my research committee.

The assistance of A. Virley in preparing photographs and photographic plates is greatly appreciated. I am also very grateful to my fellow colleagues and friends of the Plant Science Department of Macdonald College, who contributed in various ways, to Ms. C. Poirier for the French translation of the abstract, and to Mrs. W. Fogg for her excellent work in typing this thesis.

I owe debt to the Foreign Student Aid Office of McGill University and my uncle Dr. M.A. Malik, for the financial assistance which made this work possible. The research was supported, in part, from grants awarded to Dr. A.K. Watson by the Ministère de l' Agriculture, des Pêcheries et de l'Alimentation du Québec.

Last, but not least, I wish to extend my appreciation to my mother, Mrs. Z. Anas, whose moral support is always with me.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES (Cont'd)

LIST OF FIGURES

I INTRODUCTION

Russian knapweed (Acroptilon repens (L.) DC. (Canada Weed Committee, 1969) centaurée de Russie (Flore du Canada, 1974)), a member of the Centaureinae subtribe of the Cynareae (Cardueae) tribe of the Compositae family (Engler, 1964) is an introduced persistent perennial weed species which is commonly found in cultivated land in western Canada and infrequently in southern Ontario. It is listed as a prohibited noxious weed in the Seeds Act (Agric. Canada, 1967)

Russian knapweed is a native of southern Russia, Mongolia, western Turkestan, Iran, Turkish Armenia, Afghanistan and Asia Minor (Moore and Frankton, 1974) and was first introduced into Canada in the early 1900's as a contaminate of Turkestan alfalfa (Groh, 1940). Numerous introductions of Turkestan alfalfa seed occurred, but only since 1928 has Russian knapweed been recognized as a serious weed in Canada (Moore, 1969). The recognition of Russian knapweed as a "prohibited noxious weed" in 1936 probably has prevented more widespread infestations of this weed in Canada. The spread of Russian knapweed, after its initial introduction with alfalfa seed has been primarily due to the movement and sale of infested hay (Renney, 1959; Rogers, 1928). Dense infestations of Russian knapweed occur in cultivated fields of grain and alfalfa, pasture, roadsides and waste places and the weed is able to survive in almost any crop in any tillable soil (Rogers, 1928).

Actual crop losses caused by Russian knapweed have not been documented nor estimated in Canada (Watson and Harris, in press). It is reported as a serious noxious weed of dryland crops in southern U.S.S.R. and the quality of flour and other grain products is greatly reduced when contaminated with only 0.01% by weight of Russian knapweed seeds (Ivanova, 1966). The weed reduces yields of barley and maize from 5 to 10 fold and affects the quality of the yield (Agadzhanyan and Agadzhanyan, 1967). The yield of potatoes on plots infested with Russian knapweed decreased by half and both the size and quality of tubers were affected (Higgins, 1967). It is noted that Russian knapweed is the most effective competitor under dryland farming and the species has always constituted a serious hazard to crop production (Selleck, 1964). The grain yield of wheat and the foliage yield of maize is also reduced when plots are infested with Russian knapweed (Popov, Rashkin and Grudzev, 1973). Russian knapweed also has the tendency to form dense patches and to suppress the growth of other species. The allelopathic effects of Russian knapweed have been demonstrated with Russian knapweed infested soil inhibitory to tomato transplants and that plant extracts of Russian knapweed, especially the leaves and roots, inhibit the germination and growth of the radical of several plant species (Chernishov, 1967; Fletcher and Renney, 1963; Ladonin, 1968; Renney, 1958,1977; Renney and Dent, 1958). The sap from the foliage of the weed when brushed on leaves of fresh beans and oats seedlings, reduced their leaf surface area. The root sap was slightly less toxic (Berezovskii and Rashkin, 1971; Popov, Rashkin and Grudzev, 1973). In 1973 Bvetretova and co-workers found that the toxins present in the Russian knapweed are sesquiterpene lactones like repin, acroptilin and hyrcanin, and they also gave the structure of acroptilin. Seaman (1982) has reviewed sesquiterpene lactones as taxonomic characters in the Asteraceae. Russian knapweed is

also poisonous to livestock and has been shown to cause a neurological disorder and even death in horses (Young et al., 1970). No beneficial aspect of this weed has been reported (Watson, 1980). The biology of the plant has been recently reviewed by Watson (1980).

Russian knapweed, a persistent perennial, is difficult to control by cultural and chemical methods (Watson, 1975). Many different herbicides have been applied against Russian knapweed, but they have been found to be either too costly or not very effective. The control obtained by 2,4-D or dicamba has not been satisfactory (Fisyunov et al., 1977; Jones and Evans, 1973; Khodorovskii, 1969; Drumzkorov, 1974,1975, 1976; Raskin et al., 1978) when compared to 2,3,6-TBA or picloram which gave complete control (Agadzhanyan, 1968; Alley, 1976; Alley and Humburg, 1977,1978; Chernyshev, 1978; Kidrishev and Styazhkovoi, 1980; Miller, 1963; Mordovets et al., 1974). Picloram is the most widely used herbicide for Russian knapweed control, but the residual effect of the herbicide imposes a problem for sensitive crops, whereas residue in tolerant crop plants are potential health hazard to animals (Abramova, Panasyuk and Nikanorova, 1977; Berezovskii, 1974; Berezovskii and Krumzdorov, 1972; Gruzdev and Popov, 1974; Kidrishev and Styazhkovoi, 1980; Krumzdorov, 1976; Mordovets and Golovin, 1974,1976; Mordovets and Nazarenko, 1971). Therefore it is only feasible to use picloram along roads, right-of-way, ditch bank around farm and industrial buildings, etc. (Warden, 1964). Soil sterilants like fenac, benzabor, simazine and diuron + 2,4-D have also been used but they are not selective and so destroy associated desirable plant species (Alley and Chamberlain, 1964; Hopkins, 1961).

In North America, Russian knapweed is relatively free of specialized parasites and is not extensively attacked by polyphagous feeders, but in its native range, Russian knapweed is the host of a number of specialized organisms (Watson, 1980). From these parasites which have been recorded on the Russian knapweed, the potential of the leaf and stem gall nematode, Paranguina picridis Kirj. and Ivan. as a biological control agent has been recognized. The nematode produces galls on the stem, leaves and root collar of infected plants and the nematode galls cause considerable damage to the weed (Watson, 1975). In experiments with P. picridis to control Russian knapweed, Kirjanova and Ivanova (1969) showed that in plots inoculated with crushed gall material, 100% infection of knapweed was obtained and of these, 20% died and up to 30% were heavily infested. In an augmentation biological control progras in U.S.S.R. P. picridis is being sprayed in a water suspension (Kovalev et al., 1973).

Because of the reported host specificity (Ivanova, 1966) and because of its damaging effect on its host, the nematode P. picridis was imported into Canada from Alma Ata, Kazakh SSR under quarantine and investigated as a potential biocontrol agent of Russian knapweed (Watson, 1975).

The nematode was found to attack plants of the Centaureniae and Carduinae subtribes of the Cynareae tribe with the only plant susceptible to Paranguina picridis being Acroptilon repens, while other plants which formed galls ranged from intolerant to resistant to the nematode attack. When histological studies were conducted on these different galls, the variable host response to the nematode was confirmed. The galls on the Russian knapweed were the only ones which developed an extensive layer of nutritive cells with minimal necrosis, whereas in galls of other hosts the

nutritive zone was poorly developed and extensive necrosis was present (Watson, 1975,1977). Galls are pathologically developed cells, tissues or organs of plants having pronounced hypertrophy and hyperplasia which takes place due to the presence of the gall former. Nematode galls of aerial parts have a central cavity containing nematodes which is lined with a zone of nutritive cells. These cells are abundant in cytoplasm and the nematodes feed on them (Mani, 1964). It is assumed that the nutritive cells act as powerful physiological sinks attracting assimilates of the plant to the gall and gall former (Watson and Shorthouse, 1979).

Prior to unrestricted release of P. picridis in North America, additional information was requested on the effect of the nematode on the knapweed; the effect of the nematode on crop plants and the persistence of the nematode under field conditions in absence of the knapweed (Watson and Harris in press). The fears expressed against the nematode, that it might attack desirable knapweeds or even globe artichoke and become extremely difficult to erradicate have been proven groundless. The nematode has been given authorisation for release in Canada and United States (Watson and Harris in press).

The research conducted in this study had two objectives. Since P. picridis does not naturally spread great distances in the soil (Ivannikov et al. 1976; Kasimova, 1978), distribution of this biocontrol agent may require assistance (Kovalev, 1973; Watson and Harris, in press). Tissue culture methods have been utilised to obtain large quantities of some nematode species (Barker and Darling, 1965; Dasgupta et al., 1970; Eriksson, 1980; Inserra and O'Bannon, 1975; Johnson and Viglierchio, 1969a,1969b; Khera and Zuckerman, 1962; Krusberg and Babineau, 1979; Reversat, 1975), and

could be utilized to mass produce P. picridis for inundative releases. Therefore, the first objective of this research program was to determine if viable P. picridis larvae could be mass produced using tissue culture techniques.

The second objective of this research was to determine the effect of the gall forming nematode on the physiology of its host plant. Some controversy has occurred concerning the effect of gall former on their host plants (Harris, 1973; Pimentel, 1961). Harris (1973) stated that since gall formers had evolved a homeostasis with their hosts, serious damage to the host plant does not occur. Therefore studies were conducted to determine the effect of P. picridis gall formation on Russian knapweed.

II. MASS CULTURING OF NEMATODES

A. INTRODUCTION

a. TISSUE CULTURE

The concept that individual cells of an organism are totipotent is implicit in the statement of the cell theory (White, 1963). Schwann (1939) expressed the view that each living cell of a multicellular organism would be capable of developing independently if provided with the proper external conditions. A totipotent cell is one that is capable of developing by regeneration into a whole organism, and this term was coined by T.H. Morgan in 1901 (Krikorian and Berquam, 1969). In 1902, the German botanist, Gottlieb Haberlandt was the first to realize the totipotency of plant cells but he failed to obtain cell division in his cultures, which was in part due to the relatively simple nutrients and to his choice of highly differentiated mesophyll cells (Krikorian and Berquam, 1969).

Despite the fact that it has long been recognized that it is not strictly correct to refer to cultures of organs such as roots, stem, leaf and reproductive parts, cell cultures, callus cultures or embryo cultures as 'tissue culture', since they rarely are derived from or are comprised of specific tissues (Bailey, 1943), the term is still commonly used as a blanket phrase in the generic sense to cover all types of aseptic plant culture procedures (Krikorian, 1982; Street, 1977b).

The first successful organ culture was achieved by White (1934) with the demonstration of the potentially unlimited growth of excised tomato root tips. The difficulties which blocked the development of a successful method for culturing excised plant material were the problem of choosing the right plant material and the formulation of a satisfactory

nutrient medium (White, 1951).

The first plant tissue cultures, in the sense of longterm cultures of callus, involved explants of cambial tissues isolated from tobacco and carrot, were accomplished almost simultaneously by Gautheret (1939), Nobécourt (1939), and White (1939).

Skoog and Miller (1957) advanced the hypothesis that shoot and root initiation in cultured callus can be regulated by particular ratios of auxin and cytokinin. In addition to the cytokinins, there is evidence that other endogenous cell-division factors may exist in plant tissues (Wood et al. 1969).

Early experiments with shoot-apex cultures involved the degree of autonomy of this unit and the morphogenetic consequences of its isolation (Dodds and Roberts, 1982). First attempts in the culture of the shoot tip were done by White (1933) using Stellaria media (L.) Vill. Later Loo (1945) succeeded in culturing stem tips excised from Asparagus officinalis L., resulting in the formation of plantlets. The application of shoot-apex cultures in the rapid clonal propagation of plants was first realized by Morel (1960).

Several media have been developed by various workers to suit particular requirements of a cultured tissue (Narayanaswamy, 1977). The in vitro requirements for the growth and development of isolated shoot apices vary with the size of explant, the intended use of the culture, and the plant genotype. If the plant material has been cultured successfully in other laboratories, it is best to start with published methods (Dodds and Roberts, 1982). When attempting to establish a culture for the first time, the usual procedure is to test first a simple medium and then supplement as necessary (Yeoman and Forsche, 1980; Yeoman and MacLeod, 1977).

Nutritional factors play an important role in achieving success with isolated apical meristems (Morel, 1975). In plant tissue culture, the establishment of an autotropic culture has not been achieved. The basis of all nutrient media is a mixture of mineral salts combining the essential macro- and micro-elements together with a source of carbon which is almost always a sugar, and usually sucrose. The usual supplements required are vitamins, amino acids, sugar alcohols, growth regulators, a chelate such as EDTA and sometimes various natural extracts such as coconut milk, yeast extract or tomato juice (Yeoman and Macleod, 1977). Plant cells and tissues can grow on various formulations of media that are generally classified in two categories: low salt or high salt (Sharp and Larsen, 1979). A typical low salt medium is the one developed by White (1943), while the most universally used high salt mediums are MS Murashige and Skoog, 1962), and B5 (Gamborg and Wetter, 1975). The requirements in a medium for a particular organic supplement could be due to either the inability of the tissue to produce it or a new requirement resulting from a shift in metabolism (Dodds and Roberts, 1982). Additional information on the nutritional requirements of callus and suspension cultures can be found in the review by Ojima and Ohira (1978). Although agar media have been successfully used for the culture of apical meristems (Smith and Murashige, 1970), difficulty has been encountered with the culture of some isolated meristems (Romberger and Tabor, 1971).

Although explants of the apical meristem require exogenous hormones, the isolated shoot apex with primordia and emerging leaves may be independent of hormone supplements (Shabde and Murashige, 1979). The cultures require exogenous auxin, and the addition of a cytokinin may or may not be necessary (Shabde and Murashige, 1977; Smith and Murashige, 1970). Multiple shoot formation, desirable in clonal propagation, is

enhanced by supplementing the medium with high levels of cytokinins (Dodds and Roberts, 1982). Gibberellic acid provides a growth stimulus for some cultured systems, and may be required for the culture of certain shoot apices (Morel, 1975). Summaries of the problems involved in plant propagation by tissue culture have been written by de Fossard (1976) and Murashige (1974). Information on the practical aspects of the procedures involved can be obtained from the following books: Barz et al. (1976), Butcher and Ingram (1976), Davies and Hopwood (1980), Dodds and Roberts (1982), Gamborg and Wetter (1975), Gautheret (1959), Ingram and Helgeson (1980), Kruse and Patterson (1973), Merchant et al. (1960), Paul (1975), Pierik (1979), Reinert and Bajaj (1977), Rothblat and Cristofalo (1972), Street (1974,1977b), Thomas and Davey (1975), Thorpe (1978), White (1963), White and Grove (1965), Willmer (1966), and reviews by Dougherty (1975), Gautheret (1955), Hollings (1965), Krikorian (1982), Krikorian and Berquam (1969), Murashige (1974), and White (1951).

Plant tissue cultures have been used extensively for the study of cytodifferentiation (Bornman, 1974; Fukuda and Komamine, 1980; Jeffs and Northcote, 1967; Roberts, 1976; Street, 1977a; Wetmore and Rier, 1963; Wetmore and Sorokin, 1955), and experimental somatic embryogenesis has also been reported in tissues cultured from more than 30 plant families (Dodds and Roberts, 1982; Narayanaswamy, 1977).

The potential of tissue culture as a tool in plant science has been recognized, and the importance of this multi-disciplinary approach indicates exciting prospects in the fields of cell biochemistry, biochemical genetics, plant breeding, cell and plant physiology and plant pathology (Street et al. 1965). A list of old problems with new perspectives using plant tissue or cell cultures is aptly indicated by Street (1977b).

b. NEMATODE CULTURE

Plant tissue culture techniques were originally developed for the study of fundamental problems of nutrition and morphogenesis, and have led to many important advances in these fields. More recently, as techniques and media have improved, it has become increasingly clear that tissue cultures provide simplified experimental systems for use in other branches of plant science, including plant pathology (Ingram, 1980). General reviews dealing with the applications of tissue culture techniques in plant pathology have been published by Braun and Lipetz (1966); Ingram (1976, 1977, 1980), Maheshwari (1969), and White (1968).

There are many potential advantages in using tissue culture techniques to study plant diseases since the inciting organisms may be cultured together with their hosts in a controlled chemical and physical environment, free from contamination. This offers the attractive possibility of a simplified experimental system for investigating the structure and physiology of host-parasite interaction and, in the case of specialized parasites, provides a means of maintaining continuous supplies of contaminant-free propagules. In addition, metabolic inhibitors and precursors may be added to culture media and diffusible products of interaction may be extracted with comparative ease (Ingram, 1977).

One of the earliest applications of plant tissue culture involved the study of plant tumor physiology (White and Braun, 1942) and a recent summary of this field has appeared (Butcher, 1977). Other applications of plant tissue culture procedures in plant pathology with special references to nematology are outlined by Amerson and Mott (1982); Ingram (1976, 1977); Jones (1980); Krusberg and Babineau (1979), and Zuckerman (1971).

The main problem in culturing phytoparasitic nematodes is in sterilizing the original inoculum, a delicate balance is required, so that the contaminant microorganisms are killed without injury to the nematodes (Jones, 1980). Plant parasitic nematodes belonging to the order Tylenchida are usually free from internal contamination and may be rendered aseptic by simple surface sterilization of egg masses, cysts or larvae, sometimes linked with antibiotic treatment (Cairns, 1975; Zuckerman, 1971). Metcalf (1903) was apparently the first to axenize a nematode (Rhabditis brevispina (Claus, 1862) Butschli, 1873 associated with plant decay, when he obtained pure cultures by washing eggs in sterile water and then isolating uncontaminated eggs from sterile agar. Byars (1914) succeeded in carrying a root-knot nematode (Meloidogyne sp.) through a complete life cycle, in a monoxenic culture, on sterile tomato seedlings growing in test tubes on nutrient agar medium. Later Polychronopoulus and Lownsbery (1968) cultured nematodes on seedlings within enclosed containers and concluded that the technique proved unsatisfactory for long-term maintenance of nematode cultures since the plant container was soon outgrown by the plant material.

The culture of Pratylenchus minyus Sher and Allen, 1953, on sterile excised corn roots represented the next significant advance in the propagation of germfree nematodes (Mountain, 1954, 1955). Tiner (1960, 1961a,b) refined the techniques for indefinite propagation of lesion nematodes P. penetrans (Cobb, 1971) Filipjev and Schuurmans Stekhoven on corn roots in culture, and also developed a collection trap for axenic nematodes. Feder and Feldmesser (1955, 1957) reported that Radopholus similis (Cobb, 1893) Thorne 1949, the burrowing nematode, completed its life cycle in roots of citrus seedlings growing on water agar. Dasgupta et al. (1970) utilised root cultures of Sorghum vulgare, Pers. to propagate Hoplolaimus indicus Sher, 1963, and defined its embryology and life

cycle. Heterodera rostochiensis Wollenweber, 1923, the potato cyst nematode, developed to mature females in tomato roots in culture (Widdowson et al. 1958). Apparently, males required to fertilize the females were lacking in these cultures so the females produced no eggs. This economically important nematode has still not been carried through a complete life cycle in a plant tissue culture (Eriksson, 1980). Moriarty (1964) obtained development of the beet cyst nematode, Heteradera schachtii Schmidt, 1871, on excised sugar beet roots in culture, but no eggs were produced by the females because all the males settled to the bottom of the culture dishes before fertilizing the females, whereas Johnson and Viglierchio (1969b) obtained embryonated eggs produced by females of this species, but did not carry the nematodes through a second or succeeding generations on the roots. Brown (1974) found embryonated eggs in females of the oat cyst nematode, H. avenae Filipjev, 1934, growing in the roots of wheat seedlings in test tube cultures on nutrient agar medium. Reversat (1975) also found eggs in females of rice cyst nematode H. oryzae Luc and Berdon Brizuela, 1961, growing on intact rice plants in test tubes.

In 1957, Darling and co-workers propagated the potato rot nematode, Ditylenchus destructor Thorne, 1945, on callus tissues of several plants. This report initiated the current period in which many species of plant parasitic nematodes have been propagated on different plant callus tissues. Recent reviews on this topic include Ingram (1976, 1977), Krusberg and Babineau (1979), and Zuckerman (1971). It is interesting that the pattern of host-nematode specificity appears to break down in tissue culture (Ingram, 1977). Darling et al. (1957) reported that D. destructor could be grown in undifferentiated callus of potato, carrot, clover and tobacco, whereas Faulkner and Darling (1961) found that D. destructor

reproduced well on undifferentiated callus cultures of clover, but failed to reproduce on root culture of clover or tomato. Dolliver et al. (1962) found that crysanthemum nematode Aphelenchoides ritzemabosi (Schwartz, 1911) Steiner and Buhrer, 1932, grew and multiplied on callus of tobacco, carrot, periwinkle and marigold. Similarly, Webster (1966) and Webster and Lowe (1966) found that A. ritzemabosi grew on callus of a number of species, including oat, rose, potato and red clover, all nonhost of the pathogen, and also on callus of alfalfa, a normal host (Webster, 1967b).

Many clones of several varieties of clover and alfalfa have proven to be good substrates for Ditylenchus dipsaci (Kuhn, 1857) Filipjev, 1936 (Bingefors and Eriksson, 1963). Krusberg (1961) experimented with alfalfa, carrot, sweet potato, tobacco and soybean and found that alfalfa provided the best reproduction of D. dipsaci and Pratylenchus zeae Graham, 1951. The reproduction of A. ritzemabosi and D. dipsaci was better on alfalfa callus than on seedlings, whereas P. zeae reproduced well on alfalfa callus but not at all on intact seedlings, whereas Schroeder and Jenkins (1963) showed that Pratylenchus penetrans reproduced better on callus than root cultures of eleven different plants. Tylenchus agricola de Man, 1884, and Tylenchorhynchus claytoni Steiner, 1937, are seen to reproduce well on alfalfa callus, but not on callus of tomato, broccoli, carrot, cabbage, rye or corn (Khera and Zuckerman, 1962). Feder et al. (1962) found that okra callus proved better substrate than citrus seedlings for Radopholus similis, but later studies showed that alfalfa callus was superior to okra callus (Myers et al. 1965). Dolichodorus heterocephalus Cobb, 1914 reproduced on corn callus developed from root tips, but not on alfalfa callus developed from seedling (Paracer and Zuckerman, 1967). Paratylenchus projectus Jenkins, 1956, reproduced well on white clover callus but not on alfalfa callus (Townshend, 1974). However, it has been reported that some

nematodes will not infect or achieve full development in undifferentiated tissue. For example, Sayer (1958) found that Meloidogyne incognita (Kofoid and White, 1919) Chitwood, 1949, would not grow on purely undifferentiated tissues of tomato, but would grow and complete its life cycle in tissues having some degree of vascular development. Miller (1963) obtained all stages of development of Meloidogyne hapla Chitwood, 1949 in tomato callus, but vascular tissue could have been present in the callus. Furthermore, it has been observed that callus tissues of plants that are resistant to, or are nonhosts of a nematode in nature will frequently support good reproduction of that nematode (Bingefors and Bingefors, 1976; Krusberg, 1961; Krusberg and Blickenstaff, 1964; Viglierchio et al. 1973; Webster and Lowe, 1966).

Studies have been conducted on the effects of temperature (Dolliver et al.,1962; Inserra and O'Bannon, 1975; Johnson and Viglierchio, 1969a,b; Lownsbery et al.,1967; Prasad and Webster, 1967), the addition of plant growth substances (Barker and Darling, 1965; Dolliver et al., 1962; Faulkner et al.,1964; Krusberg, 1961; Lownsbery et al.,1967; McClure and Viglierchio, 1966a, b; Sandstedt and Schuster, 1966a,b; Webster, 1966, 1967a,b; Webster and Lowe, 1966), the addition of inhibitors (Webster, 1967b), and the effect of different nutrient media or varying the constituents in the medium (Barker and Darling, 1965; Bingefors and Bingefors, 1976; Dolliver et al.,1962; Faulkner et al., 1974; Johnson and Viglierchio, 1969b; Krusberg, 1961; Krusberg and Blickenstaff, 1964; McClure and Viglierchio, 1966a,b; Riedel and Foster, 1970; Riedel et al., 1973; Schroeder, 1963; Schroeder and Jenkins, 1963; Tamura and Maniya, 1975) on nematode growth in callus cultures.

For the establishment of a successful meristem culture in which differentiation and plant development takes place, it is necessary to determine the choice of medium, culture conditions and the growth hormone ratio (Gamborg and Wetter, 1975). On the other hand, for the culturing of the nematodes, a reasonable balance between nematode growth and reproduction and tissue growth is needed for successful long term maintenance of the association in culture (Jones, 1980). Medium solidified with agar have usually been found to be better substrates than liquid cultures (Sayre, 1958).

Paraneuina picridis Kirj. and Ivan, is a highly specialized nematode and its potential as a biological control agent of Russian knapweed has been demonstrated (Watson, 1975; Watson and Harris, in press). It has been shown that plant endoparasitic nematodes have evolved the ability to induce morphological changes in the host cells to form feeding sites (Jones, 1981), and the nutritive cells present in these sites act as powerful "physiological sinks" attracting assimilates to the gall and gall former (Jankiewicz et al., 1969). The nutritive cells and and syncytia in nematode galls serve in the same manner (Bird and Loveys, 1975; Watson, 1975). The mechanism by which the nematodes trigger the development of these cells is not known (Skinner et al., 1980).

It has been reported that migratory nematodes (e.g. Ditylenchus spp., Aphelenchoides spp., Pratylenchus spp.) are being maintained routinely in 'nematode banks' on suitable callus tissues. In addition attempts to maintain sedentary types of endoparasitic nematodes (e.g. Globodera spp., Heterodera spp.) are continuing (Bingefors and Bingefors, 1976; Eriksson, 1980). However, nematodes forming galls on aerial parts of the plant have never been axenically cultured. Since P. picridis does not naturally spread great distances in the soil (Ivannikov et al., 1976; Kasimova, 1978) its

distribution may require assistance (Kovalev, 1973; Watson and Harris, in press). The primary objective of the research conducted was, therefore, to develop a defined axenic culture system using tissue culture for the mass culture of viable P. picridis larvae for inundative releases. The use of different nutrient media, the use of different ratios of added growth regulators and even the combination of different techniques for surface sterilizing the nematode inoculum were all components of the objective. Furthermore, it is suggested that subsequent studies should be conducted to investigate the nutritional and hormonal factors involved in the host-parasite interaction.

II.B. MATERIALS AND METHODS

a. AXENIZATION (NEMATODE STERILIZATION)

A major problem in culturing phytoparasitic nematodes is the sterilization of the original inoculum. For successful results, clean preparations of active nematodes are required. Axenization procedures reported in literature have certain limitations and no uniform, consistent method has been devised. Therefore, in this study different methods were tried to obtain large quantities of sterilized nematodes for inoculation of Russian knapweed tissue cultures. The procedures followed are presented in Table 1.

TABLE 1. TECHNIQUES FOR AXENIZING PLANT PARASITIC NEMATODES

PROCEDURE			REMARKS	REFERENCES
Pre sterilisation	Sterilisation	Post Sterilisation		
OLD GALLS				
Scrub clean in sterile water. Submerge in 70% ethanol 10-15 sec. Wash in sterile dist. H_2O.	Submerge for 10-15 min. in 2% sodium hypochlorite, agitate. Wash 4-5 times in sterile dist. H_2O.	Place gall on agar medium containing .05g/L malachite green + .05g/L streptomycin sulfate	Contamination after 3 days	Dodds & Roberts, 1982 Fenwick, 1956 Gamborg & Wetter, 1975 Lapage, 1933 Weinstein & Jones, 1956 Yeoman & Macleod, 1977
FRESH GALLS				
As above	As above	As above	Contamination when galls cut open after 5 days	As above
INDIVIDUAL NEMATODES				
1. Old galls cut open and agitated in water by passing air 3-4 hours. Nematodes collected by Baermann's funnel technique under sterile conditions. Washed 3-4 times in sterile distilled water.	2 serial transfers to streptomycin sulfate, 5-6 transfers of sterile dist. water.	Nematodes transferred to agar	40 to 50% contamination	Barker & Darling, 1965

TABLE 1. (Cont'd.)

PROCEDURE			REMARKS	REFERENCES
Pre sterilization	Sterilization	Post Sterilization		
ii. As above	Batches of 100 nematodes in 100 ppm $HgCl_2$ + 1% streptomycin sulfate 2 minutes	As above	30% contamination	Dolliver et al., 1962
iii. As above	Nematodes passed singly through 5-6 baths of 20 ppm malachite green + 1000 ppm streptomycin sulfate (3-4 hrs).	As above	No contamination. Time consuming. Method not suitable for large quantities of nematode	Krusberg, 1961 Chen et al., 1961
BULK NEMATODES				
Nematodes collected by Baermann's funnel technique (20 ppm malachite green + 1000 ppm streptomycin sulfate in incubation water). Nematodes washed 3-4 times in sterile dist. H_2O.	Nematodes washed in 100 ppm $HgCl_2$ for 2 minutes. Then 3-4 times with streptomycin sulfate (1000 ppm) + malachite green (30 ppm). Finally 4-5 times with sterile distilled water	Nematodes transferred to agar plates. If nematodes not utilized for inoculation, they were stored at room temp. on agar medium containing .05g/L malachite green + .05 g/L streptomycin sulfate.	No contamination No contamination	Barker & Darling, 1965 Chen et al., 1961 Christie & Crossman, 1936 Dolliver et al., 1962 Krusberg, 1961 Lownsbery & Lownsbery, 1956 Mountain & Patrick, 1959

b. RUSSIAN KNAPWEED AND NEMATODE CULTURE

1. PLANT MATERIAL

Russian knapweed roots were collected from a field site on the Macdonald Campus of McGill University, Ste. Anne de Bellevue, Quebec, and after washing, they were cut into approximately 7-8 cm. segments. The root segments were potted in vermiculite in 10 cm diameter pots. The pots were placed in a growth chamber, with the following environmental conditions: temperature night 10°C, day 20°C; day length 14 hr; light intensity 75 microeinsteines ($m^{-2}s^{-1}$) (one microeinsteine = 6.023×10^{7} photons); relative humidity 30%. The pots received an excess of modified Hoagland's solution (Hoagland and Aron, 1938-Appendix A), with 10.5 ppm nitrogen supplied as ammonium nitrate (NH_4NO_3) every second day, and on alternate days received sufficient distilled water to reach saturation.

When the plants were 1½ months old, the terminal bud was cut off to remove apical dominance. After a week the lateral buds, which were 0.5 to 1.0 cm long, were removed from the parent plant with a sharp razor blade.

2. PREPARATION OF STERILE TISSUE

All operations were performed aseptically in a laminar flow cabinet under sterile conditions. The instruments (scalpels, needles and forceps) were sterilized by immersing them in 70% ethanol and dried with sterilized filter/blotting paper. Before using each instrument, it was dipped in 95% ethanol and flamed and allowed to cool.

Shoot apices were placed in a Syracuse watch glass and the leaves were removed. Apices were then submerged in 70% ethanol for 20-30 seconds, transferred to 50% (v/v) commercial bleach (Javex) solution for 5-10 minutes, and washed 5-6 times in sterile distilled water.

3. DISSECTION OF MERISTEMATIC DOMES

The dissection of the meristematic domes followed a procedure adapted from Gamborg and Wetter (1975), and were performed aseptically using a binocular microscope within the laminar air flow cabinet. The meristematic domes are enclosed within many whorls of leaves and are usually free of contamination.

The disinfected shoot apex was held with a pair of forceps with one hand under a workable magnification (10 x 50X) of the microscope. The outer whorls of leaves were removed with sharp sterile scalpel until the meristematic dome was reached. The meristematic domes contain portions of procambial tissue and two to three leaf primordia. After four cuts at the base of the meristematic domes at right angles to each other, the dome was gently removed and immediately placed on the nutrient agar medium.

4. NUTRIENT AGAR MEDIUM

The media, MS of Murashige and Skoog (1962) (Appendix B) and B5 of Gamborg and Wetter (1975) or its modifications (Appendix C), were used in the subsequent studies. The MS and B5 media have been formulated to support the growth of a wide variety of tissues of both monocotyledons and dicotyledons (Narayanaswamy, 1977).

5. GROWTH HORMONES

In the following experiments, the cytokinin used was benzyladenine (BA), α-naphthaleneacetic acid (NAA) was the auxin employed and gibberellic acid (GA_3) was the gibberellin used. The gibberellic acid was filter sterilized by passing through Nalgene$^{(R)}$ filter unit (Sybron/

Nalge) with a 0.45 micron size filter and was added under sterile conditions after autoclaving the medium.

6. TYPE AND SIZE OF CULTURE APPARATUS

Twenty ml of medium were dispensed into 250 ml Erlenmeyer flasks, the openings covered with aluminum foil and the flasks autoclaved at 15 psi and 115°C for 15 minutes. After autoclaving, the flasks were allowed to cool at room temperature and the medium could be stored for 6-8 weeks at 4°C.

7. GROWTH CONDITIONS

The cultures were incubated on a growth bench in the laboratory at 25 ± 2°C and 16 hr day length with a light intensity of 65 micro-einsteines ($m^{-2}s^{-1}$) using fluorescent 'cool white' light.

8. HISTOLOGICAL METHODS

i. FIXATION

Cultures were removed from the flask and any agar sticking to the callus was carefully removed. The callus or plant tissues were cut into pieces not larger than 1.0 cm^3 and transferred into vials containing FAA fixative (formalin-acetic-alcohol) 'Pempel's mixture' (Appendix D). The vials were evacuated to remove trapped air in the callus or plant tissues. Tissue specimens were kept in fixative at room temperature for at least 48 hr.

ii. DEHYDRATION AND EMBEDDING

Specimens were removed from the fixative and washed thoroughly 2 to 3 times with water at room temperature and were left in water for at least 2 hr or preferably overnight. The water was replaced by transferring

through an ethanol series and eventually to Tertiary Butyl Alcohol (TBA). The dehydrating sequence through to TBA is listed in Appendix E. Specimen in 100% TBA should be kept at temperature higher than 26°C because TBA solidifies at 25.5°C. The tissues were placed in a 1:1 solution of TBA:parafin oil, then 1/3 of the TBA:parafin oil was poured off, and melted paraplast (60°C) added and specimens were left overnight in the oven at 60°C. The TBA:parafin oil:paraplast was replaced by pure paraplast and exchanged 2 times during one day with specimens remaining in the oven at 60°C. Specimens were embedded in pure paraplast and the blocks were trimmed for sectioning (Berlyn and Miksche, 1976; Jensen, 1962; O'Brien and McCully, 1981).

iii. SECTIONING

Sections of 25 μ thickness were cut using a AO Spencer No. 820 Rotary Microtome. Ribbons with groups of 4-5 sections were floated on water on clean slides with very thin layer of Mayer's adhesive (Albumen and Glycerin). Ribbons were gradually stretched on a warm plate (40°C). The slides were drained and then left overnight for drying on the warm plate.

iv. STAINING AND MOUNTING

When preparations were perfectly dry, they were stained in Safranin (1% in 50% ethanol) and Fast Green FCF (0.5% in 95% ethanol) and cleared in carbol-xylene and xylene (Berlyn and Miksche, 1976; O'Brien and McCully, 1981) (Appendix F). The stained specimens were mounted in Canada balsam, covered with a coverslip dried overnight in an oven at 30-40°C.

EXPERIMENT 1

The selection of the nutrient medium was the initial step in the tissue culture study. MS and B5 medium were prepared without any hormones and 20 ml were dispensed into 250 ml Erlenmeyer flasks and autoclaved.

Shoot tips of about 1 cm long were collected from Russian knapweed plants growing in growth chambers and after surface sterilization they were put on either MS or B5 medium and incubated on growth benches in the laboratory. Observations were made over a period of five weeks on the growth of the young shoots to determine which medium was superior in supporting Russian knapweed growth.

EXPERIMENT 2

Using B5 medium with 5 levels of auxin (NAA) and cytokinins (BA), 0.01, 0.1, 1.0, 5.0 and 10.0 mg/L, 25 combinations were obtained and following the procedures mentioned before, meristem cultures were made. Observations were made over a period of 5 weeks on the growth of the cultures.

In a similar experiment, the established cultures (1 week old) were inoculated with an aliquot of sterile nematode inoculum containing approximately 50 nematodes in 1 ml of water, under sterile conditions to avoid contamination. After one month, the cultures were fixed in FAA (formalin-acetic-alcohol) 'Pempel's mixture' (Appendix D), and dehydrated through TBA (Tertiary Butyl Alcohol) series and embedded in paraplast (Appendix E). Sections were cut at 25 μ using a AO Spencer No. 820 Rotary Microtome and were stained with Fast Green FCF and Safranin (Appendix F).

EXPERIMENT 3

Since nematode penetration occurred in none of the previous cultures, another experiment was conducted taking in account the possible environmental and nutritional conditions which play a role in the penetration of the nematode in the host tissue. It is known that the penetration of P. picridis in nature takes place when the Russian knapweed is emerging or is at the soil level (Watson, personal communication). B5 medium with 1.0 mg/L of BA and 0.01 mg/L of NAA was selected, because shoot multiplication and development was best with this hormonal ratio. Once the cultures were established (10 days) they were covered with moist, sterile perlite or vermiculite which had been autoclaved and inoculated with an aliquot of sterile nematode inoculum containing approx. 50 nematodes in 1 ml of water, as in the previous experiment. Since plant growth was slow and the plants did not appear through perlite or vermiculite, GA_3 was applied to some of the cultures at a rate of 0.5 mg/L. Fifteen days after GA_3 application half the plants from each treatment were taken out, fixed in FAA and sectioned as in the previous experiment.

It has been mentioned previously that the modification of the culture medium should be done according to the objectives of the experiment. In the second part of this experiment, the B5 medium was therefore modified based on the information, which suggested increased penetration and reproduction of the nematodes in tissue culture (Dolliver et al., 1962; Krusberg and Blickenstaff, 1964; McClure and Viglierchio, 1966b).

Two mediums, B5 mod. with higher concentrations of macronutrients, sucrose and vitamins and low iron chelate and B5½ mod. with low concentrations of macronutrients, sucrose, vitamins and FeEDTA

(Appendix C) were formulated and culture procedures were followed as mentioned above. Hormones were supplied at a rate of 1.0 mg/L BA and 0.01 mg/L NAA. The inoculated cultures were incubated for one month, fixed in FAA, embedded in paraplast and after sectioning, stained with safranin and fast green.

EXPERIMENT 4

Since the penetration of the nematodes still posed a problem in this study, it was decided that beside nutritive factors, gibberellic acid might be playing a role in the penetration of the nematodes in cultures.

In this experiment, using B5 medium with 3 levels of BA, NAA and GA_3 which were 0.01, 0.1 and 1.0 mg/L, 27 combinations were obtained and meristem culture was performed following the procedures mentioned above. In the second part of this experiment, the cultures were inoculated with nematodes and incubated for 1 month. The cultures were fixed in FAA and sectioned following the above mentioned procedures.

II.C. RESULTS

EXPERIMENT 1.

The cultures made using short apices on B5 medium of Gamborg and Wetter (1975) and MS medium of Murashige and Skoog (1962) without any growth hormones, showed the following growth pattern over a period of five weeks. The data summary is presented in Table 2.

TABLE 2. OBSERVATIONS MADE ON CULTURES OF SHOOT APICES ON B5 and MS MEDIUM WITHOUT GROWTH HORMONES OVER A PERIOD OF 5 WEEKS

MEDIUM	WEEK 1			WEEK 2			WEEK 3			WEEK 4			WEEK 5		
	# of plants	Height of mother plant	Remarks	# of plants	Height of mother plant	Remarks	# of plants	Height of mother plant	Remarks	# of plants	Height of mother plant	Remarks	# of plants	Height of mother plant	Remarks
MS	1	1 cm	Plant looking normal. No change	1	1 cm	Plant looking normal. No change	2	1.5 cm	Slight callus at base of shoot. Leaves thickening and fleshy	3	3.2 cm	Callus enlarged 1 cm. Leaves fleshy, thick, shining and long	4	5 cm	Callus turning slightly brown. Leaves fleshy, thick, shining and long
B5	1	1 cm	Plant looking normal. No change	1	1.5 cm	Plant slightly grown. Normal. No change	2	2 cm	Slight callus at base .5 cm Leaves green. Normal	2	3 cm	Slight callus leaves green and normal	3	3.5 cm	Slight callus Leaves green and normal

EXPERIMENT 2.

Meristem cultures were made on B5 medium with 5 levels of cytokinin (BA) and auxin (NAA) which in total gave 25 combinations. In a similar experiment, the cultures were inoculated with nematodes and after one month the cultures were fixed, sectioned, and stained. The results are presented in Table 3.

From Table 3 the results show that the cultures on medium with high levels of BA (5 or 10 mg/L) did not give a positive response. There was very little shoot development and callus formation. The cultures started browning after one month. There was also very little or no differentiation in time of callus.

Medium with high levels of NAA (5 or 10 mg/L) and with lower levels of BA (0.01, 0.1 or 1.0 mg/L) had callus formation but there was no differentiation of tissue in the callus.

When the levels of BA in comparison to NAA were higher (e.g. 0.1 or 1.0 mg/L BA with 0.01 or 0.1 mg/L NAA) the medium supported very good shoot multiplication and development (Figure 1) and the plantlets could be subcultured and established (Figure 2). The callus in these cultures also showed tissue differentiation.

In the medium with the lowest level of BA (0.01 mg/L) and with equal or slightly higher levels of NAA (0.01, 0.1 or 1.0 mg/L) there was slight callus formation which had little tissue differentiation. Root development was observed in medium with 0.1 or 1.0 mg/L NAA.

There was no nematode penetration in any of the cultures and therefore no gall formation took place.

TABLE 3. B5 MEDIUM WITH 5 LEVELS OF CYTOKININ AND AUXIN (25 COMBINATIONS). INOCULATED WITH APPROXIMATELY 50 NEMATODES AND FIXED AFTER 1 MONTH

B5 medium mg/L BA	B5 medium mg/L NAA	Differentiation of time in callus †	Number of plantlets	Cultures without nematodes (control) formation of callus, roots, plant initiation or plant development	Nematode penetration (in section) ††	Gall formation
.01	.01	+	1	Slight callus, little shoot development	-	None
.01	.1	+	3	Slight callus formation, slight root formation	-	"
.01	1.0	+	2	Slight callus formation, root development after 15 days	-	"
.01	5.0	-	1	Callus formation	-	"
.01	10.0	-	1	Slight callus formation	-	"
.1	.01	++	4	Little shoot multiplication and development	-	"
.1	.1	++	3	Little shoot multiplication and development	-	"
.1	1.0	+	2	Slight callus formation, little shoot development	-	"
.1	5.0	-	1	Slight callus formation	-	"
.1	10.0	-	1	Slight callus formation	-	"
1.0	.01	++	7	Shoot multiplication and development*	-	"
1.0	.1	++	6	Slight callus formation, shoot multiplication and development	-	"
1.0	1.0	++	4	Callus formation, little shoot multiplication and development	-	"
1.0	5.0	+	2	Slight callus formation	-	"
1.0	10.0	-	1	Slight callus formation	-	"
5.0	.01	++	3	Slight shoot development	-	"
5.0	.1	+	2	Slight shoot development	-	"
5.0	1.0	-	1	Slight shoot development	-	"
5.0	5.0	-	1	Very slight callus formation	-	"
5.0	10.0	-	1	Very slight callus formation	-	"
10.0	.01	-	1	Very slight shoot development	-	"
10.0	.1	-	1	Very slight shoot development	-	"
10.0	1.0	-	1	Very slight shoot development	-	"
10.0	5.0	-	1	Very slight shoot development	-	"
10.0	10.0	-	1	Very slight shoot development	-	"

† - = not differentiated
+ = slightly to moderately differentiated
++ = highly differentiated

†† - = no penetration
+ = penetration

* Photograph of plant showing shoot multiplication

EXPERIMENT 3.

Using B5 medium or its modifications with 1.0 mg/L of BA and 0.01 mg/L of NAA, the following results, presented in Table 4, were obtained.

All the cultures developed callus to some degree and there was slight differentiation of cells in the callus tissue in cultures on B5/perlite and B5/vermiculite which were incubated for 15 days. Cultures from the modified B5 medium (B5 mod) with higher concentrations of macronutrients, sucrose and vitamins and low FeEDTA which were incubated for 15 or 30 days had highly differentiated tissue in the callus whereas the rest of the cultures had moderately differentiated tissues in their callus (Table 4). Nematode penetration did not take place in any of the cultures which had perlite or vermiculite, with or without GA_3 at after 15 or 30 days of incubation. Only one culture (Figure 3) from the modified B5 medium (B5 mod) had nematode penetration (Figure 4) and there was very slight cavity formation around the nematodes.

TABLE 4. B5 MEDIUM OR ITS MODIFICATIONS, INOCULATED WITH NEMATODES

B5 MEDIUM 1.0 mg/L BA: 0.01 mg/L NAA	Differentiation of tissue in callus *	Plant or Callus Growth	Nematode ** penetration	Gall formation
B5/perlite				
(15 days)	+	Leaves thin, transparent, slight callus, plant did not emerge	-	None
(30 days)	++	" " " " " " " " "	-	"
B5/vermiculite				
(15 days)	+	" " " " " " " " "	-	"
(30 days)	++	" " " " " " " " "	-	"
B5+GA/perlite				
(15 days)	++	Leaves long, slight callus, plant emerged	-	"
(30 days)	++	" " " " " "	-	"
B5+GA/vermiculite				
(15 days)	++	" " " " " "	-	"
(30 days)	++	" " " " " "	-	"
B5 with higher concentrations of macronutrients, sucrose and vitamins and low FeEDTA (B5 mod)				
(15 days)	+++	Slight callus and plant multiplication	-	"
(30 days)	+++	Slight callus, plant multiplication and development ***	+ (one culture only)	slight cavity formation
B5 with low concentrations of macronutrients sucrose, vitamins and FeEDTA (B5½ mod)				
(15 days)	++	Slight callus and little plant multiplication	-	None
(30 days)	++	Slight callus, little plant multiplication and development	-	"

* - = no differentiation
\+ = slightly differentiated
++ = moderately differentiated
+++ = highly differentiated

** - = no penetration
\+ = penetration

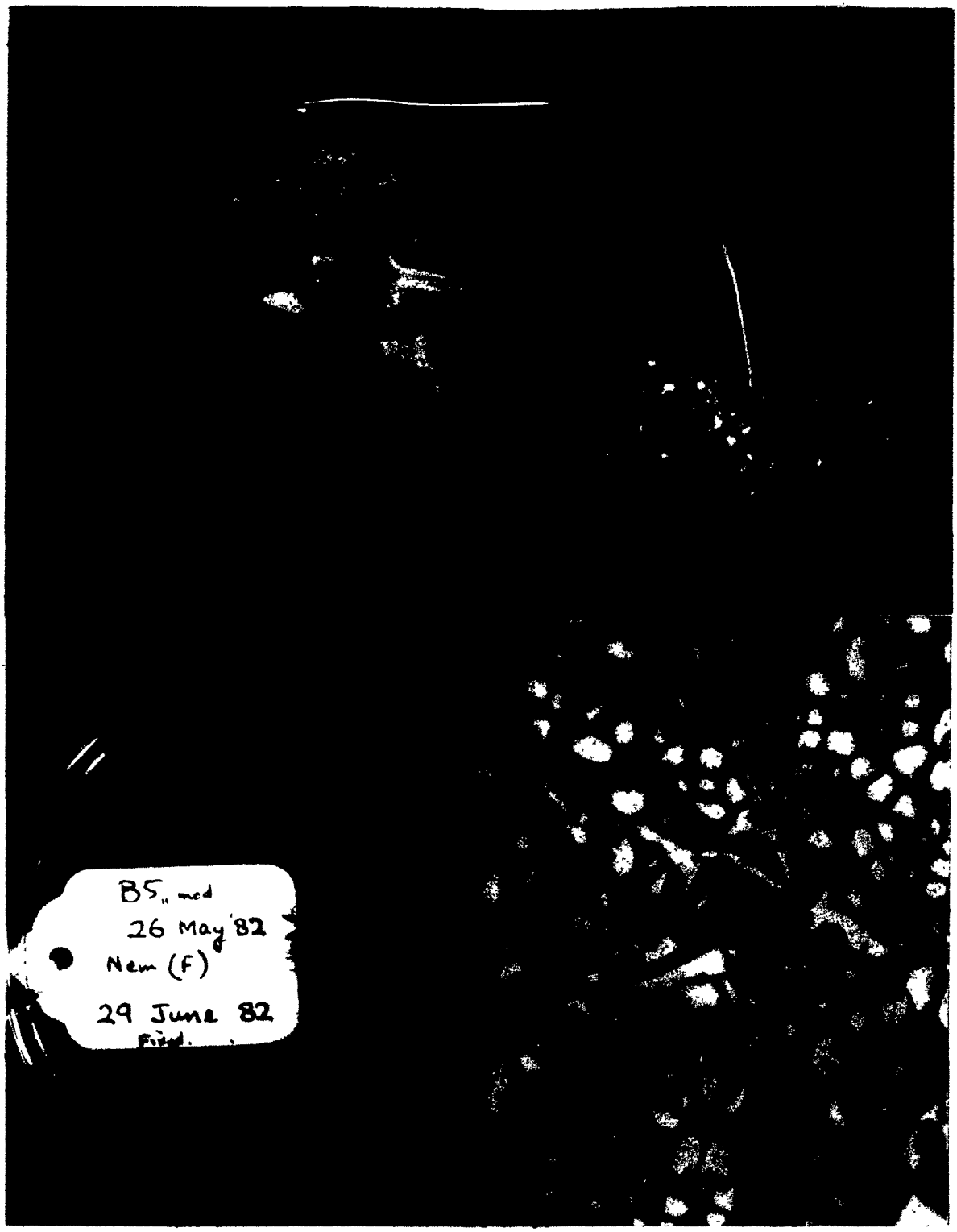
*** Photographs of cultures with nematodes

Figure 1. Meristem culture of Russian knapweed on B5 medium with 1.0 mg/L BA and 0.01 mg/L NAA, showing shoot multiplication. 18X.

Figure 2. Plantlet from B5 medium with 1.0 mg/L BA and 0.01 mg/L NAA, subcultured and established. 0.75X.

Figure 3. Culture of Russian knapweed on modified B5 medium (B5 mod) with 1.0 mg/L BA and 0.01 mg/L NAA, inoculated with nematodes. 4.5X.

Figure 4. Section of Russian knapweed culture on modified B5 medium (B5 mod) showing nematodes with slight cavity formation around them. 162X.


B5 " med
26 May 82
New (F)
29 June 82
Fixed

EXPERIMENT 4

Meristem cultures were grown on B5 medium with 3 levels of cytokinins (BA), auxin (NAA) and gibberellic acid (GA_3) which gave 27 combinations. In a similar experiment, the cultures were inoculated with nematodes and after one month, the cultures were fixed, sectioned and stained. Results are presented in Table 5.

When gibberellic acid (GA_3) was added to the medium, nematode penetration took place in most of the cultures (Table 5). Callus formation took place in all the cultures and the callus was slightly to moderately differentiated on medium with 0.01 mg/L BA, 0.01 mg/L NAA; 0.1 mg/L BA, 0.1 ml/L NAA and 1.0 mg/L BA, 0.01 mg/L NAA, whereas callus on medium with 0.1 mg/L BA, 0.01 mg/L NAA was highly differentiated. Plant multiplication and development was also more in the medium with 0.1 mg/L BA, 0.01 mg/L NAA when compared to other BA and NAA combinations.

In the medium with 0.01 mg/L BA, 1.0 mg/L NAA and 0.1 mg/L GA_3 (Figure 5) the nematodes penetrated the callus. There was no tissue differentiation or cavity formation around the nematodes with dedifferentiation of the tissues and the formation of nutritive cells and the nematodes were in the intercellular spaces (Figure 6). A similar type of response was observed in the medium with 1.0 mg/l BA, 0.1 mg/L NAA and 0.1 mg/L GA_3 where the callus was light green with formation of plantlets (plant initiation) (Figure 20) and no tissue differentiation or dedifferentiation or cavity formation around the nematodes occurred (Figure 21).

The medium which supported the highest plant multiplication and development (0.1 mg/L BA, 0.01 mg/L NAA) (Figures 7, 10, 12) also had the highest nematode penetration, which was about 16 to 20% (Figures 8, 11, 13). The callus tissue was differentiated and the nematodes were

TABLE 5. B5 MEDIUM WITH 3 LEVELS OF BA, NAA and GA_3 (27 COMBINATIONS). INOCULATED WITH APPROXIMATELY 50 NEMATODES AND FIXED AFTER 1 MONTH.

B5 MEDIUM mg/l			Differentiation of tissue in callus *	Number of plantlets	Height of plantlets in cm	REMARKS Formation of callus, roots, plant initiation, multiplication or development	Nematode penetration (in section)**	Nematodes in cavity
BA	NAA	GA_3						
.01	.01	.01	+		.5	light callus, twisted thin leaves	+	No
.01	.01	1	+		1.1	light callus, leaf margin curled inside	+	No
.01	.01	10			0.5	slight callus formation, leaf margin curled inside, slight root formation	+	No
.01	1	.01		1	.6	callus, leaves thin, slight root formation	+	No
.01	1	1		1	1	callus, leaves thin, root formation	+	No
.01	1	10		1	0.5	callus, slight plant initiation, slight root formation	+	No
.01	10	.01		1	0.1	callus	+	No
.01	10	1		1	0.5	callus ***	++	No
.01	10	10		1	1	callus	+	No
1	.01	.01	++	10	1.0	callus, plant multiplication and development ***	+++	Yes
1	.01	1	++	10	1.0	callus, plant multiplication and development ***	+++	Yes
1	.01	10	+	11	1.	callus, plant multiplication and development ***	+++	Yes
1	1	.01	+	3	1	callus, plant multiplication	++	No
1	1	1	+	4	1.	callus, plant multiplication twisted thin leaves	++	No
1	1	10		3	1.	callus, plant multiplication twisted thin leaves	++	No
1	10	.01		1	1	callus	+	No
1	10	1		1	1	callus	+	No
1	10	10				callus	+	No
10	.01	.01	+		1.	slight callus, plant initiation ***	+	No
10	.01	1	+	1	1	slight callus, plant initiation, twisted thin leaves	++	Yes
10	.01	10	+		1.0	light callus, plant initiation, twisted thin leaves	++	Yes
10	1	.01		1	1	callus dark green, slight plant initiation	+	No
10	1	1		1	0.5	callus light green, slight plant initiation ***	++	No
10	1	10		1		slight callus, slight plant initiation, very twisted leaves	+	No
10	10	.01		1	1.0	callus twisted leaves		No
10	10	1			0	callus		No
10	10	10		1	0.5	callus		No

* Differentiation
+ fully to moderately differentiated
++ not fully differentiated
*** Photographed with nematodes

** Penetration
+ No penetration
++ Slight penetration
+++ Heavy penetration

present in a cavity with pronounced necrosis around the walls but there was no redifferentiation of the tissues in the callus which would have resulted in the formation of nutritive cells. The nematodes were also present intracellularly (Figure 1[illegible]). In the medium with 0.1 mg/l BA, 0.01 mg/l [illegible] and [illegible] mg/l [illegible], one of the cultures had nematode penetration [illegible] leaf (Figure [illegible]) but no nutritive cells were present. In the medium with [illegible] mg/l [illegible], [illegible] mg/l [illegible] and 1.0 mg/l BA, nematodes were present [illegible] sections of meristems [illegible] plantlets but in these sections, no [illegible] penetration of the tissues was observed (Figure [illegible]).

[illegible], there [illegible] little callus [illegible] callus tissue was different [illegible] mg/l and 1.0 mg/l, the cultures with [illegible] (Figures [illegible]) and the nematodes were present intercellularly [illegible] with walls having pronounced necrosis (Figures 1[illegible], 1[illegible]).

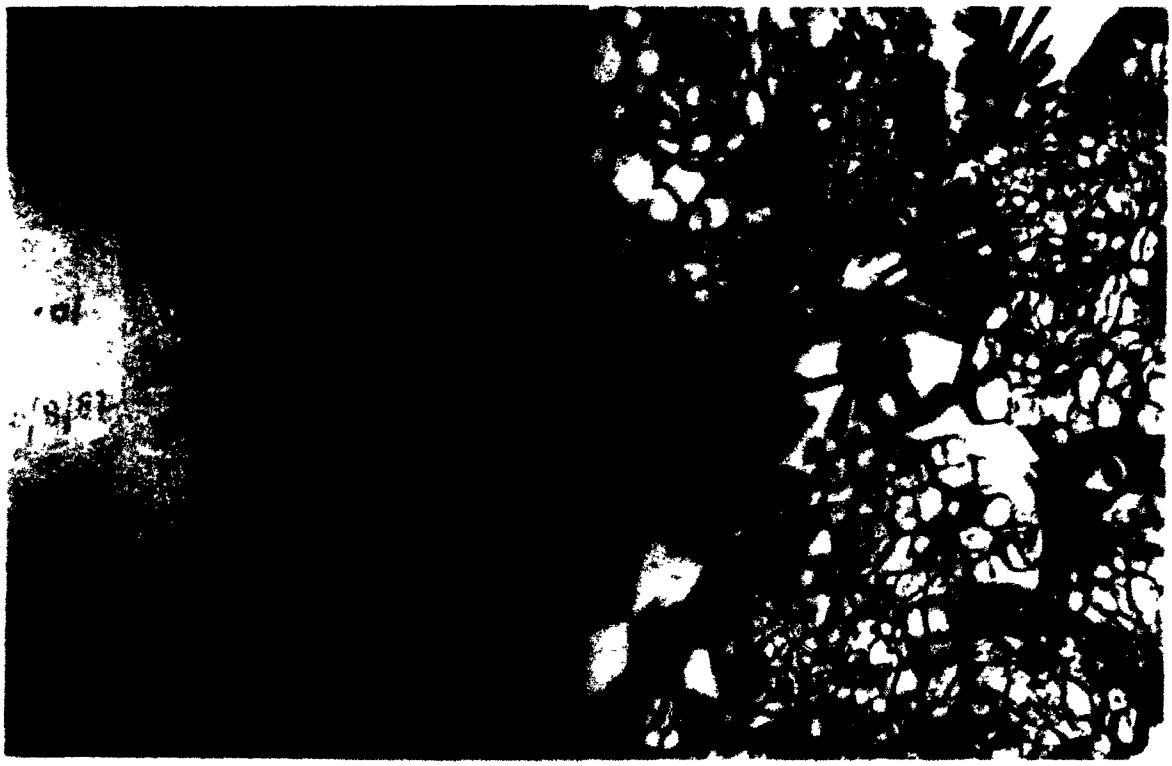
Figure 5. Culture of Russian knapweed on B5 medium with 0.01 mg/L BA, 1.0 mg/L NAA and 0.1 mg/L GA_3, inoculated with nematodes. Callus formation. 4.5X.

Figure 6. Section of Russian knapweed culture on B5 medium with 0.01 mg/L BA, 1.0 mg/L NAA and 0.1 mg/L GA_3. Nematodes in undifferentiated tissue. 162X.

Figure 7. Culture of Russian knapweed on B5 medium with 0.1 mg/L BA, 0.01 mg/L NAA and 0.01 mg/L GA_3 inoculated with nematodes. Callus formation, plant multiplication and development. 4.5X.

Figure 8. Section of Russian knapweed culture on B5 medium with 0.1 mg/L BA, 0.01 mg/L NAA and 0.01 mg/L GA_3. Nematodes in cavity, tissues differentiated. 102X.

Figure 9. Section of a leaf with nematode from Russian knapweed culture on B5 medium with 0.1 mg/L BA, 0.01 mg/L NAA and 0.01 mg/L GA_3. 102X.



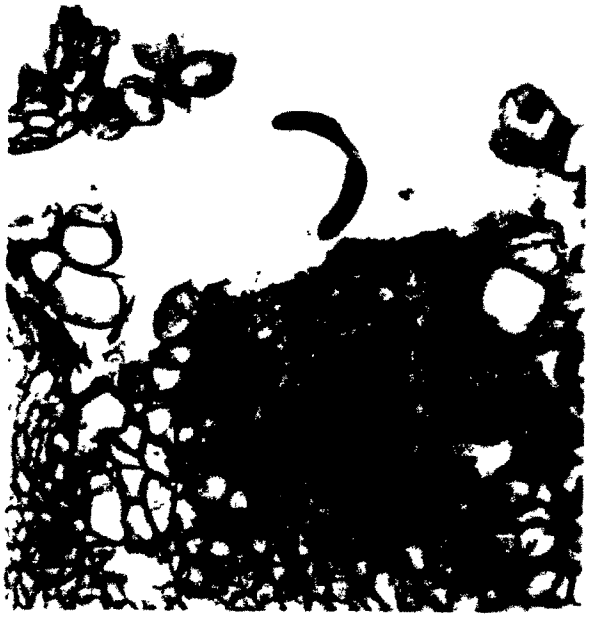


Figure 10. Culture of Russian knapweed on B5 medium with 0.1 mg/L BA, 0.01 mg/L NAA and 0.1 mg/L GA_3, inoculated with nematodes. Callus formation, plant multiplication and development. 5X.

Figure 11. Section of Russian knapweed culture on B5 medium with 0.1 mg/L BA, 0.01 mg/L NAA and 0.1 mg/L GA_3. Nematodes present in cavity and cells. Time differentiated. 162X.

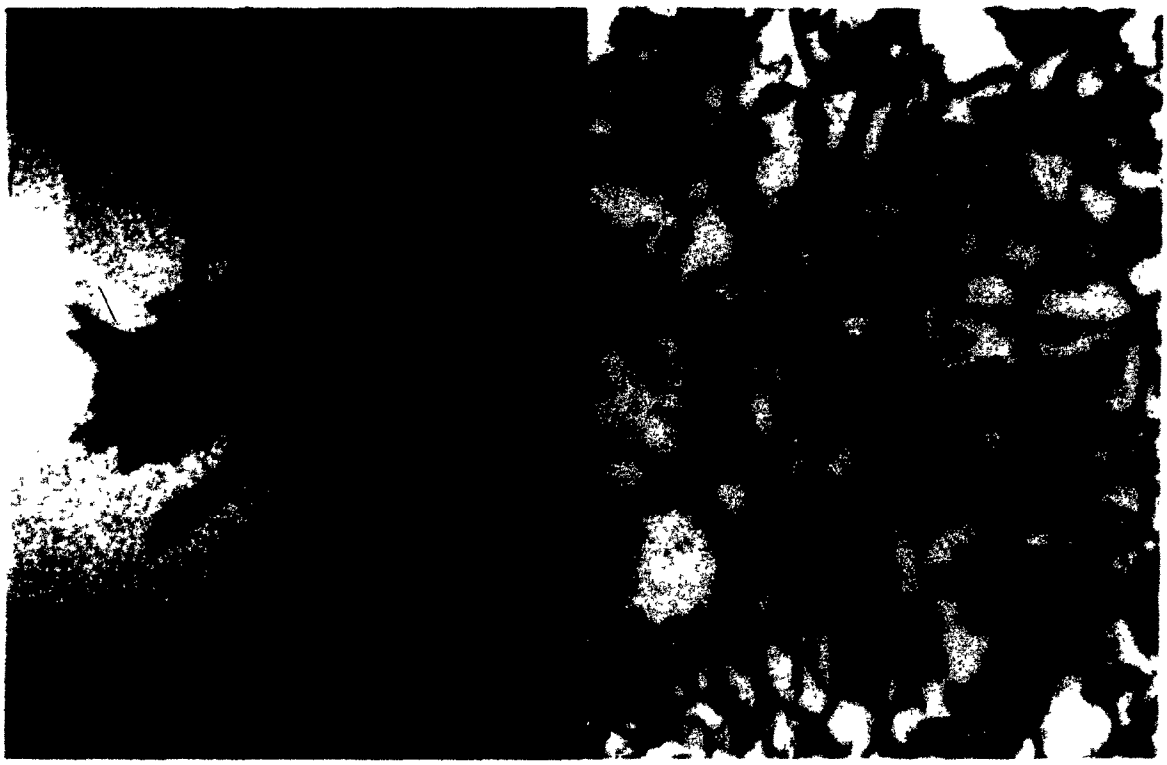


Figure 12. Culture of Russian knapweed on B5 medium with 0.1 mg/L BA, 0.01 mg/L NAA and 1.0 mg/L GA_3. Callus formation, plant multiplication and development. 4.5X.

Figure 13. Section of Russian knapweed culture on B5 medium with 0.1 mg/L BA, 0.01 mg/L NAA and 1.0 mg/L GA_3. Nematodes present in cavity and cells. Time differentiated. 102X.

Figure 14. Section of Russian knapweed culture on B5 medium with 0.1 mg/L BA, 0.1 mg/L NAA and 1.0 mg/L GA_3. Nematodes present in cell. 180X.

Figure 15. Section of a meristem with nematodes from Russian knapweed culture on B5 medium with 0.1 mg/L BA, 0.01 mg/L NAA and 1.0 mg/L GA_3. The nematodes have not penetrated the tissue. 102X.

Figure 16. Culture of Russian knapweed on B5 medium with 0.1 mg/L BA, 0.01 mg/L NAA and 0.1 mg/L GA_3. Callus formation and plant initiation. 4.5X.

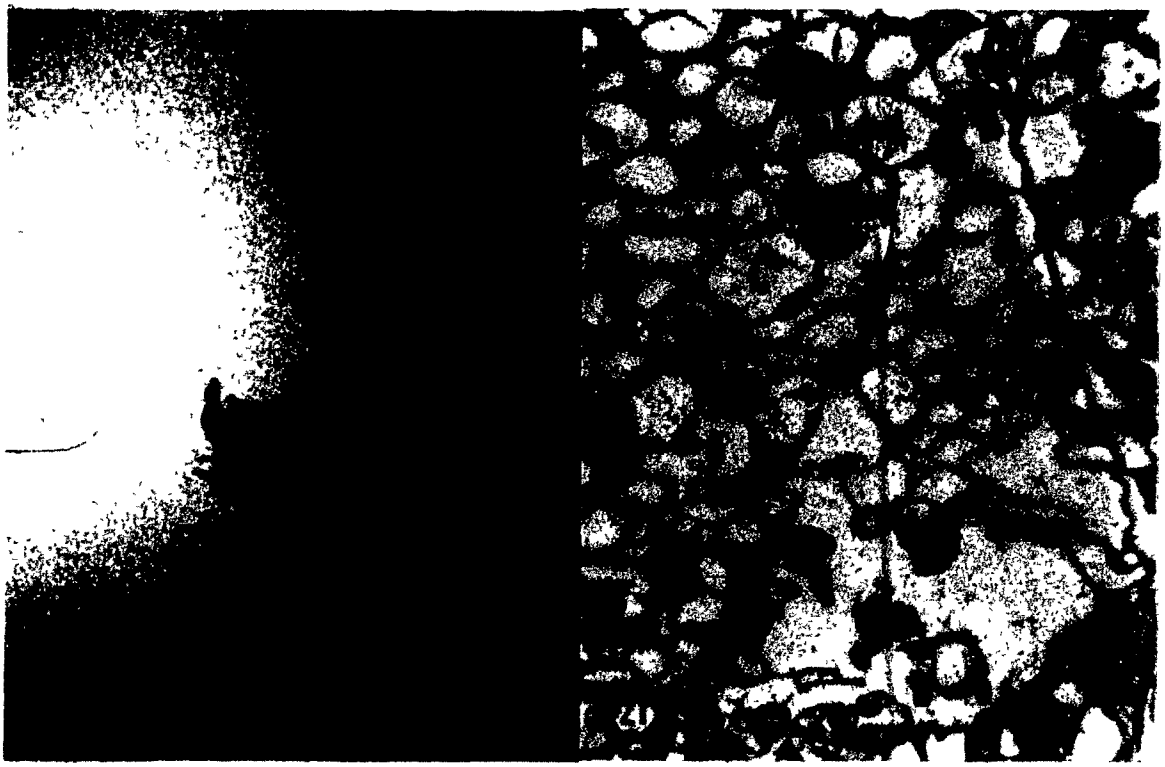
Figure 17. Section of Russian knapweed culture on B5 medium with 1.0 mg/L BA, 0.01 mg/L NAA and 0.1 mg/L GA_3. Nematodes present in cavity. Tissue differentiated. 68X.

Figure 18. Culture of Russian knapweed on B5 medium with 1.0 mg/L BA, 0.01 mg/L NAA and 1.0 mg/L GA_3. Callus formation and plant initiation. 4.75X.

Figure 19. Section of Russian knapweed culture on B5 medium with 1.0 mg/L BA, 0.01 mg/L NAA and 1.0 mg/L GA_3. Nematodes present in cavity. Tissue differentiated. 80X.

Figure 20. Culture of Russian knapweed on B5 medium with 1.0 mg/L BA, 0.1 mg/L NAA and 0.1 mg/L GA_3. Callus formation and very little plant initiation. 4.75X.

Figure 21. Section of Russian knapweed culture on B5 medium with 1.0 mg/L BA, 0.1 mg/L NAA and 0.1 mg/L GA_3. Nematodes in undifferentiated tissue. 91X.



II.D. DISCUSSION

Nematodes which form galls on aerial parts of the plant are found in a central cavity which is lined by a layer of "nutritive cells" abundant in cytoplasm. The formation of these nutritive cells takes place by dedifferentiation [illegible] the [illegible] gall form[illegible] [illegible]se, 19[illegible]. [illegible] the plants belonging [illegible] [illegible]areae tribe [illegible] [illegible]terod-eridae [illegible] [illegible]todes have been [illegible] [illegible]duction. Brown, [illegible] [illegible] and Vigliercn[illegible] [illegible] Miller, 1963; [illegible] [illegible]re, 1958; Schuster [illegible] [illegible] *et al.*, 19[illegible]. Attempts have not been made [illegible] galls on the aerial parts of the plants. [illegible] represents the first attempt to culture *P. picridis* [illegible] cultures.

AXENIZATION OF [illegible]

The primar[illegible] in culturing phytoparasitic nematodes is sterilizing the [illegible] inoculum. The axenization procedures reported in the literature [illegible] effect[illegible] and had their limitations. When fresh or [illegible] hypo-chlorite, there was still contamination. Fungi and bacteria were present inside the nematode galls, therefore it was necessary that the nematodes

be in direct contact with the sterilant. However, the use of sodium hypochlorite should be avoided since it has been shown to dissolve nematode stylets [illegible].

When streptomycin sulfate was used to axenize individual nematodes, there was [illegible] contamination in the agar plates on which the nematodes had been transferred. [illegible] and Pitcher [illegible] and Pitcher [illegible] effective [illegible] obtained using [illegible] additional serial treatment [illegible] have not generally [illegible] Zuckerman, 1971).

Mercuric chloride has been used as an antiseptic and disinfectant and inhibits the growth of both fungi and bacteria (Brewer, 1968). When mercuric chloride was used for axenizing individual nematodes following the procedures outlined by Dolliver et al. (1962) there was 30% fungal contamination on the agar plates. Nematodes were treated for only about 2 minutes with 100 ppm $HgCl_2$. Longer treatments may damage the nematodes due to the corrosive and toxic nature of this disinfectant.

Although the use of malachite green with streptomycin sulfate (Chen et al., 1961; Krusberg, 1961) proved to be very effective and no contamination occurred, the procedure was not feasible for obtaining sterile nematodes in bulk because it was very time consuming. However, a modified procedure proved to be very effective for obtaining bulk nematode inoculum. The nematodes were collected by Baermann's funnel technique with the incubation water containing 20 ppm malachite green and 1000 ppm streptomycin sulfate, washed three to four times in sterile distilled water,

soaked in 100 ppm [illegible] for two minutes, rinsed three to four times with malachite green [illegible] ppm [illegible] streptomycin sulfate 1000 ppm and finally rinsed four to five times with sterile distilled water. This was done under sterile conditions in a laminar air flow cabinet. The sterilized nematodes obtained through the procedure described above could be stored on agar plates at room temperature [illegible] inoculation [illegible].

RUSSIAN [illegible]

[illegible] important factor in the [illegible] a different [illegible] optimum [illegible] Medium [illegible] nematode [illegible] Webster [illegible] may not always [illegible] Krusberg and Blickenstaff, [illegible] Webster [illegible] this study, [illegible] of Russian knapweed grew best [illegible] medium was used [illegible].

NEMATODE [illegible]

The term callus [illegible] nematology, loosely means a [illegible] mass of plant cells arising from excised plant tissues that have been plated on agar medium containing plant growth promoters. These tissues contain many differentiated as well as undifferentiated plant cells (Krusberg and Babineau, 1979). Thus the term callus tissue as used here is considerably different from that defined [illegible] (1973) who [illegible] derived the term from the fact that cell proliferation is induced in the explants by injury of tissue caused by excision, suggesting

that the tissue formed can be equated with wounded callus. The determinative factors leading to proliferation of the cells in callus may be the appropriate nutrients and growth-regulating substances and the plant tissue from which the callus arises is the primary object of concern.

According to Bingefors and Eriksson (1963), the plant cultures should be in the log phase of growth when the nematodes are added. Callus that [illegible] three weeks to two months provides low, or no, nematode reproduction. [illegible] cultures [illegible] inoculated with nematodes before they [illegible] (Faulkner and Darling, [illegible]; Krusberg and Blickenstaff, [illegible]; [illegible] et al., [illegible]). [illegible] for [illegible] cultures were inoculated after [illegible] weeks.

[illegible] nematode [illegible], the amount of [illegible] varied from one or two nematodes per culture (Krusberg and [illegible]), [illegible] hundred or more ([illegible] et al., [illegible]) with the [illegible] number [illegible] about fifty nematodes (Johnson and Viglierchio, [illegible]; Krusberg, [illegible]). Therefore in this study all cultures were inoculated with approximately fifty nematodes per culture.

Cultures were incubated at [illegible] in the tissue culture laboratory. [illegible] have been made of the effects of temperature on nematode cultures (Faulkner et al., [illegible]; [illegible]serra and O'Bannon, [illegible]; Johnson and Viglierchio, [illegible]; Krusberg et al., [illegible]; Prasad and Webster, [illegible]) with the optimum temperature for the tissue growth also being the best for nematode culturing.

The penetration of the nematodes in the cultures appeared to be a major problem in this study. In nature, the nematode infects young shoots emerging through the soil, which suggested the use of meristem culture. Attempts to provide an environment suitable for nematode penetration

[illegible] the placement of sterilized perlite or vermiculite over the cultures of [illegible] and inoculated with the nematodes. But, no [illegible] penetration occurred in all of the cultures after fifteen or thirty days. Most of the nematodes migrated into the agar medium and [illegible] the cultures [illegible] of the agar medium, the nematodes [illegible] trapped, and if this was the case, the nematodes would [illegible] observation was made by [illegible] with *Aphelenchoides ritzemabosi*. In some studies [illegible] peat moss [illegible] [illegible] commended that the agar surface [illegible] sprinkled with sterile sand [illegible] penetration and to increase surface [illegible] problems.

[illegible] probable that the time period of fifteen or thirty days [illegible], because under natural conditions the nematodes need [illegible] for about two months in soil before they become [illegible]. The reason why the cultures were incubated [illegible] was due to the fact that after five weeks the callus started [illegible]. The incubation period of one month [illegible] for the *P. [illegible]* larvae to become infective [illegible] cultures should have been incubated for a longer period of time.

[illegible] suggested that methods for long-term preservation of cultures [illegible] storage at low temperatures [illegible], should be investigated [illegible] culture life (Krusberg and Babineau, [illegible]).

In the second part of experiment 3, the effect of nutritional factors on the penetration of the nematodes was examined. Penetration of nematodes occurred in only one culture which was formulated with higher concentrations of macronutrients, sucrose and vitamins and low concentrations of iron chelates.

It has been established that the addition of chelating agents to [illegible] media will increase the reproductive rates of nematodes in culture, therefore the media were formulated with low EDTA concentrations (Oliver et al., 1962; Grisberg and Blickenstaff, [illegible], but due to the toxicity of EDTA to nematodes indirect reasons for the observed inhibition of reproduction by this compound have been advanced (Oliver et al., 1962). EDTA chelates cations, reducing their availability to the medium (Oliver, 1962). Further suggested that reproduction of the nematodes required a higher available concentration of one or more nutrients than is necessary for plant growth (Oliver et al., 1962).

It is a matter of speculation, however, as to why penetration of H. [illegible] larvae should be affected by the concentrations of the nutrients in the medium. The concentration of nutrients may influence penetration, either through the host or directly on the nematodes themselves (Wallace and Mitchell, 1960b,). The level of host nutrition can affect the degree of host attraction and direct absorption of nutrients from the medium. The nematodes may affect the parasite's ability to survive and penetrate either through chemical interactions or physical manifestations such as osmotic pressure or electrolyte imbalance. Johnson and Mitchell (1960a, found that the growth of aseptic Beta vulgaris root explants was essentially the same at all concentrations of macronutrient salts, vitamins or sucrose tested. They concluded that the effect

on penetration cannot be explained by change in root growth alone, therefore, the physiology of the host root must be involved and the concentrations of the nutrients in the media may make penetration sites more susceptible to the infective Heterodera schachtii larvae.

More tissue differentiation occurred in callus on medium with low concentrations of macronutrients, sugar and vitamins B5 mod., than on media with low macronutrients, sugar and vitamins B5 mod. Gall forming nematodes seem to require vascular development in plant tissues in order to complete their life cycle and the results of Jaworski (1968) support this hypothesis. He found that M. incognita would not grow on undifferentiated tissues of tomato but would grow and complete its life cycle on tissues having some degree of vascular development.

The effects of growth regulators has been demonstrated in the culturing of plant parasitic nematodes. When different levels of BA and NAA (0.01, 0.1, 1.0, 5.0 and 10.0 mg/L) were employed in experiment 2, culture characteristics were different Table 3, p.31,. But still, no nematode penetration or any indication of gall formation took place in the cultures. In the mediums with high levels of NAA (5.0 or 10.0 mg/L, and low levels of BA (0.01, 0.1 or 1.0 mg/L), the callus formed was a friable mass of loose tissue with no cell differentiation. Whereas in mediums with lower levels of NAA (0.01, 0.1 or 1.0 mg/L, in combination with high levels of BA (5.0 or 10.0 mg/L) there was only slight shoot development. Medium which had low levels of both BA and NAA supported callus formation and plantlets were initiated from the callus. When sections of the callus from mediums with low levels of BA and NAA were examined, the tissue was found to have vascular differentiation. The amount of vascular differentiation in the tissue varied. Medium with low

-A and higher BA ratios had more differentiated tissue than medium with low BA and high NAA ratios.

Studies dealing with the effects of growth promoters on nematode reproduction in plant tissue cultures demonstrate that plant growth substances may affect nematodes directly as well as indirectly through influence on plant tissues. Krusberg and Blickenstaff 1964) reported that when kinetin was added to the medium, which also contained 2,4-dichlorophenoxyacetic acid 2,4-D reproduction of Pratylenchus penetrans and P. zeae on alfalfa tissue was inhibited, but reproduction of D. dipsaci was unaffected. They also reported that NAA could be omitted from the medium with no adverse effects on nematode reproduction. However, kinetin in the medium did not affect reproduction of P. vulnus on alfalfa tissue (Lownsbery et al., 1963). Barker and Darling 1965) reported that Aphelenchus avenae reproduced poorly on tissue cultures of several plants growing on synthetic medium supplemented with kinetin and IAA.

In this study, when the nutrient medium contained only BA and NAA, penetration of the nematodes in tissue cultures was not achieved, but when GA_3 was added to the medium in combination with BA and NAA, nematode penetration was observed in most of the cultures whether or not differentiation of the tissue had occurred Table 5, p.37). The meristem cultures were fixed in FAA, one month after they had been inoculated with the nematodes, embedded in paraffin and sectioned at 25 using a rotary microtome, were stained with Safranin and Fast Green FCF. The sections of the cultures were observed under the microscope in a serial manner to estimate the percent nematode penetration. In cultures with no tissue differentiation the penetration was from 4 to 10% whereas in tissues with vascular development, the penetration was from 16 to 20%.

Cell wall thickness may be an important factor in the susceptibility of callus tissue. The cell walls of young callus tissues appear to be very thin and are unprotected by epidermal cells which probably enables the nematodes to penetrate. The increased resistance of older callus may be due primarily to increased cell wall thickness (Barker and Darling, 1965). There is clear evidence that GA_3 has a synergistic effect with other growth regulators on the cell walls of the treated tissues (Zeroni and Hall, 1980) and this could account for the penetration of the nematodes in cultures when GA_3 was supplemented in the media.

The medium which supported the highest differentiation of tissue in callus (0.1 mg/L BA and 0.01 mg/L NAA in combination with three levels of GA_3) also had the highest number of plants (Figures 7, 10 and 12) and nematode penetration (Figures 8, 11 and 13). The nematodes in these cultures were present either intercellularly (Figure 14) or were contained in a cavity which had necrosis along its walls. In a laboratory host specific study, the galls formed by P. picridis on some nonhost plants had a variable response, with a central cavity containing nematodes but there was pronounced necrosis along the walls of the cavity instead of cells containing abundant cytoplasm (Watson, 1975). The host response to nematodes in cultures in this study was somewhat similar to the gall tissue of the nonhost plants observed by Watson (1975). The only difference in the cultures in this study was that the necrosis was not as pronounced. In one of the cultures on the medium containing 0.1 mg/L BA; 0.01 mg/L NAA and 0.01 mg/L GA_3, there was a slight indication of gall formation on one of the leaves, but only one nematode was present in the cavity and there were no nutritive cells (Figure 9). In medium containing

0.1 mg/L BA, 0.01 mg/L NAA and 1.0 mg/L GA_3 there were nematodes present in the meristematic region but had not penetrated the tissues (Figure 15) indicating that P. picridis may be attracted to the growing meristem.

In cultures on medium with 1.0 mg/L BA and 0.01 mg/L NAA with 0.1 mg/L GA_3 (Figure 17) and 1.0 mg/L GA_3 (Figure 19) the host response to the nematodes was similar to the cultures on medium containing 0.1 mg/L BA and 0.01 mg/L NAA in combination with the three levels of GA_3. The nematodes were present in a cavity with some necrosis, but fewer nematodes were present in these cultures compared to the cultures on medium containing 0.1 mg/L BA and 0.01 mg/L NAA in combination with the three levels of GA_3. The reduced number of nematodes could be explained due to the slight vascular differentiation of the tissue, with only plant initiation and the leaves formed were thin and twisted (Figures 16 and 18).

The mediums containing 0.01 mg/L BA and 1.0 mg/L NAA, 0.1 mg/L BA and 1.0 mg/L NAA, 1.0 mg/L BA and 1.0 mg/L NAA in combination with three levels of GA_3 only supported callus growth (Figures 5 and 20). There was no differentiation of tissue in the callus and when the nematodes entered the callus, there was no cavity formation nor necrosis of the tissue around the nematodes (Figures 6 and 21). It was probable that the host tissue did not react to the penetration of the nematodes, therefore no cavity formation took place.

Webster (1967b) performed a series of growth regulator studies using Aphelenchoides ritzemabosi and alfalfa as the host plant. In medium lacking 2,4-D nematode reproduction increased when kinetin, GA_3, IAA or tryptophan were added to the medium. Furthermore the combinations GA_3 +

IAA and GA_3 + tryptophan were better than GA_3 alone. The concentrations of GA_3 in his study were 10 or 50 mg/L and were much higher than the ones used in this study.

The use of tryptophan in plant tissue cultures have produced contradictory results (Gautheret, 1955). Nickell and Burkholder (1950) and Riker and Gutsche (1948) obtained no stimulation in the plant tissues with this amino acid but Kulescna and Gautheret (1949) discovered that it displays stimulatory and formative properties similar to those of auxins. It has been demonstrated that tryptophan is the primary precursor in the pathway of IAA biosynthesis both in microorganisms and higher plants. This suggestion is supported by the close chemical similarity of tryptophan and IAA, as well as their ubiquitous occurrence as natural constituents of higher plants (Sembdner et al., 1980). The formation of IAA from tryptophan has been studied both in intact plant tissues and in cell-free preparations (Libbert et al., 1970; Schneider and Wightman, 1974, 1978), and it has also been found that its activity is about one hundred times less than that of IAA, that is, it causes the same reactions but at concentrations a hundred times higher in plant tissue cultures (Gautheret, 1955). It has been found that nematode secretions, which contain neither auxins nor cytokinins, induce actively growing tissue to form syncytia which usually occur in the form of a cluster of multinuclear cells with hypertrophy and hyperplasia (Sandstedt and Schuster, 1966a). The auxins are also not freed from the plant tissue. Instead, it is suggested that nematodes enable the tissue to retain and use endogenous auxins that otherwise would have been transported to the basal ends of the segments (Sandstedt and Schuster, 1966b). Webster (1967b) suggested that the nematodes secrete proteolytic enzymes that release amino acids such as

tryptophan in galled tissues but not in healthy tissues and concluded that IAA may be involved in the galling mechanism, where some of this free tryptophan could easily be converted to IAA by enzymes in the nematode or plant. If this occurred IAA could be the actual cause of galling and the nematodes might cause the plants to accumulate IAA in the vicinity of the nematodes.

In culturing of the plant parasitic nematodes, it has been demonstrated that when tryptophan was added to the medium in combination with GA_3 or IAA or both, the nematode reproduction increased significantly (Webster, 1967b), where substances such as GA_3, that stimulate cell activity and growth, also provide a favourable nutritional environment for nematode multiplication.

Another possible reason why the penetration of the nematodes took place in the cultures in experiment 4, in which the medium contained BA, NAA and GA_3, was that the nematodes had been in a free living state on agar plates for a month and a half before they were inoculated in the Russian knapweed meristem cultures. Watson (1975) had suggested that P. picridis larvae should remain in the soil for about two months before they become infective. It is probable that the nematodes in this free living stage build up some sort of enzyme mechanism which makes them infective, because hydrolytic enzymes have long been considered to be involved in the parasitism of plants by nematodes (Chitwood and Krusberg, 1977; Deubert and Rohde, 1971; Giebel, 1974; Krusberg, 1960, 1967; Miller and Sands, 1977; Morgan and McAllan, 1962; Myers, 1965; Tracey, 1958).

Culture studies with A. ritzemabosi suggest that unless all conditions involving the nematode, the host tissue and the environment are favourable, maximum reproduction was not achieved and this conclusion has also been true for Heterodera rostochiensis which has failed to reproduce in undifferentiated potato callus or cause syncytia to form in the callus (Webster and Lowe, 1966). In this study, the penetration of the nematodes in the cultures was achieved, but no nematode reproduction occurred in any of the cultures. It is suggested that further studies on environmental conditions, nutrition of the host tissue in combination with the hormonal factors should be conducted.

III HOST PARASITE RELATIONSHIP

A. INTRODUCTION

It is not uncommon for diseased plants to manifest as part of their pathological syndrome some type of exaggerated growth disorder, leaf epinasty, adventitious root formation, thickening or elongation of plant parts, lack of branching, bending of stems, stunting and disorganized growth characteristics of tumors and galls. Although there is a great diversity in the organisms that effect such growth disorders on higher plants, the groups of principal importance are microorganisms, insects and nematodes (Mani, 1964; Viglierchio, 1971).

Galls are pathologically developed cells, tissues or organs of plants that have risen mostly by hypertrophy (over growth) and hyperplasy (cell proliferation) under the influence of these parasitic organisms (Mani, 1964). They represent the growth reaction of plants to the attack of the parasite and are in some way related to the feeding activity and nutritional physiology of the parasite (Küster, 1911). Nematode galls on aerial parts of the plant develop by hypertrophy and hyperplasia of parenchyma tissue and usually have a central cavity containing nematodes (Dropkin, 1969; Goodey, J.B., 1939,1948; Goodey, T., 1934,1935,1938), and a zone of cells with abundant cytoplasm that line the cavity (Mani, 1964). The cells which line the cavity are the so called nutritive zone, and are usually cytoplasmically rich, contain fragmented vacoules, exhibit nuclear and nucleolar hypertrophy, rich in mitochondria, and contain high concentrations of lipids, hydrolytic enzymes, and amino acids (Maresquelle and Meyer, 1965; Meyer, 1969). Plant endoparasitic nematodes have evolved the ability to induce morpho-

logical changes in the host cells to form feeding sites. The mechanisms by which the host cells modify vary, but in functional terms the end results are similar [illegible] nutrients [illegible] these nutrients are [illegible] the nematodes are [illegible] les of the modified cells [illegible] between the gall-inducing [illegible] the gall-maker that apparently [illegible] differs loss of substances. [illegible] substances in the sap flow, premature [illegible] at the cost of essential, and other [illegible] believed that the formation of a gall is probably [illegible] part of the plant to protect itself from an injury which is not sufficient to cause death, and that it is a gradual process of mutual adaptation between the gall-maker and the gall bearing plant (Cook, 1923; Zweigelt, 1942).

According to Viglierchio (1971) nematode galls are simply pathological swellings or enlargements of plant parts, irrespective of histological organization or structure. He stated that syncytia (giant cells) are not present and no specialized cell types occur except in galls on Andropogon pertusus Willd. caused by Nothanguina cecidoplastes (Goodey, 1934) Whitehead, 1959 . These galls have a well differentiated structure with distinctive cell types (Goodey, 1934). There is an innermost nutritive zone surrounded by a zone of strong walled cells which show secondary thickenings. The nutritive cells close to the gall cavity are enlarged, rounded to rhomboidal in shape and containing granular cytoplasm. The nucleus is enlarged with 2 to 3 nucleoli. The cells are vacuolated with no intercellular spaces. They have thin walls and no chloroplasts. Outside

the nutritive zone are elongated cells with reticulate secondary thickenings. Their contents are also granular and contain large nuclei. Small groups [illegible] the thick[illegible] cells [illegible]. The morpho[illegible] Nutt. and [illegible] been described [illegible] variation[illegible] epidermis, and size [illegible] numerous vascular [illegible] the galls, and the [illegible] histologically examined the developmental morphology of the galls [illegible] susceptible, intolerant and resistant hosts of [illegible]. picridis and observed the cellular response of the different hosts to the parasite's attack. Skinner et al. (1980) examined the histopathogenesis of the foliar galls induced by Nothanguina phyllobia Thorne in Solanum elaeagnifolium Cav. They concluded that the exceptional biomass of the galls support the logic that S. elaeagnifolium granular cells transfer cell-like in nature and make up a tissue that functions as a physiological sink. The mechanisms by which the nematodes trigger the development of granular cells is not known.

Harris (1973) designed a scoring system for determining the relative effectiveness of biological control agents, and his approach was to select for minimum host-parasite homeostasis. He stated that since gall formers have evolved a homeostasis with their hosts makes them incapable of causing serious damage. Parasites that have had a long and close association with a host do not severely restrict its abundance as there has been a selection for resistance (Pimentel, 1961). This

criteria of Harris may not apply to all gall formers, especially nematodes formin[illegible] the aerial parts of the plant[illegible] with rac[illegible] act as [illegible] and ga[illegible] in the [illegible] of the [illegible] effect.

The [illegible] concept was originally advanced by Münch [illegible], Zimmermann [illegible] has placed much emphasis on the metabolic aspect of such a mechanism, where leaves with their photosynthetic capacity typically constitute the source (an exporting storage organ would also constitute a source, and any non photosynthesizing, growing or metabolizing tissue might constitute the sink. Roots, developing fruits, flowers, growing stem tips and galls are common examples. The bulk of translocated material, about nine-tenths or more, consists of carbohydrates (Zimmermann, 1960) with sucrose and oligosaccharides the predominant translocated sugars (Biddulph and Cory, 1957; Kursonov et al., 1958; Pristupa, 1959; Swanson and El-Shishiny, 1958). Besides sucrose, glucose and fructose have been reported in smaller amounts (Zimmermann, 1960).

The granular cytoplasm and pronounced nuclear activity are characteristics that were observed in the nutritive tissue in the galls of numerous cecidozoans (Dropkin, 1969; Goodey, J.B., 1948; Goodey, T., 1934, 1938; Kostoff and Kendall, 1929; Mani, 1964; Rebois et al., 1975; Skinner et al., 1980; Watson and Shorthouse, 1979; Westphal, 1977). Closer examination of several nematode species with electron microscopy revealed

that the [illegible] ribosoma [illegible] matri [illegible] North [illegible] enlarged [illegible] [illegible] associated with the acti[illegible] occur[illegible] transfer [illegible]. These [illegible] enriched granular cells are transfer cell-like in nature and make up a tissue that functions as a physiological sink. The mechanism by which the nematodes trigger the development of granular cells is not known (Skinner *et al.*, 1980).

It has been suggested that syncytia (giant cells) induced by nematodes are multinucleate form of transfer cells (Jones and Northcote, 1972a,1972b). Normal transfer cells are thought to supply organic nutrients to plant tissues which are actively growing or secreting but nematode-induced transfer cells are thought to supply organic nutrients to the nematode (Bird and Loveys, 1975). Pate and Gunning (1972) in their review of the transfer cell pointed out that there is little information on their physiological role. Bird and Loveys (1975) showed that the nematode-induced transfer cells and the nematode act as 'sinks'. The uptake of carbon compounds in these nematode-induced cells increased at a time when there was maximum growth and activity of transfer cell-like syncytia and their components. It was also observed that the organic nutrients required by these nematodes originated, at least in part, from the products of current photosynthesis and would thus presumably be translocated to the nematode via the phloem (Bird, 1962; Bird and Loveys, 1975). Some work on histochemistry of nematode-induced transfer cells has been done by Gommers and Dropkin (1977), but little work has been done on biochemical changes in plant tissues injected with nematodes.

were also reported higher rates of glycolysis in galls and higher rates of intermediary metabolism, particularly that leading to protein and nucleic acid synthesis. Lewis and McClure (1975) found differences in several free amino acids after infection with proline increasing nearly 20 fold in susceptible plants. Owens and Specht (1966) also reported increases in proline and showed that giant cells were especially rich in ninhydrin-positive compounds. DeMott (1965) estimated that hexose monophosphate pathway was 14 to 18 times more active in galled roots than in non-infected tissue.

Paranguina picridis is a highly specialized parasite. The nematode has a histospecific and exceptionally pronounced effect on the morphology of its host. Kirjanova and Ivanova (1969), and Watson (1975) have proposed using P. picridis as a biological control agent of Acroptilon repens. Subsequent studies on host specificity, virulence, distribution and behavior of the nematode have demonstrated its suitability as a bio-control agent (Watson, 1975; Watson and Harris, in press).

This study was performed to investigate the effect of the galls on the plant. The nutritive zone in the galls is massive compared with that of most gall-forming nematodes, and although chlorophyll occurs in the outer layer of the wall surrounding the central cavity, it probably contributes but a small fraction of the photosynthates required for gall development. It is assumed that the nutritive cells act as powerful 'physiological sinks' attracting assimilates of the plant to the gall and gall-maker. If it is correct, the galls induced by P. picridis on Russian knapweed are constructed largely from translocated assimilates and impose a considerable energy drain on the plant. The carbohydrate accumulation by the galls was investigated with the aid of radioactive tracers.

CHAPTER III. MATERIAL AND METHODS (GENERAL)

PLANT MATERIAL

Russian knapweed roots were collected from the field study site on the Macdonald Campus of McGill University, Ste. Anne de Bellevue, Quebec, and after washing, they were cut into 10 cm segments. The root segments were potted in vermiculite in 15 cm diameter pots. The pots were placed in a growth chamber with the same environmental conditions as described in the previous section. The pots received an excess of modified Hoagland's solution every second day and on alternate days received sufficient distilled water to reach saturation. Once the plants emerged, they were inoculated with nematodes in a manner similar to that described by Watson (1975). Crushed gall material was mixed in 500 ml of distilled water and was continuously agitated for about 4 hr by bubbling air through it. Once the nematodes emerged, the suspension was filtered through a cheese cloth to obtain a clean nematode suspension. Ten ml of this suspension containing about 2000 nematodes was applied to the vermiculite surface. After about one month, the top growth was removed from all the pots. Gall formation was recorded on the shoots that subsequently emerged. At least one to three galled plants appeared in each pot. The galled plants were then separated and repotted in vermiculite in 8 cm diameter pots, making sure that the volume of the roots and also the size of the shoots were approximately of the same size. The plants were left to grow in the growth chamber for about a month for the galls to attain maturity. If secondary plants emerged, they were cut off. Control plants were also grown in the same way, the difference being that they were not inoculated with the nematodes, and hence there was no gall formation. When these plants were repotted in vermiculite in 8 cm diameter pots, their root

volume and size of the shoot was approximately the same as in galled plants. All plants were watered with modified Hoagland's solution as mentioned before.

[illegible]

[illegible]

During the plant development, two types of leaf form were observed in Russian knapweed which was probably due to heteroblastic development. Plants were classified on the basis of leaf margin and position of the gall. Plants with only one gall were selected for this study. The groups were:

Galled plants:

A - plants with lobed leaf margins and lyrate leaf, gall on leaf.

B - plants with entire leaf margins and spatulate leaf, gall on petiole.

C - plants with entire leaf margins and spatulate leaf, gall on leaf.

Nongalled plants:

A - plants with lobed leaf margins and lyrate leaf, no gall (control).

B - plants with entire leaf margins and spatulate leaf, no gall (control).

C - plants with entire leaf margins and spatulate leaf, no gall (control).

Three replicates were assigned per group. Visual condition of the gall was noted on a scale of 1 to 4 where 1 stands for green fleshy galls, 2 stands for green non-fleshy galls, 3 stands for brown non-fleshy galls and 4 stands for brown dry galls (Table I - Appendix).

The plants were taken to the radiotracer laboratory and 0.005 ml of Fructose D- [^{14}C(U)] - was injected into the stem of each plant with a microsyringe ($\approx$0.185 MBq/plant). The isotope was obtained from New England Nuclear with a specific activity of 200-300 mCi/mmol. with a concentration of 1 mCi in 10 ml ethanol:water (9:1). The plants were taken back to the growth chamber and placed under conditions specified above. One plant from each treatment was taken and incubated for 12, 24, and 36 hr, respectively. After the allotted time for translocation in each plant, the plants were harvested and separated into:

GALL - total galls

LEAF - total leaves

ROOT - total roots

STEM.X - total stem of plant in which radioactivity was injected.

The fresh weights of each plant part (i.e. gall, leaf, root or stem.() was recorded. The fresh plant tissues were dried overnight in an oven at 60°C and the dry weights were recorded (Table I - appendix). Each plant part was finely ground and samples of 10 mg dry weight were prepared for liquid scintillation counting (for procedure see Appendix G). If the plant part was larger than 50 mg dry weight (e.g. leaf, root or stem.X) then at least three samples were prepared which were from top, middle or bottom, and for parts larger than 10 mg dry weight but less than 20 mg dry weight (e.g. galls) one sample was prepared. Each sample was counted four times with a Nuclear Chicago ISOCAP/300 liquid scintillation counter and the counts obtained were corrected for background. Since there was no significant difference between the counts obtained in samples from the same plant part, their means were used for further analysis.

EXPERIMENT 2. ($^{14}CO_2$)

Since the injection of the radioactive material into the plant in experiment 1 was done by a microsyringe, it was thought that the injury caused by injecting might be affecting the translocation in the plant (R.L. Pelletier, personal communications). Therefore another experiment was designed in which the whole plant was put in a chamber through which $^{14}CO_2$ was passed. The circuit was a closed system in which $^{14}CO_2$ was generated when 50% lactic acid solution reacted with $Ba^{14}CO_3$ in the CO_2 generator. The system had a pump (Portable Masterflex Sampling Pump) which regulated the flow of $^{14}CO_2$ and it also had a $^{14}CO_2$ absorber which contained NaOH (Figure 22).

Fifteen plants of approximately the same age (45 to 50 days old) were selected and were divided into three groups on the basis of number of galls per plant. The approximate age of the galls was 30 to 40 days. All plants had spatulate leaves with entire leaf margins. The groups were:

Galled plants:

A' - plants with one gall in the meristem region.

B' - plants with two galls; one gall in the meristem region and another gall on a leaf.

Nongalled plants:

C' - plants with no galls (control).

$BA^{14}CO_3$ (hot) was obtained from New England Nuclear with a specific activity of 5 mCi/mmol, the concentration obtained was 1 mCi in 39.5 mg of $Ba^{14}CO_3$. To this, 960.5 mg of $BaCO_3$ (cold) was added so that when 5 mg of $BaCO_3$ + $Ba^{14}CO_3$ was taken, it contained approximately 0.185 MBq of radioactivity. Fifteen $^{14}CO_2$ generators (injection bottles) were prepared, each containing 5 mg of $BaCO_3$ + $Ba^{14}CO_3$.

The plants were taken to the radiotracer laboratory. A plant was placed in the $^{14}CO_2$ chamber (plant chamber), and the chamber was sealed air tight using vaseline. Stop cock grease was used to seal any other connections in the system. Rubber tubing was avoided because of its rather high permeability to CO_2. Therefore 'Tygon' tubing was used (Weiser et al. 1962). Bends and joints in glass tubing were also checked for leaks. Extreme care was taken not to handle the plants excessively and plants were well watered at all times to prevent water potential decreases (water stress increases) which result in stomatal closure. This effect can predominate over low CO_2 and bright light (Salisbury and Ross, 1969). Once the plant was in the chamber, valve I was closed and valve II opened. The pump was turned on, air circulated through NaOH and CO_2 present in the system was absorbed (low CO_2 partial pressure). The lights were also turned on at this point. The Lamba LI-185 instrument with quantum sensor was used to measure quanta in the Photosynthetically Active Radiation (PAR) spectrum between 400 and 700 nm (visible wave length) range received on a plane surface. It was found to be 57 microeinsteines ($m^{-2}s^{-1}$) on the photosynthesing leaves (where one microeinsteines equals 6.034×10^7 photons). The system was left running for 30 minutes to ensure that the stomata were open. Stomata typically close in darkness and open in light. Opening in light appears to be brought about by a photosynthetic lowering of CO_2 partial pressure within the leaf. The CO_2 inside the leaf controls this response (Salisbury and Ross, 1969). After 30 minutes valve I was opened, valve II closed and 3 ml of 50% lactic acid solution was injected into the $^{14}CO_2$ generator with a hypodermic syringe. The system was left running for a period of 15 minutes. After 15 minutes, the lights were turned off, valve I was closed and valve II opened, and NaOH was injected into the $^{14}CO_2$ generator. The system was left running for another 15

minutes for excess $^{14}CO_2$ to be absorbed. The plant was removed and the procedure was repeated with all 15 plants. Treated plants were returned to the growth chamber and placed under normal conditions for 24 hr for translocation to take place. After 24 hr the plants were harvested and separated into gall in meristem region (GALL 1), gall on leaf (GALL 2, leaves (LEAF), roots (ROOT) and stem (STEM), and were treated in the same manner as in experiment 1. After the fresh and dry weights of the tissues and condition of the galls was recorded (Table II - Appendix) each plant part was finely ground and samples of 10 mg dry weights were prepared for liquid scintillation counting. In the previous experiment, since there was no significant difference between the counts obtained in samples from the same plant part, therefore only one sample per plant part was prepared.

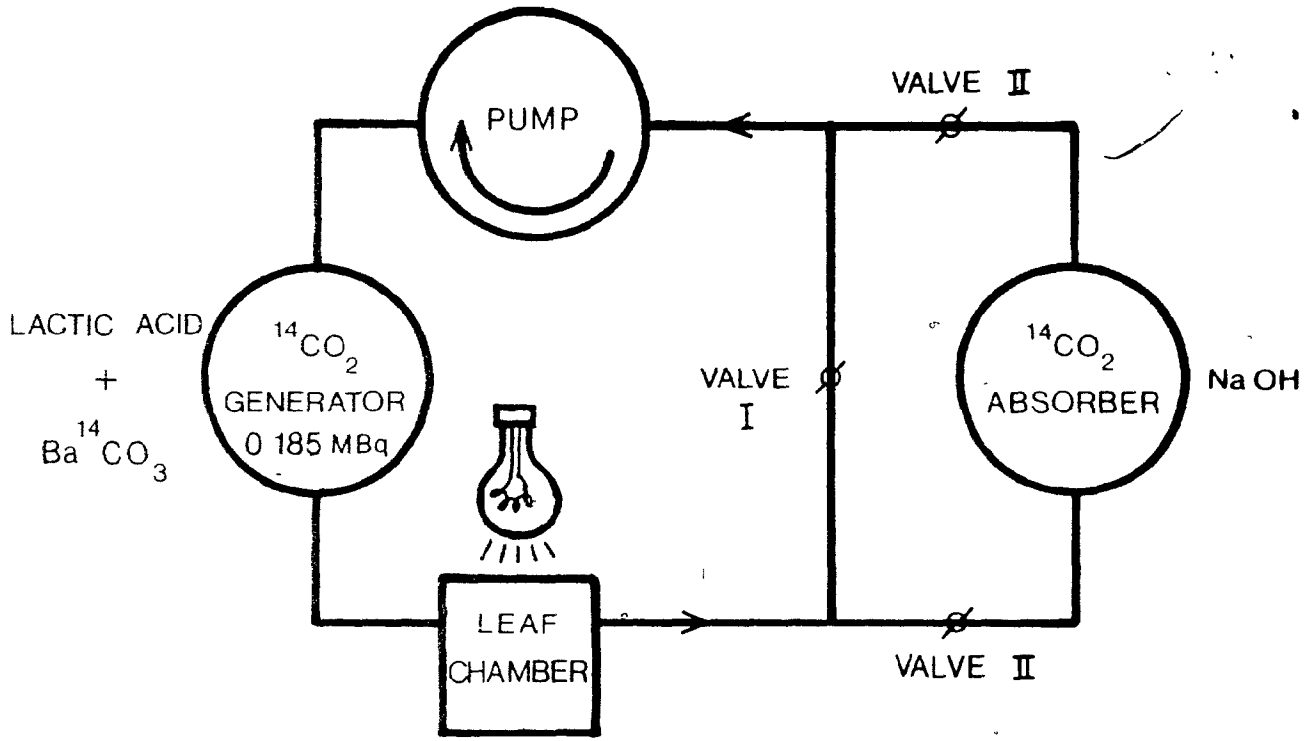


Figure 22. Circuit of a closed system in which $^{14}CO_2$ is generated and plants photosynthesize in it.

EXPERIMENT 3. ($C^{14}O_2$)

Although galls contain some chlorophyll in the outer layer of the wall surrounding the central cavity, it is assumed that it contributes but a small fraction of the photosynthates required for the gall development (Skinner et al. 1980). This experiment was conducted to demonstrate that the galls induced by P. picridis on Russian knapweed are constructed largely from the translocated assimilates and impose a considerable energy drain on the plant.

Forty plants of approximately the same age (50 to 60 days) were selected and were divided into four groups (10 plants/group) on the basis of the number of galls per plant. All plants had spatulate leaf with entire leaf margins. If the plants had galls, the age of the galls was 35 to 40 days. The condition of the galls was recorded (Table III of Fresh wt/Dry wt. - Appendix). The groups were:

Galled plants:

A" - plants with one gall in the meristem region.

B" - plants with two galls; one gall in the meristem region and another gall on a leaf.

C" - plants with three galls; one gall in the meristem region, second gall on a leaf below meristem, third gall on leaf below the second gall.

Nongalled plants:

D" - plants with no galls (control).

The procedures outlined in experiment 2 were followed with one exception. Instead of putting the whole plant in the $^{14}CO_2$ chamber only one leaf without gall was treated with $^{14}CO_2$. The leaves which receive this treatment are called presentation leaf, and are indicated by X.LEAF.

EXPERIMENT 4 (AUTORADIOGRAPHY)

Since the data obtained from liquid scintillation counting gives only the quantitative representation of the results, a similar type of experiment using autoradiographic techniques was conducted to give qualitative representation of results in which 16 plants of approximately the same age (50 to 60 days) which were grown under the conditions outlined in experiment 1, were selected. Half of these plants had galls and the age of the galls was from 35 to 40 days.

The plants were taken to the radiotracer laboratory and one leaf (presentation leaf) from the plants was put in the $^{14}CO_2$ chamber with an activity of 0.185 MBq. The procedures outlined in experiment 3 were followed.

Once all the plants received this treatment, they were taken back to the growth chamber and placed under normal conditions for 24 hours for translocation to take place. After 24 hours, 4 plants with galls and 4 without galls were killed by pressing them on a hot plate and the other half were harvested and separated into gall, leaf, root, stem and presentation leaf and fixed in FAA. Once the tissues were fixed, they were dehydrated through a TBA series and embedded in paraffin wax following the procedures outlined in Appendix E. Sections of the embedded material were cut at 25 using a rotary microtome and adhered to microscope slides using Myer's albumen. The thickness of tissue must be kept in mind when comparing stem, root, leaf and gall autoradiographs, as comparisons can only be made between tissues of similar thickness.

The pressed plant specimens and the sectional material were taken to a darkroom and placed in contact with KODAK SAFETY FILM NS using film holders and left in the dark. To protect the film from damage a layer of 'SARAN WRAP' was put in between the specimen and film.

The pressed plants were left in contact with the film for 7 days and the sections for 21 days. After the allotted time, the film was removed from the holders and developed in D-19 developer at 20°C for 4 minutes.

ANALYSIS OF DATA

The data were analysed as a completely randomized design to evaluate the effects of the galls on the plant. The number of galls per plant were assigned as treatments and the plant part was considered as a variable. Since the coefficient of variability was very high in counts per minute per 10 mg dry weight sample and disintegration per minute per plant part, square root and natural log transformations were applied to the data. Analysis of variance was conducted and Duncan's Multiple Range Test was used to locate significant differences among the variables within treatments on the original and transformed data. Student pair 't' tests were used to locate differences between treatment means of galled and nongalled plants within variables. A correlation was also done between the counts per minute or disintegration per minute and the fresh weight/dry weight ratio, taking in account the position and condition of the galls.

III.C. RESULTS

The counts obtained from the samples are the measurements of radiation, and are the external indication of a device designed to enumerate ionizing events. It may refer to a single detected event or to the total registered in a given period of time. The term is loosely used to designate a disintegration, ionizing event or voltage pulse. The average rate of occurrence of ionizing events as observed by means of a counting system (liquid scintillation counter) is the counting rate and is usually expressed as counts per minute (CPM).

Although most radioactivity work involves relative or comparative measurements for a series of samples, it is sometimes desirable to know the actual amount of radioactivity material present. The quantity of a radionuclide present in a source can be expressed as a weight, a number of atoms or a disintegration rate. The disintegration rate is the rate of decay of radioactive substances and it is usually expressed as disintegration per unit time.

Using samples with known amount of radioactivity (Table IV - Appendix) a Carbon-14 Standard Quench Curve is plotted (Figure I - Appendix) with the counts obtained for the Automatic External Standard (AES) as the dependent variable Y and efficiency of the counter as the independent variable X. Using these values in the regression equation $Y = \beta_0 + \beta_1 X$, the Y-intercept ($\hat{\beta}_0$) and the slope of the regression line ($\hat{\beta}_1$) is obtained.

The efficiency of the plant samples is then determined (Table IV - Appendix). The efficiency is considered to be a measure of the probability that a count will be recorded when radiation is incident on the detector. The usage varies considerably, therefore it is important to know which

factors (window transmission, sensitive volume, energy dependence, etc.) are included in a given case and therefore in these experiments the conversion factor was found to be 1.67.

The counts obtained for the automatic external standard for the samples used in these experiments were used to find the efficiency from the Carbon-14 Standard quench curve and dividing the counts per minute from channel B which is for C^{14} with the efficiency gives the disintegration per minute (DPM) for 10 mg dry weight samples. This value of the disintegration per minute when multiplied with the dry weight (mg) of the plant part gives the total disintegration per minute for the plant part.

An important part of a report of the results of any experiment is an indication of the reliability of the results. This is particularly important when slightly different results would lead to major changes in the interpretation of the results. The results obtained for the plant parts were in counts per minute for the sample of 10 mg dry weight. The very nature of the process of radioactive decay and the properties of the radiation emitted lead to errors in radioactivity measurements. If all the sources of error are taken into consideration, the next problem which arises is whether all the radioactivity which is supplied to the plant, enters it or not, which means that whether the stomata of the plant were open or not at the time when the leaves were in the $^{14}CO_2$ plant chamber. There is no certainty as to the number of stomata which were open at the time when the leaves were left to photosynthesize in $^{14}CO_2$ chamber. The counts obtained for the same plant part from different plants in the same experiment (replicates) had a very high coefficient of variability, therefore the data was presented as percent counts per minute and square root and log transformations were also applied to the data. Since the counts per minute or the transformed data gives only the

quantitative representation of the results, therefore, for comparative studies, frequency bar charts were plotted which gives a better visual presentation of this study, using the means of percent counts per minute, square root of counts per minute, natural log of counts per minute, percent disintegration per minute, square root of disintegration per minute and natural log of disintegration per minute with a ZETA PLOTTER using the Statistical Analysis System.

EXPERIMENT 1

Since time (12, 24 or 36 hr) did not play a significant role in the translocation of the assimilates, all the plants in each treatment were treated as one.

The fresh and dry weights of different parts of the galled and nongalled Russian knapweed plants injected with Fructose D [^{14}C(U)] - is shown in Table I of the Appendix and the radioactivity recorded from these parts for 10 mg sample corrected for background in counts per minute is shown in Table V of Appendix.

A correlation between counts per minute recorded in 10 mg dry weight gall samples or disintegration per minute recovered in galls with fresh weight/dry weight ratio of the gall parameters shows that the correlation coefficient for counts per minute was 0.77241 with a probability level of 0.0147 and the correlation coefficient for disintegration per minute was 0.64207 with a probability level of 0.0623.

The radioactivity in counts per minute for 10 mg dry weight samples in different parts of galled and nongalled Russian knapweed plants which were injected with Fructose D [^{14}C(U)] - (Table V - Appendix) had 71.0 to 95.7 percent coefficient of variability. The data was therefore transformed and average values were taken from the transformed data. Table 6 shows the mean distribution of radioactivity in the galled and nongalled plants as percent counts per minute. Square root of counts per minute and Natural log of counts per minute. Student pair 't' test was performed on the data comparing different parts of galled and nongalled plants and it was found that the leaf was only plant part significantly different at the 5% level. The galls of the Russian knapweed plants show the highest accumulation of radioactivity on mean percent counts per

minute basis for 10 mg dry weight sample which was about 64.8%. This percentage indicates the 'sink activity' of the galls. Figures 23, 24, and 25 show the distribution of radioactivity in galled and nongalled Russian knapweed plants and the significant differences between the plant parts at 5 percent level, when the values in Table 6 are used to plot the frequency bar charts.

The total amount of radioactivity recovered in galled and non-galled Russian knapweed plant parts in disintegration per minute is shown in Table 7. The highest radioactivity recovered in the plant part after treatment with Fructose D [$^{14}C(U)$] - was in the leaves. When comparisons between the galled and nongalled plant parts were made using student pair 't' test, the leaves of the galled and nongalled plants were significantly different at the 5% level. The galls accumulate about 23% of the radio-activity which indicates the 'sink strength' of the galls. The trans-location of radioactivity towards the galls was mainly from the leaves. Using the values in Table 7, frequency bar charts were plotted (Figures 26, 27 and 28).

Table 6. Distribution of radioactivity in galled and nongalled Russian knapweed plants injected with Fructose D [$^{14}C(U)$] -. These average values are obtained from the data presented in Table V of Appendix. (Experiment 1)

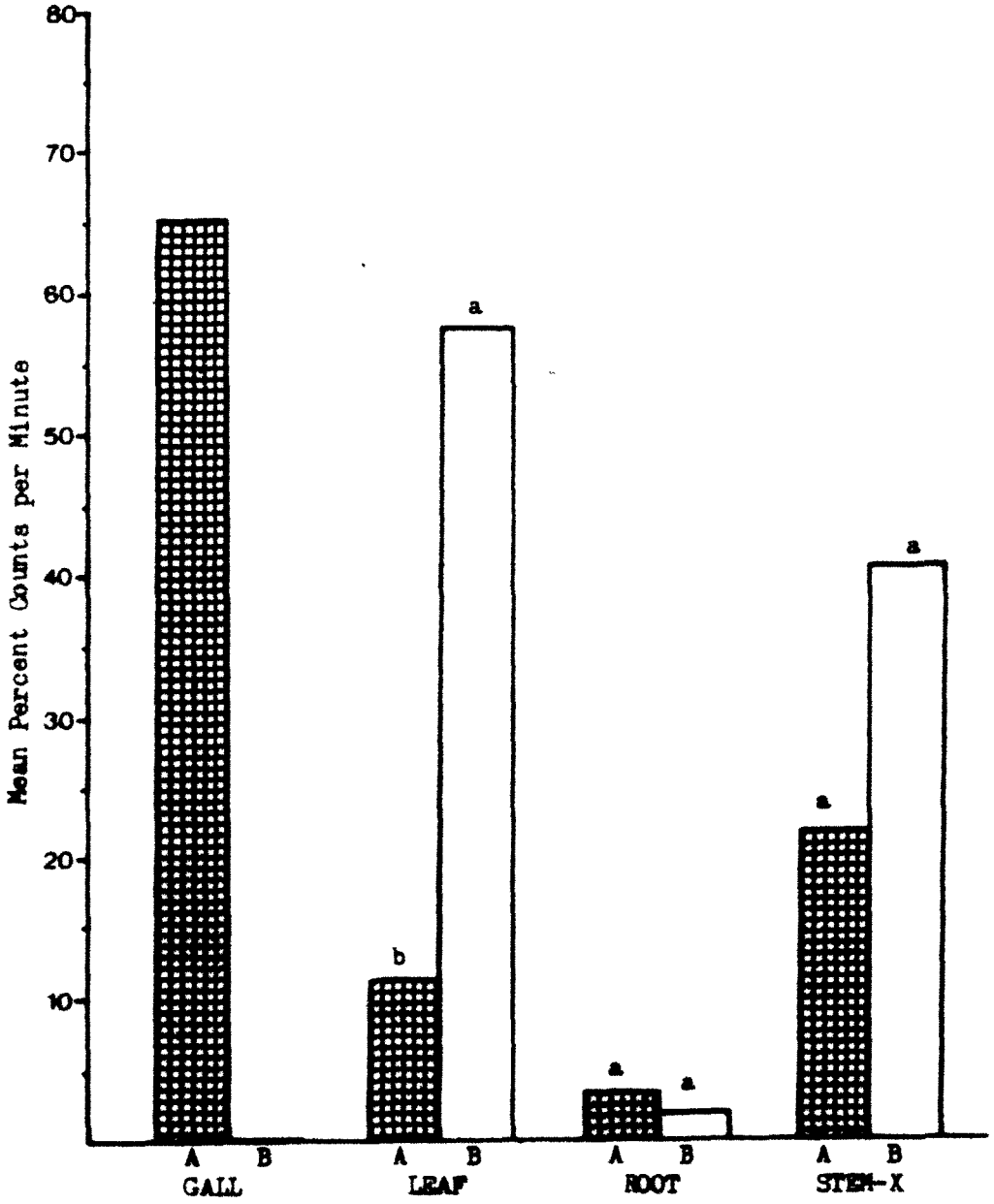
Group* (Type of plant)		Counts per minute (CPM) recorded in 10 mg dry wt. samples after treatment**			
		GALL	LEAF	ROOT	STEM.X
Galled	Mean % CPM	64.79	10.16	3.40	21.64
Nongalled		-	57.31	2.12	40.57
Galled	Mean sq.rt. of CPM	153.56	43.80	21.42	76.45
Nongalled		-	144.51	25.48	123.33
Galled	Mean natural log of	9.59	7.19	5.83	8.40
Nongalled	CPM	-	9.81	6.40	9.41

* Group Galled - plants with lobed or entire leaf margin, lyrate or spatulate leaf, one gall on leaf or petiole.

Nongalled - plants with lobed or entire leaf margin, lyrate or spatulate leaf, no gall (control)

** Student pair 't' test comparing galled and nongalled plants parts. Means within a column are not significantly different at a 5% level sharing the same line within a section.

Figure 23. Comparing the distribution of radioactivity using means of percent counts per minute recorded in 10 mg dry weight samples (Table 6), in galled and nongalled Russian knapweed plants injected with Fructose D [^{14}C(U)] -. Bars topped with same letter are not significantly different at the 5% level using a Student pair 't' test, when galled and nongalled plant parts are compared.

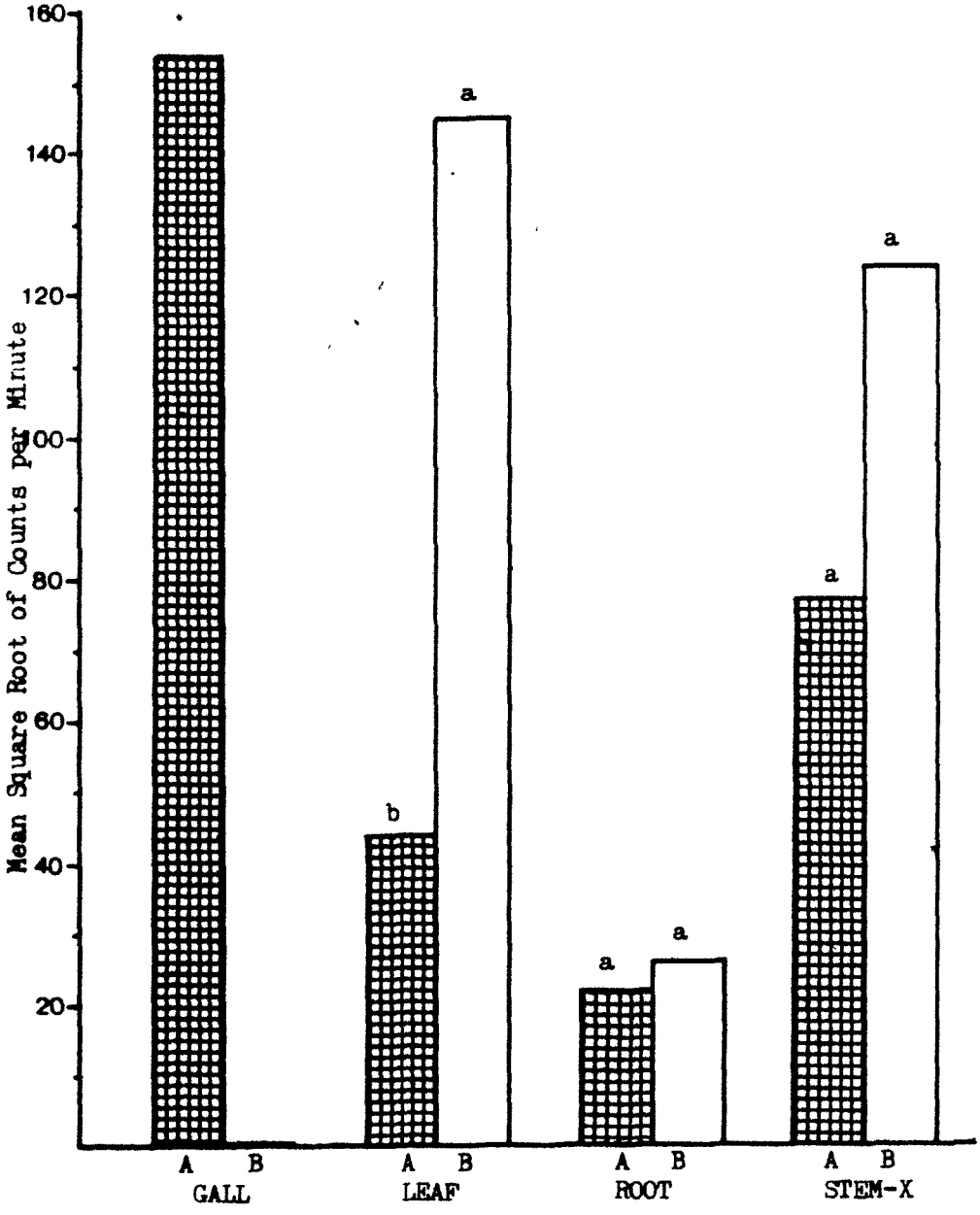


A - Plants with one gall on leaf or petiole.

B - Plants with no gall (control)

Figure 23

Figure 24. Comparing the distribution of radioactivity using means of the square root of counts per minute recorded in 10 mg dry weight samples (Table 6) in galled and nongalled Russian knapweed plants injected with Fructose D [$^{14}C(U)$] -. Bars topped with same letter are not significantly different at the 5% level using a Student pair 't' test, when galled and nongalled plant parts are compared.

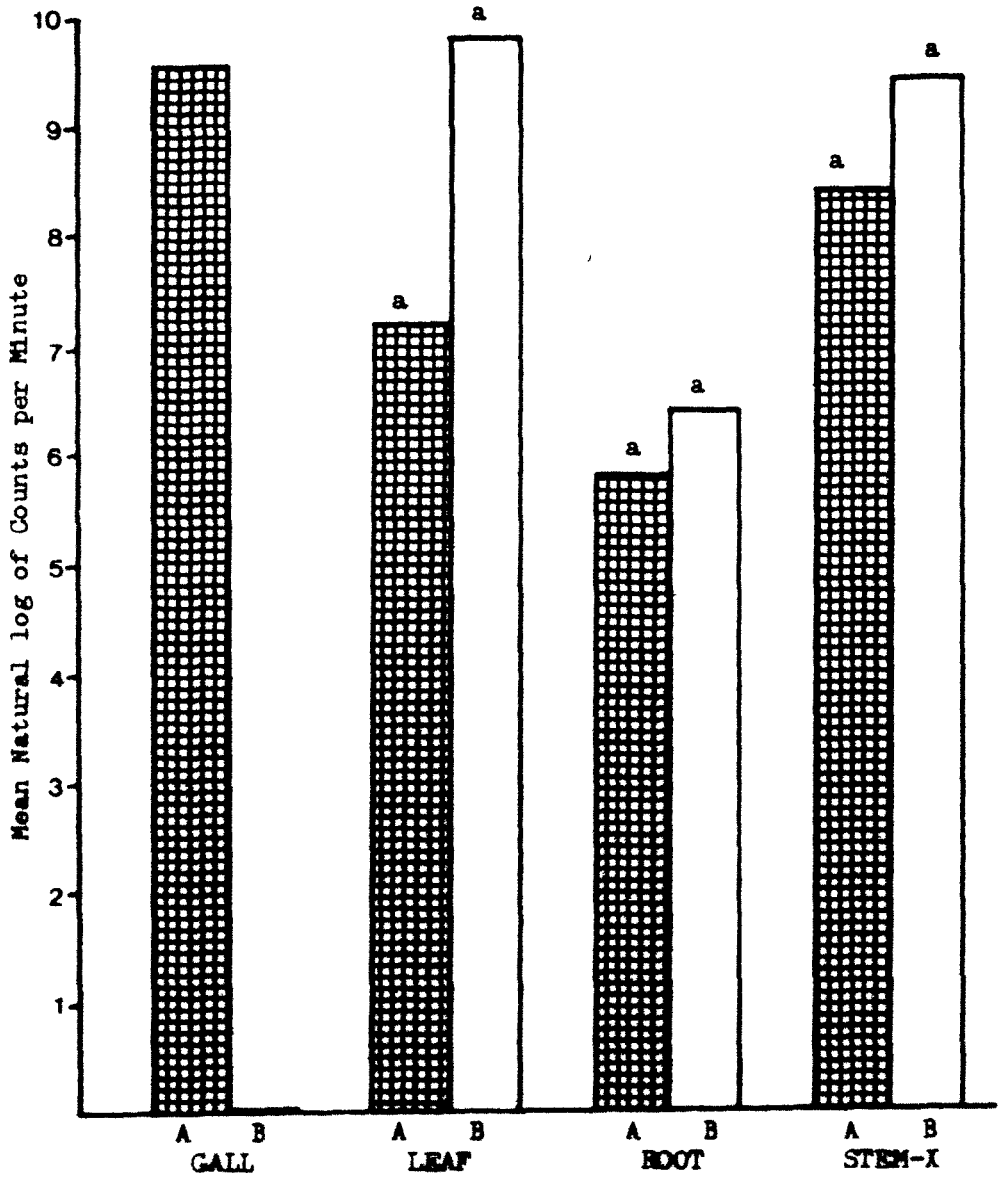


A - Plants with one gall on leaf or petiole.

B - Plants with no galls (control).

Figure 24

Figure 25. Comparing the distribution of radioactivity using means of the natural log of counts per minute recorded in 10 mg dry weight samples (Table 6) in galled and nongalled Russian knapweed plants injected with Fructose D [^{14}C(U)] -. Bars topped with same letter are not significantly different at the 5% level using a Student pair 't' test, when galled and nongalled plant parts are compared.



A - Plants with one gall on leaf or petiole.

B - Plants with no gall (control).

Figure 25

Table 7. Total radioactivity recovered in galled and nongalled Russian knapweed plants injected with Fructose D [^{14}C(U)] -. These average values are obtained by calculating the disintegration per minute (DPM) from the data presented in Tables I and V of Appendix (Experiment 1)

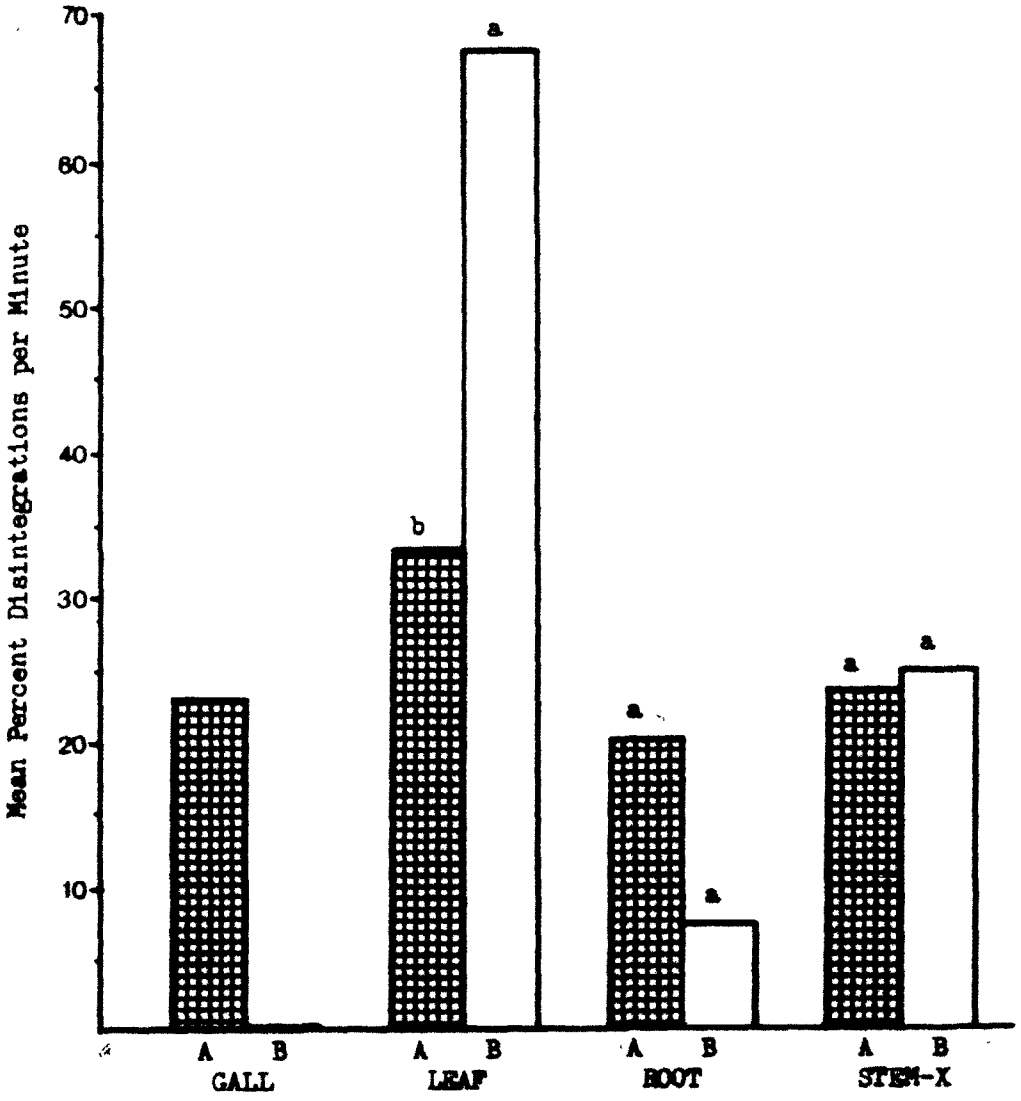
Group* (Type of plant)		^{14}C activity recovered in plant part after treatment**			
		GALL	LEAF	ROOT	STEM.X
Galled	Mean % DPM	23.04	33.35	20.04	23.56
Nongalled		-	67.75	7.47	24.78
Galled	Mean sq.rt. of DPM	251.45	291.24	169.76	262.01
Nongalled		-	715.10	230.01	395.73
Galled	Mean natural log of DPM	10.53	10.89	9.95	10.80
Nongalled		-	13.09	10.81	11.59

* Group Galled - Plants with lobed or entire leaf margin, lyrate or spatulate leaf, one gall on leaf or petiole.

Nongalled - Plants with lobed or entire leaf margin, lyrate or spatulate leaf, no gall (control).

** Student pair 't' test comparing galled and nongalled plant parts. Means within a column are not significantly different at the 5% level sharing the same line within a section.

Figure 26. Comparing the distribution of total radioactivity using means of percent disintegrations per minute recovered in the plant part (Table 7) in galled and nongalled Russian knapweed plants injected with Fructose D [$^{14}C(U)$] -. Bars topped with same letter are not significantly different at the 5% level using a Student pair 't' test when galled and nongalled plant parts are compared.

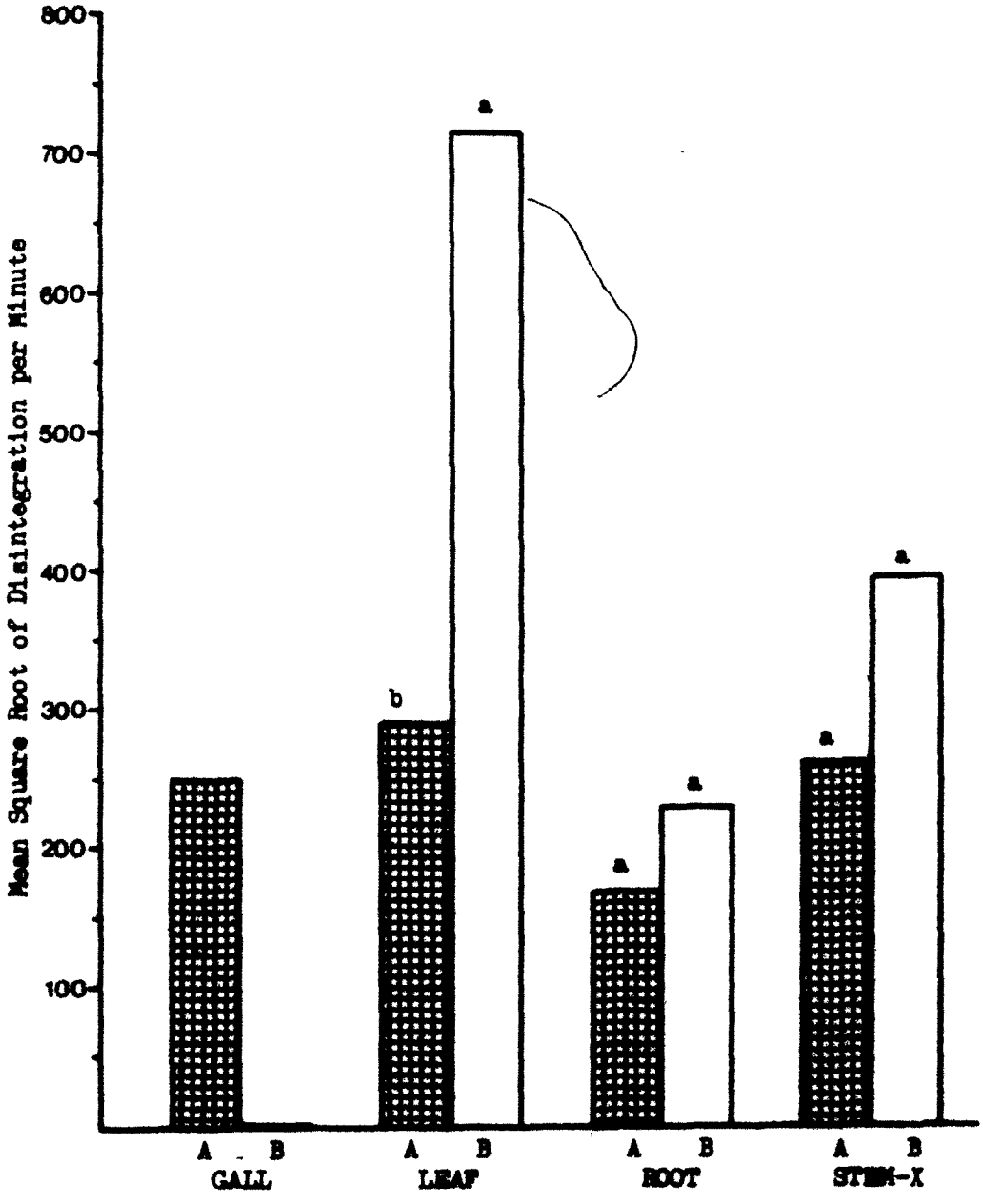


A - Plants with one gall on leaf or petiole.

B - Plants with no gall (control).

Figure 26

Figure 27. Comparing the distribution of total radioactivity using means of the square root of disintegrations per minute recovered in the plant part (Table 7) in galled and nongalled Russian knapweed plants injected with Fructose D [$^{14}C(U)$] -. Bars topped with same letter are not significantly different at the 5% level using a Student pair 't' test when galled and nongalled plant parts are compared.

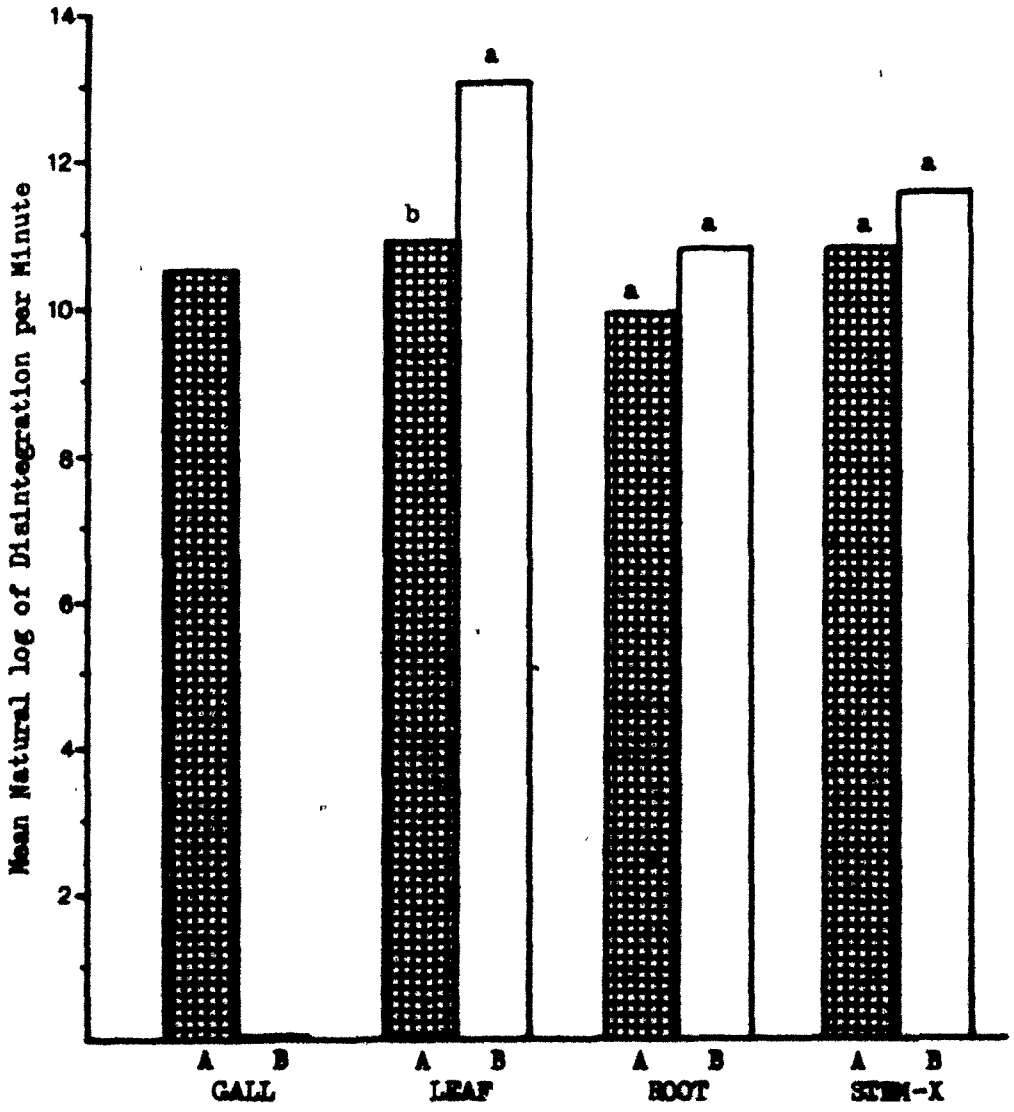


A - Plants with one gall on leaf or petiole.

B - Plants with no gall (control).

Figure 27

Figure 28. Comparing the distribution of total radioactivity using means of the natural log of disintegrations per minute recovered in the plant parts (Table 7) in galled and nongalled Russian knapweed plants injected with Fructose D [$^{14}C(U)$] -. Bars topped with same letter are not significantly different at the 5% level using a Student pair 't' test when galled and nongalled plant parts are compared.



A - Plants with one gall on leaf or petiole.
B - Plants with no gall (control).

Figure 28

EXPERIMENT 2

The fresh and dry weights of different parts of the galled and nongalled Russian knapweed plants which were treated with radioactive CO_2 in the $^{14}CO_2$ chamber is recorded in Table II of Appendix, and the radioactivity measured from these parts for 10 mg dry weight samples corrected for background in counts per minute is shown in Table VI of Appendix.

Since the whole plant was treated with $^{14}CO_2$ in the $^{14}CO_2$ chamber, about 54% of the radioactivity was retained by the leaves (Table 8 and Figures 29, 30 and 31), and the leaves do not show a significant difference at 5% level when a Student pair 't' test was performed comparing galled and nongalled plants, whereas roots and stem show a significant difference at the 5% level. The percent counts per minute recorded in the 10 mg dry weight samples of the galls was about 42%, which indicates a high 'sink activity'.

Table 9 and Figures 31, 32 and 33 show the total amount of radioactivity recovered in the galled and nongalled Russian knapweed plant parts in disintegration per minute. The leaves had retained most of the radioactivity compared to the roots and stem. On the basis of percent Disintegration per minute recovered in the plant parts, a student pair 't' test comparing galled and nongalled parts shows no significant difference at the 5% level but when square root and natural log transformations were applied to the data, roots and stem of all galled plants and leaves of plants with two galls show significant difference at the 5% level when compared with nongalled plants. The galls accumulated about 20% of the radioactivity which was translocated from roots and stem.

Table 10 indicates a positive correlation between the counts per minute or disintegration per minute with the fresh weight/dry weight ratios of the galls, but it was not significant at the 5 percent level.

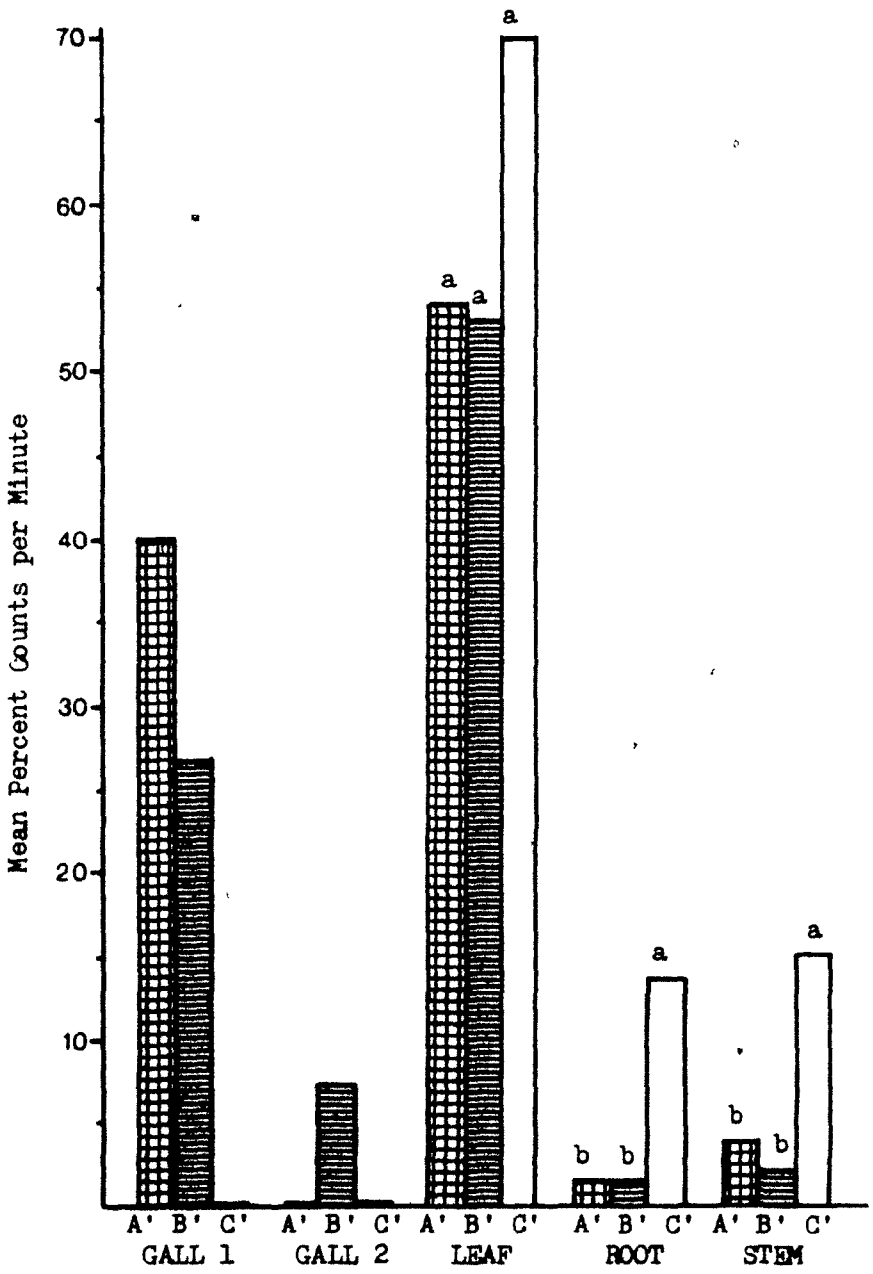
Table 8. Distribution of radioactivity in galled and nongalled Russian knapweed plants which photosynthesised in $^{14}CO_2$ chamber. These average values are obtained from the data presented in Table VI of Appendix. (Experiment 2)

Group* (Type of plant)			Counts per minute (CPM) recorded in 10 mg dry weight samples after treatment**				
			GALL 1	GALL 2	LEAF	ROOT	STEM
GALLED	Plants with 1 gall A'	Mean % CPM	40.26	-	54.15a	1.58b	4.00b
	Plants with 2 galls B'		35.40	7.42	53.35a	1.48b	2.35b
NONGALLED	C'		-	-	70.60a	14.05a	15.35a
GALLED	Plants with 1 gall A'	Mean sq. root of CPM	123.73	-	152.18a	22.44b	32.65b
	Plants with 2 galls B'		130.74	52.85	157.12a	21.35b	30.50b
NONGALLED	C'		-	-	116.75a	45.16a	53.63a
GALLED	Plants with 1 gall A'	Mean natural log of CPM	9.60	-	9.92a	6.10a	6.77a
	Plants with 2 galls B'		9.64	7.76	10.02a	5.62b	6.59b
NONGALLED	C'		-	-	9.47a	7.48a	7.96a

* Group (Plants with entire leaf margin and spatulate leaf)
Galled - A' - Plants with one gall in the meristem region.
B' - Plants with two galls, one gall in the meristem region and another gall on a leaf.
Nongalled - C' - Plants with no galls (control).

** Student pair 't' test comparing galled and nongalled plant parts. Means within a column followed by the same letter within a section are not significantly different at the 5% level.

Figure 29. Comparing the distribution of radioactivity using means of percent counts per minute recorded in 10 mg dry weight samples (Table 8) in galled and non-galled Russian knapweed plants which photosynthesised in $^{14}CO_2$ chamber. Bars topped with same letter are not significantly different at the 5% level using a Student pair 't' test, when galled and nongalled plant parts are compared.



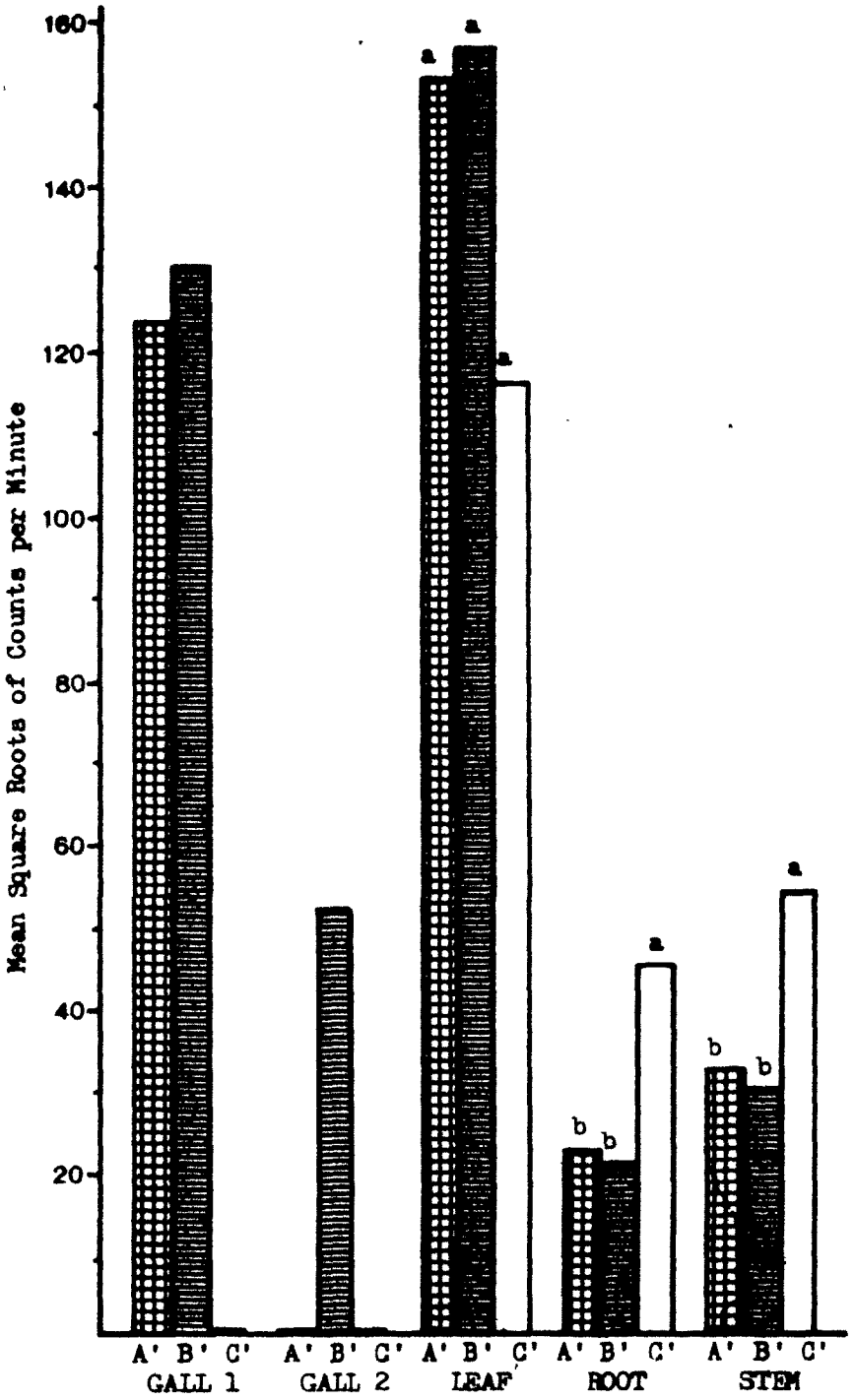
A' - Plants with one gall in meristem region.

B' - Plants with two galls, one gall in meristem region and another gall on a leaf.

C' - Plants with no galls (control).

Figure 29

Figure 30. Comparing the distribution of radioactivity using means of the square root of counts per minute recorded in 10 mg dry weight samples (Table 8) in galled and nongalled Russian knapweed plants which photosynthesised in $^{14}CO_2$ chamber. Bars topped with same letter are not significantly different at the 5% level using a Student pair 't' test, when galled and nongalled plant parts are compared.



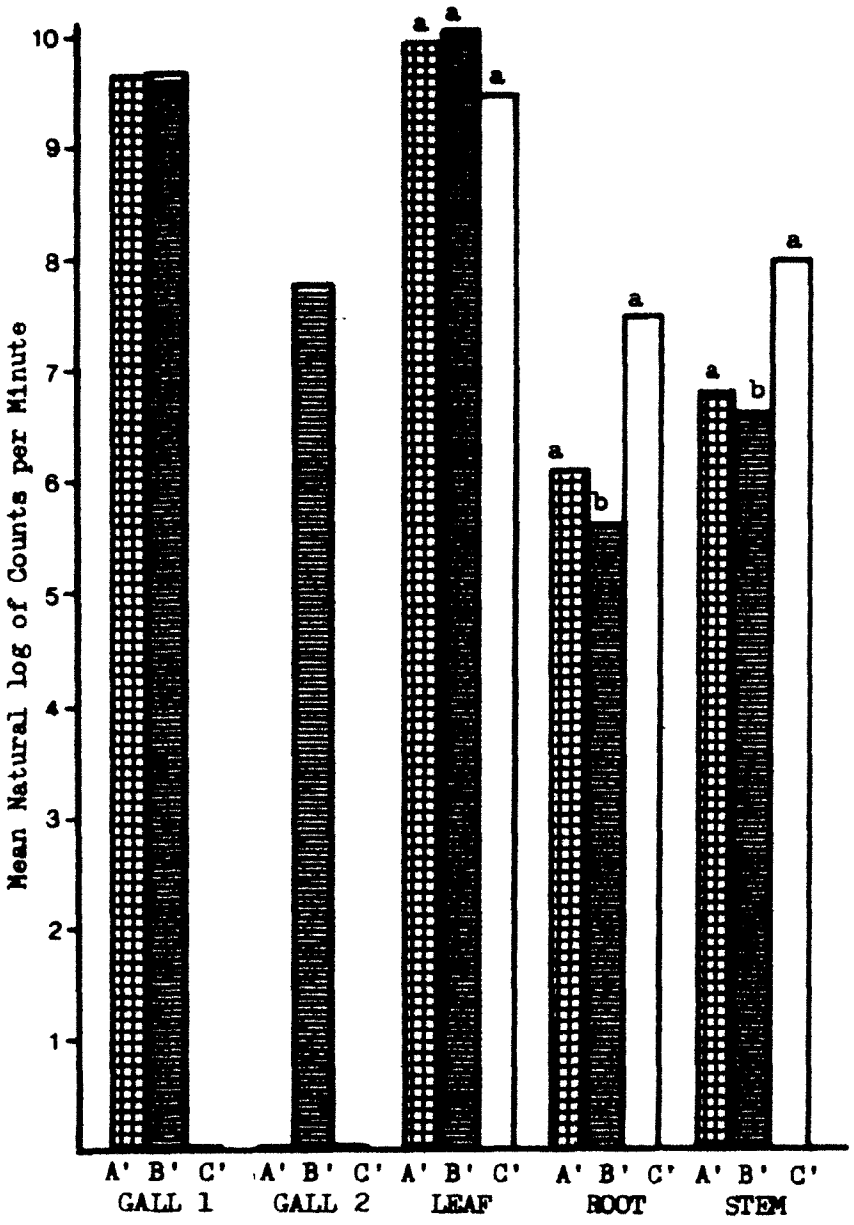
A' - Plants with one gall in meristem region.

B' - Plants with two galls, one gall in meristem region and another gall on leaf.

C' - Plants with no galls (control).

Figure 30

Figure 31. Comparing the distribution of radioactivity using the means of natural log of counts per minute recorded in 10 mg dry weight samples (Table 8) in galled and nongalled Russian knapweed plants which photosynthesised in $^{14}CO_2$ chamber. Bars topped with same letter are not significantly different at the 5% level using a Student pair 't' test, when galled and nongalled plant parts are compared.



A'- Plants with one gall in meristem region.

B'- Plants with two galls, one gall in meristem region and another gall on a leaf.

C'- Plants with no galls (control).

Figure 31

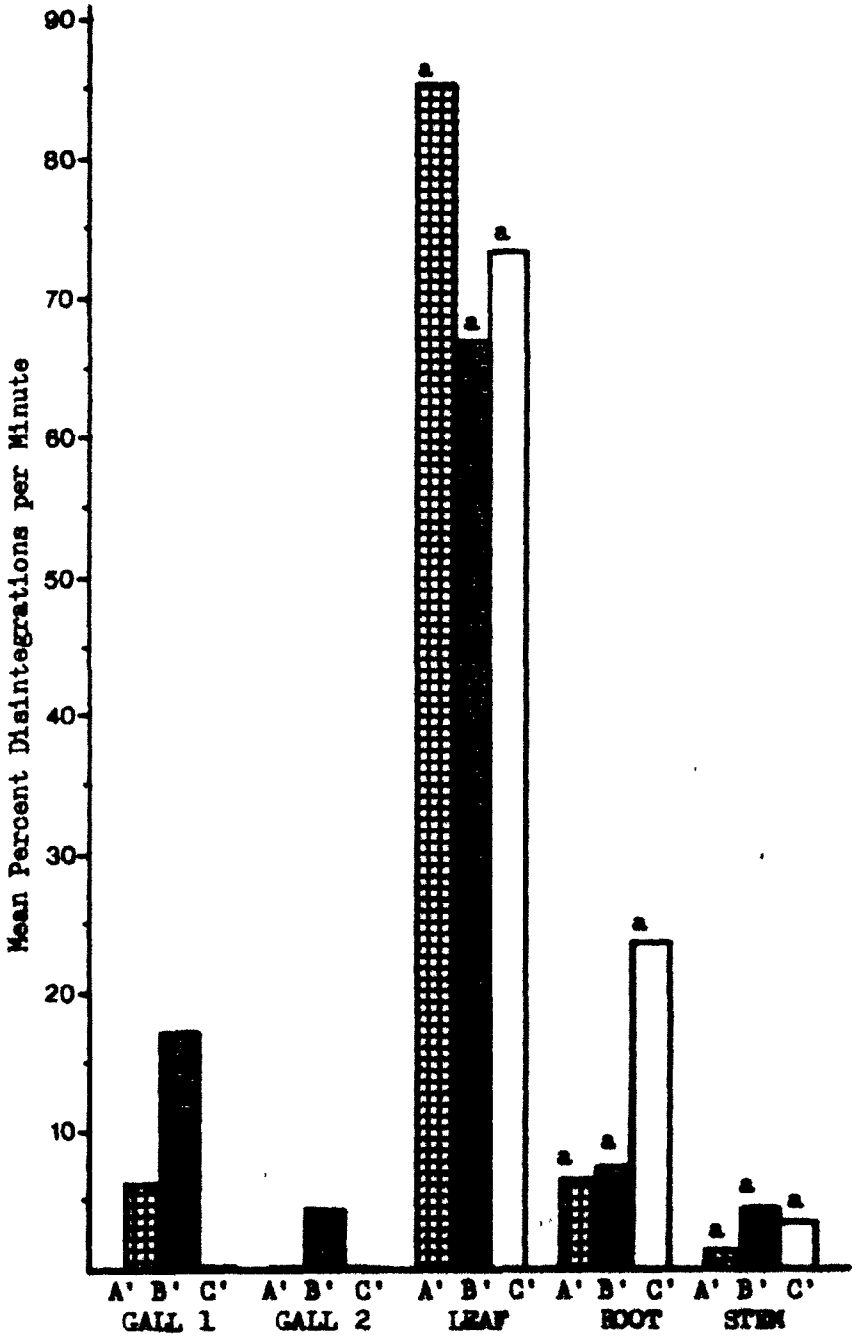
Table 9. Total radioactivity recovered in galled and nongalled Russian knapweed plants which photosynthesised in $^{14}CO_2$ chamber. These average values are obtained by calculating the disintegration per minute (DPM) from the data presented in Tables III and VI of Appendix. (Experiment 2)

Group* (Type of plant)			^{14}C activity recovered in plant part after treatment**				
			GALL 1	GALL 2	LEAF	ROOT	STEM
GALLED	Plants with 1 gall A'	Mean % DPM	6.24	-	85.68a	6.77a	1.31a
	Plants with 2 galls B'		17.07	4.31	66.77a	7.39a	4.45a
NONGALLED	C'		-	-	73.04a	23.63a	3.33a
GALLED	Plants with 1 gall A'	Mean sq. root of DPM	172.93	-	692.67a	161.29b	68.90b
	Plants with 2 galls B'		180.41	73.05	404.79b	104.98b	79.25b
NONGALLED	C'		-	-	698.72a	336.37a	148.35a
GALLED	Plants with 1 gall A'	Mean natural log of DPM	10.24	-	13.01a	10.09b	8.30b
	Plants with 2 galls B'		10.25	8.37	11.86b	8.77b	8.50b
NONGALLED	C'		-	-	13.04a	11.74a	9.97a

* Group (Plants with entire leaf margin and spatulate leaf)
Galled - A' - Plants with one gall in the meristem region.
B' - Plants with two galls, one gall in the meristem region and another gall on a leaf.
Nongalled - C' - Plants with no galls (control).

** Student pair 't' test comparing galled and nongalled plant parts. Means within a column followed by the same letter within a section are not significantly different at the 5% level.

Figure 32. Comparing the distribution of total radioactivity using means of percent disintegrations per minute recovered in the plant part (Table 9), in galled and nongalled Russian knapweed plants which photosynthesised in $^{14}CO_2$ chamber. Bars topped with same letter are not significantly different at the 5% level using a Student pair 't' test when galled and nongalled plant parts are compared.



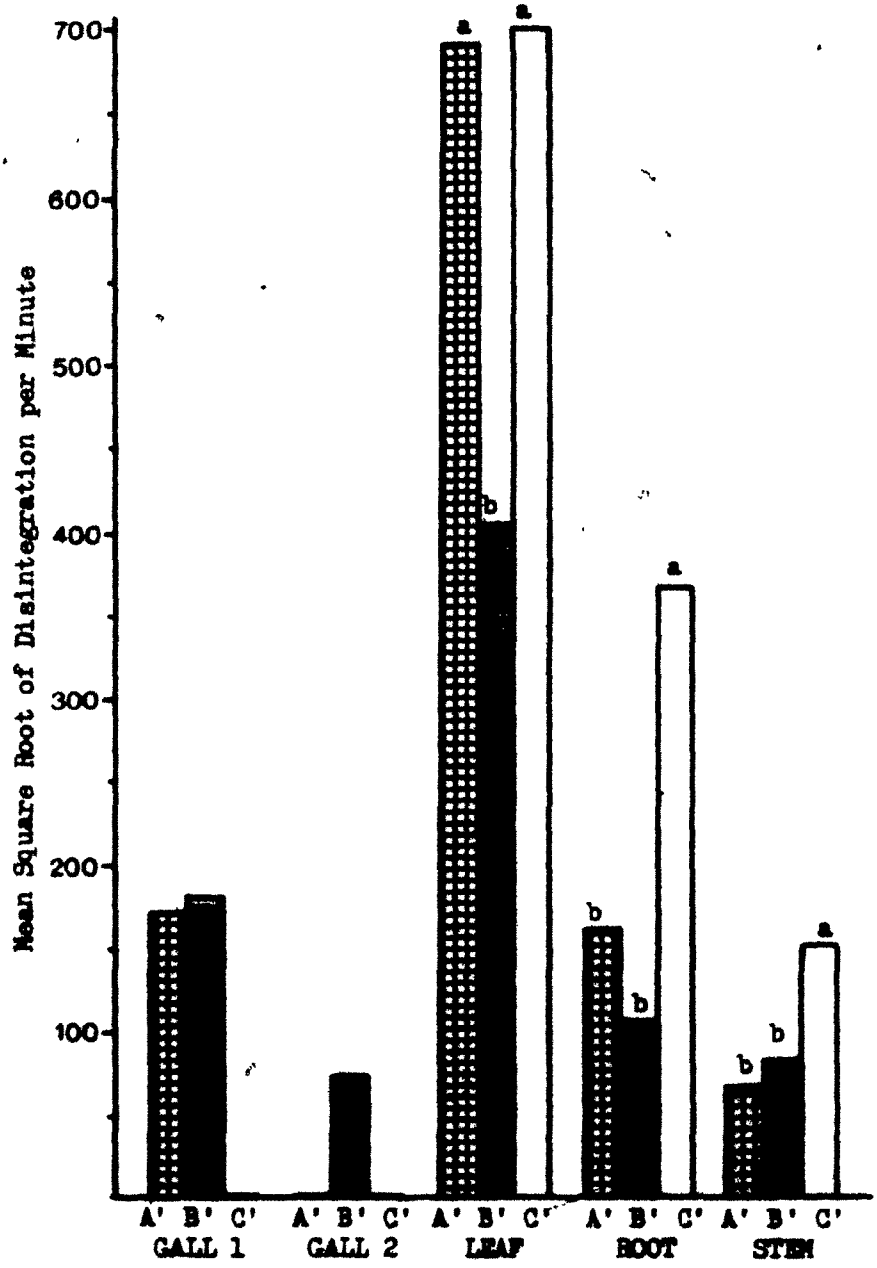
A' - Plants with one gall in meristem region.

B' - Plants with two galls, one gall in meristem region and another gall on a leaf.

C' - Plants with no galls (control).

Figure 32

Figure 33. Comparing the distribution of total radioactivity using means of the square root of disintegrations per minute recovered in the plant part (Table 9) in galled and nongalled Russian knapweed plants which photosynthesised in $^{14}CO_2$ chamber. Bars topped with same letter are not significantly different at the 5% level using a Student pair 't' test when galled and nongalled plant parts are compared.



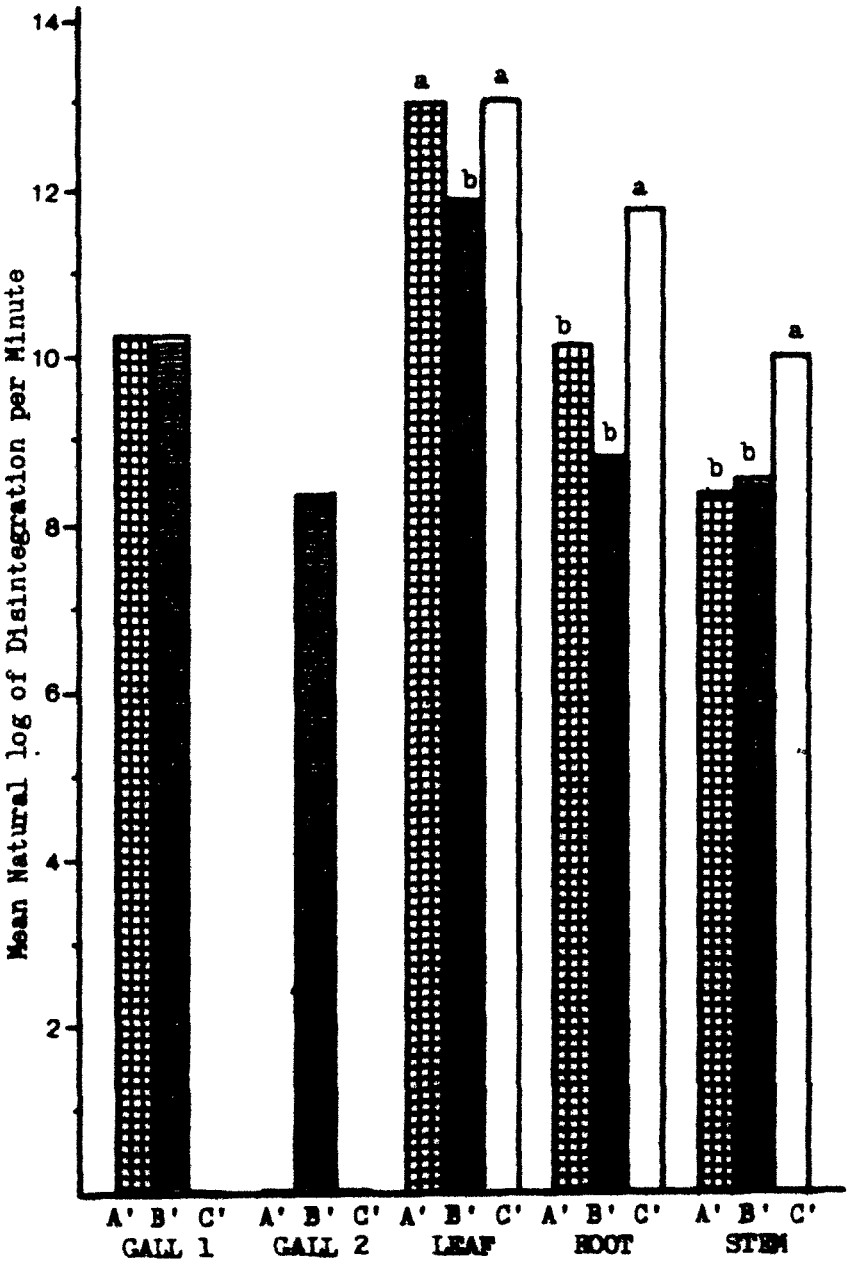
A' - Plants with one gall in meristem region.

B' - Plants with two galls, one gall in meristem region, and one gall on a leaf.

C' - Plants with no galls (control).

Figure 33

Figure 34. Comparing the distribution of total radioactivity using means of the natural log of disintegrations per minute recovered in the plant parts (Table 9) in galled and nongalled Russian knapweed plants which photosynthesized in $^{14}CO_2$ chamber. Bars topped with same letter are not significantly different at the 5% level using a Student pair 't' test when galled and nongalled plant parts are compared.



A' - Plants with one gall in meristem region.

B' - Plants with two galls, one gall in meristem region, and one gall on a leaf.

C' - Plants with no galls (control).

Figure 34

Table 10. Correlation coefficients of Russian knapweed parameters (Experiment 2)

Russian knapweed parameters		Counts per minute recorded in 10 mg dry wt. gall samples	Disintegration per minute recovered in total dry weights of galls
Plants with 1 gall	Fresh/Dry weight ratio of the gall in meristem region	0.35788	0.41048
		0.5543 *	0.4924
Plants with 2 galls	Fresh/Dry weight ratio of the gall in meristem region	0.82712	0.70730
		0.0840	0.1815
	Fresh/Dry weight ratio of gall on a leaf	0.70492	0.68815
		0.1837	0.1990

* Probability level

EXPERIMENT 3

The data obtained was treated basically in the same way as experiment 2. Table III of the Appendix shows the fresh and dry weights of different parts of the galled and nongalled Russian knapweed plants from which one leaf without a gall was treated with radioactive CO_2 in the $^{14}CO_2$ chamber. The radioactivity in counts per minute, corrected for background for 10 mg dry weight samples measured from the different plant parts is shown in Table VII of the Appendix.

In this experiment, only the 'presentation leaf' (X.LEAF) was treated with radioactive $^{14}CO_2$, and the translocation of the radioactivity to the other parts of the plant was from the 'presentation leaf'. Table 11 and Figures 35, 36 and 37 show the distribution of radioactivity in counts per minute for galled and nongalled Russian knapweed plant parts of 10 mg dry weight samples. The 'presentation leaf' retains about 53% of the radioactivity in the galled plants and about 17% in the nongalled plants, the difference is significant at 5 percent level. The indication of the 'sink activity' of the galls is noticed from the results. The galls accumulate from 30% to 45% of the radioactivity, which was mainly translocated from the leaves in all galled plants and also from the root and stem in plants with more than one gall. When a student pair 't' test was performed comparing the galled and nongalled plant parts, the difference was significant at 5 percent level.

When the total amount of radioactivity translocated and recovered in the galled and nongalled Russian knapweed plant parts in disintegration per minute was calculated (Table 12 and Figures 38, 39 and 40), the 'presentation leaf' (X.LEAF) retained most of the activity in the plants with two galls, but the difference was not significant at the 5 percent level.

In the nongalled plants the leaves accumulated the highest amount of radioactivity which was about 50% and when compared with galled plants, the difference was significant at the 5 percent level, whereas roots and stem of the galled and nongalled plants were not significantly different. The galls accumulated 16 to 35 percent of the radioactivity which was translocated from the leaves mainly.

When square root transformations were applied to the data and comparisons done between galled and nongalled parts, beside leaves, 'presentation leaf' of plants with one gall and stem of plants with three galls were also found to be significantly different at the 5 percent level, and when natural log transformation was applied to the data then stem of plants with two galls was also found significantly different at the 5% level along with the others mentioned above.

When a correlation was done between the counts per minute obtained for 10 mg sample or disintegration per minute for the whole plant part with the fresh weight/dry weight ratios of the galls, only the galls in the meristem region of the plants with two galls showed a negative correlation (Table 13).

Out of the 0.185 MBq of the radioactivity ($^{14}CO_2$) only 0.063 MBq was recovered in the nongalled plants and 0.050 MBq in plants with one gall, 0.043 MBq in plants with two galls and 0.033 MBq in plants with three galls. The reason for this low recovery was that only one leaf was made to photosynthesize in the presence of $^{14}CO_2$. The photosynthetic rate also decreased from 20 to 40 percent in the galled plants when compared to the nongalled plants.

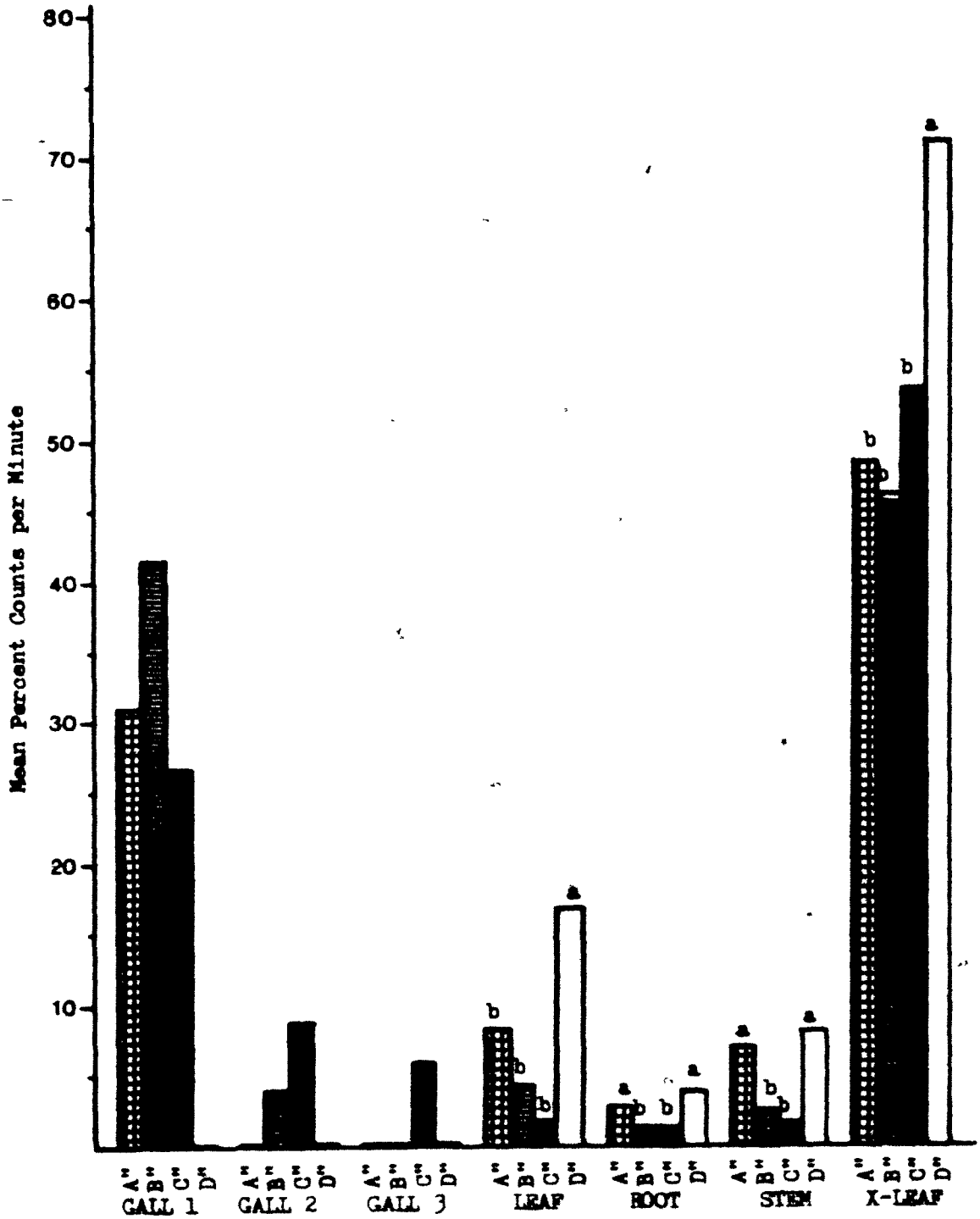
Table 11. Distribution of radioactivity in galled and nongalled Russian knapweed plants from which only presentation leaf (X.LEAF) photosynthesised in $^{14}CO_2$ chamber. These average values are obtained from the data presented in Table VII of the Appendix. (Experiment 3)

Group* (Type of plant)		Counts per minute (CPM) recorded in 10 mg dry weight samples after treatment**						
		GALL 1	GALL 2	GALL 3	LEAF	ROOT	STEM	X.LEAF
GALLED	plants with 1 gall A"	31.19	-	-	8.32b	2.85a	7.11a	48.37b
	plants with 2 galls B" Mean	41.81	4.10	-	4.25b	1.35b	2.61b	45.88b
	plants with 3 galls C" % CPM	26.60	8.95	6.01	1.83b	1.41b	1.62b	53.58b
NONGALLED	D"	-	-	-	16.86a	4.00a	8.07a	71.06a
GALLED	plants with 1 gall A"	115.75	-	-	54.34a	31.51a	52.68a	149.02a
	plants with 2 galls B" Mean	188.28	55.61	-	37.61b	29.84a	44.93a	202.77a
	plants with 3 galls C" square root of CPM	141.45	77.91	49.89	29.37b	32.70a	35.35a	210.87a
NONGALLED	D"	-	-	-	74.27a	30.86a	49.66a	157.09a
GALLED	plants with 1 gall A"	9.13	-	-	7.29a	6.67a	7.70a	9.91a
	plants with 2 galls B" Mean	10.40	7.47	-	6.61b	6.61a	7.43a	10.51a
	plants with 3 galls C" natural log of CPM	9.61	8.14	7.19	6.44b	6.70a	6.86a	10.56a
NONGALLED	D"	-	-	-	8.47a	6.65a	7.76a	10.08a

* Group (Plants with entire leaf margin and spatulate leaf)
Galled - A" - Plants with one gall in the meristem region.
B" - Plants with two galls, one gall in the meristem region and another gall on a leaf.
C" - Plants with three galls, one gall in the meristem region, second gall on a leaf below meristem, third gall on a leaf below the second gall.
Nongalled - D" - Plants with no galls (control).

** Student pair 't' test comparing galled and nongalled plant parts. Means within a column followed by the same letter within a section, are not significantly different at the 5% level.

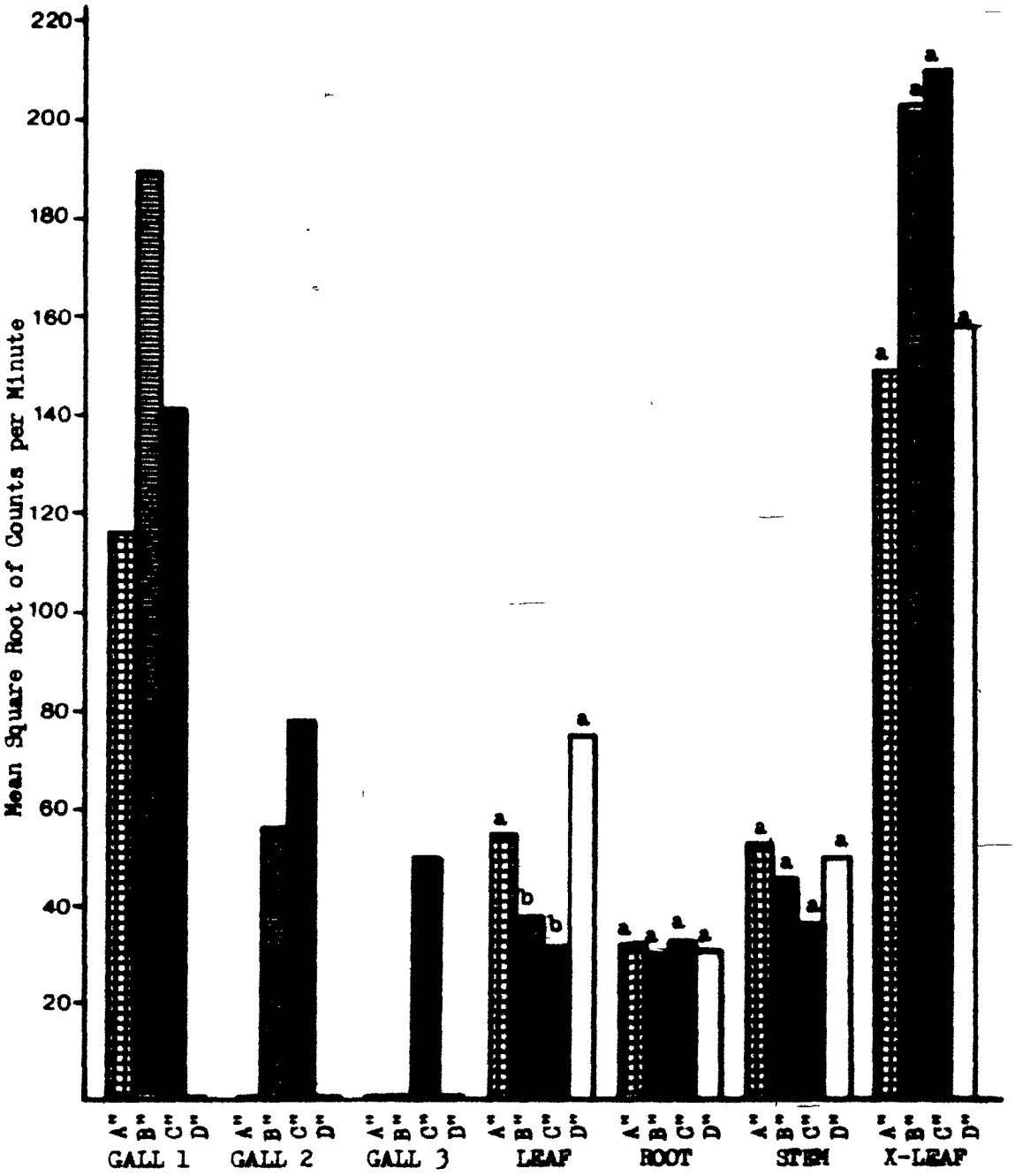
Figure 35. Comparing the distribution of radioactivity using means of percent counts per minute recorded in 10 mg dry weight samples (Table 11), in galled and nongalled Russian knapweed plants from which only presentation leaf (X-LEAF) photosynthesised in $^{14}CO_2$ chamber. Bars topped with same letter are not significantly different at the 5% level using a Student pair 't' test, when galled and nongalled plant parts are compared.



A" - Plants with one gall in the meristem region.
B" - Plants with two galls, one gall in the meristem region and another gall on a leaf.
C" - Plants with three galls, one gall in the meristem region, second gall on a leaf below meristem, third gall on a leaf below the second gall.
D" - Plants with no galls (control).

Figure 35

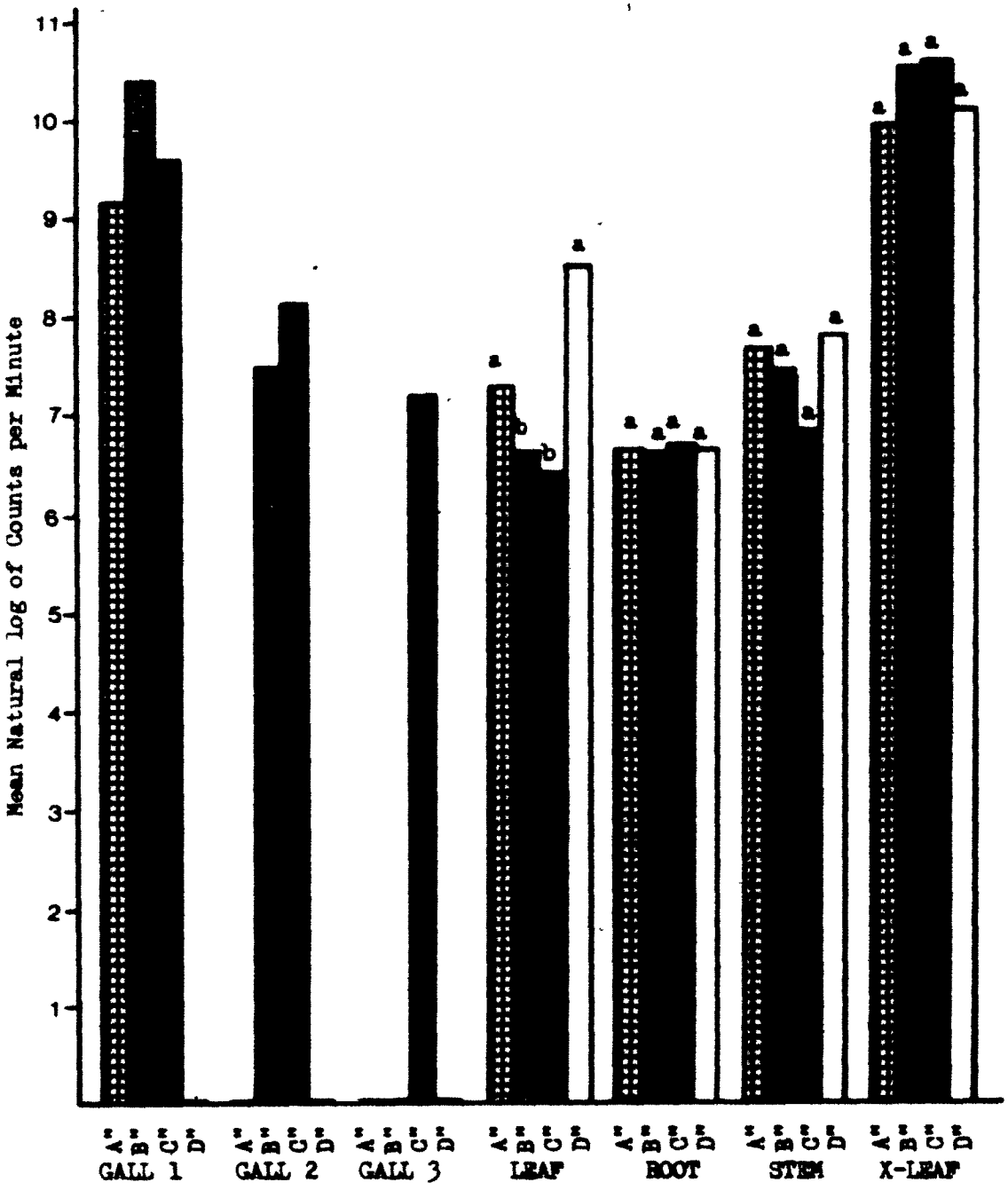
Figure 36. Comparing the distribution of radioactivity using means of the square root of counts per minute recorded in 10 mg dry weight samples (Table 11), in galled and nongalled Russian knapweed plants from which only presentation leaf (X-LEAF) photosynthesised in $^{14}CO_2$ chamber. Bars topped with same letter are not significantly different at the 5% level using a Student pair 't' test, when galled and nongalled plant parts are compared.



A" - Plants with one gall in the meristem region.
B" - Plants with two galls, one gall in the meristem region and another gall on a leaf.
C" - Plants with three galls, one gall in the meristem region, second gall on a leaf below meristem, third gall on a leaf below second gall.
D" - Plants with no galls (control).

Figure 36

Figure 37. Comparing the distribution of radioactivity using means of natural log of counts per minute recorded in 10 mg dry weight samples (Table 11), in galled and nongalled Russian knapweed plants from which only presentation leaf (X-LEAF) photosynthesized in $^{14}CO_2$ chamber. Bars topped with same letter are significantly different at the 5% level using a Student pair 't' test, when galled and nongalled plant parts are compared.



A" - Plants with one gall in the meristem region.
B" - Plants with two galls, one gall in the meristem region and another on a leaf.
C" - Plants with three galls, one gall in the meristem region, second gall on a leaf below meristem, third gall on a leaf below second gall.
D" - Plants with no galls (control).

Figure 37

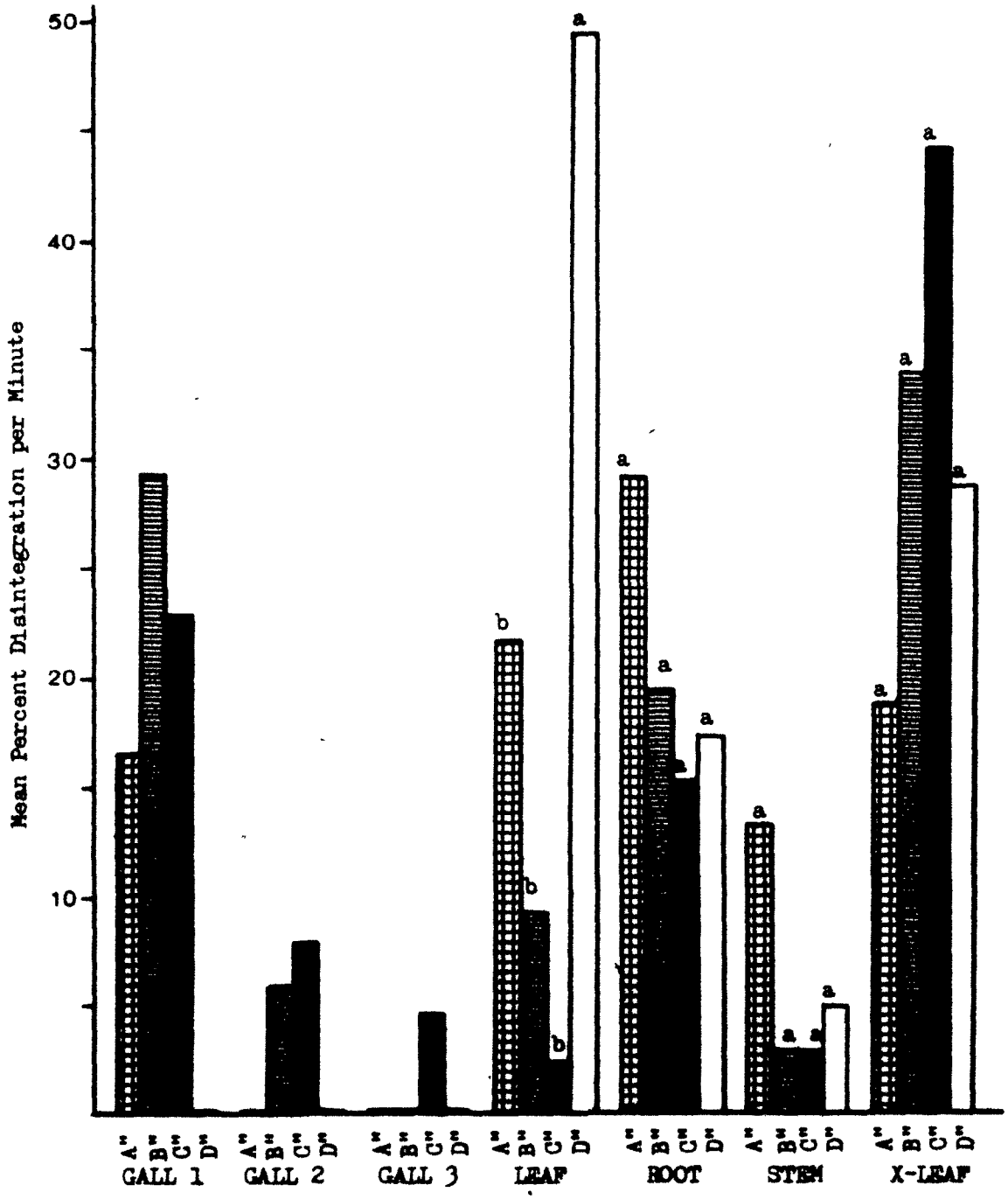
Table 12. Total radioactivity recorded in galled and nongalled Russian knapweed plants from which only presentation leaf (X.LEAF) photosynthesised in $^{14}CO_2$ chamber. These average values are obtained by calculating the disintegration per minute (DPM) from the data presented in Tables IV and VII of the Appendix. (Experiment 3)

Group* (Type of Plant)		^{14}C activity recovered in plant part after treatment**						
		GALL 1	GALL 2	GALL 3	LEAF	ROOT	STEM	X.LEAF
	Plant with 1 gall A"	16.46	-	-	21.61b	30.47a	13.21a	18.88a
GALLED	Plant with 2 galls B" Mean % DPM	29.18	5.67	-	9.27b	19.08a	2.98a	33.80a
	Plant with 3 galls C"	22.77	7.77	4.72	2.39b	15.35a	2.78a	44.26a
NONGALLED	D"	-	-	-	49.21a	17.28a	4.81a	28.70a
	Plant with 1 gall A"	197.79	-	-	222.74b	269.93a	172.29a	210.16b
GALLED	Plants with 2 galls B" Mean square root of DPM	258.91	100.66	-	105.44b	109.49a	81.57a	287.45a
	Plants with 3 galls C"	192.66	105.09	64.84	49.71b	161.10a	68.44b	279.24a
NONGALLED	D"	-	-	-	428.44a	218.94a	125.79a	309.20a
	Plant with 1 gall A"	10.04	-	-	10.33b	10.92a	10.05a	10.61b
GALLED	Plants with 2 galls B" Mean natural log of DPM	11.04	8.44	-	8.80b	10.42a	8.66b	11.22a
	Plants with 3 galls C"	10.26	8.76	7.74	7.50b	9.92a	8.09b	11.11a
NONGALLED	D"	-	-	-	11.99a	10.59a	9.63a	11.42a

* Group (Plants with entire leaf margin and spatulate leaf)
Galled - A" - Plants with one gall in the meristem region.
B" - Plants with two galls, one gall in the meristem region and another gall on a leaf
C" - Plants with three galls, one gall in the meristem region, second gall on leaf below meristem, third gall on a leaf below the second gall.
Nongalled - D" - Plants with no galls (control).

** Student pair 't' test comparing galled and nongalled plant parts. Means within a column followed by the same letter within a section, are not significantly different at the 5% level.

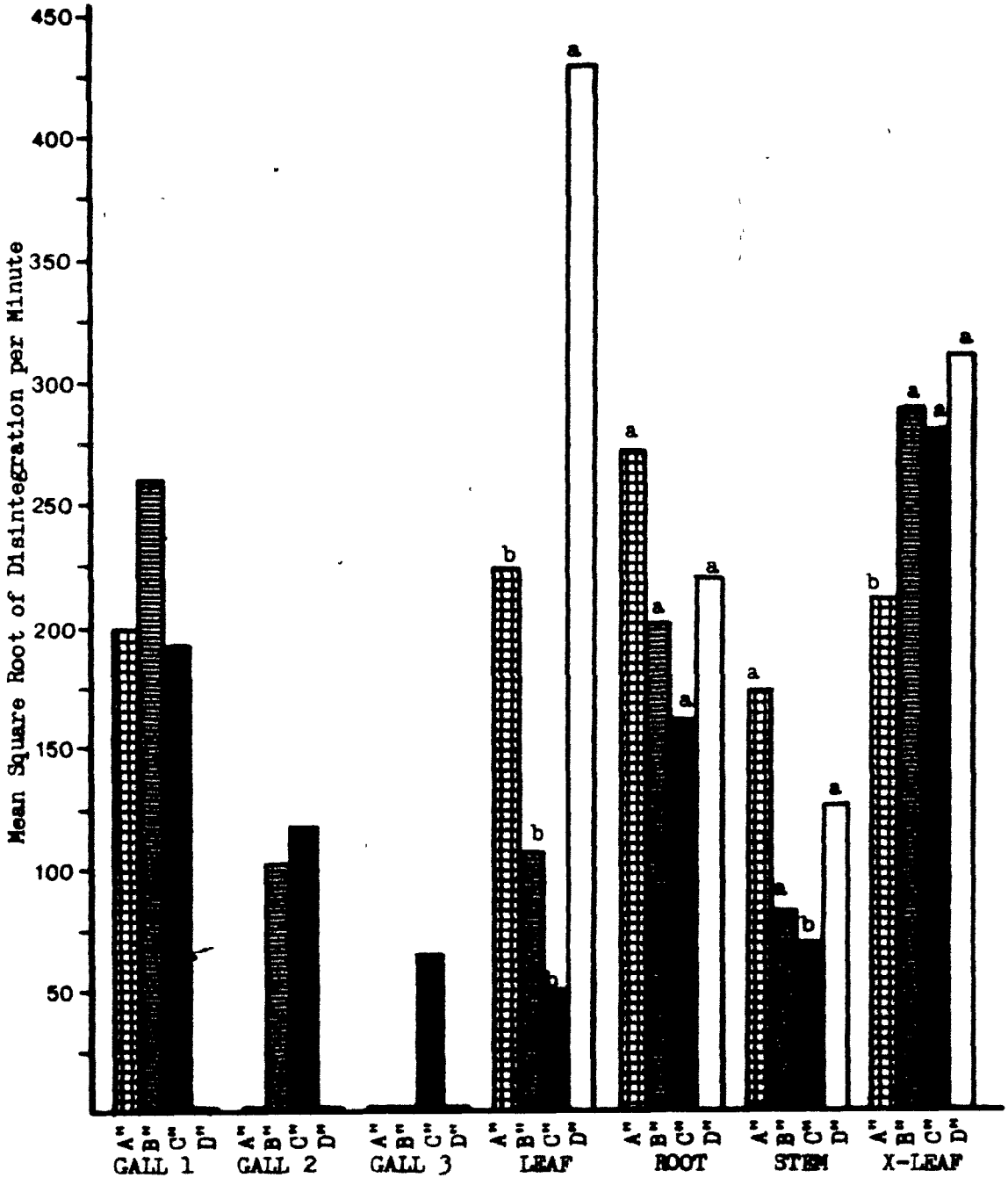
Figure 38. Comparing the distribution of total radioactivity using means of percent disintegrations per minute recovered in the plant part (Table 12), in galled and nongalled Russian knapweed plants from which only presentation leaf (X-LEAF) photosynthesised in $^{14}CO_2$ chamber. Bars topped with same letter are not significantly different at the 5% level using a Student pair 't' test, when galled and nongalled plant parts are compared.



A" - Plants with one gall in the meristem region.
B" - Plants with two galls, one gall in the meristem region and another on a leaf.
C" - Plants with three galls, one gall in the meristem region, second gall on a leaf below meristem, third gall on a leaf below second gall.
D" - Plants with no galls (control).

Figure 38

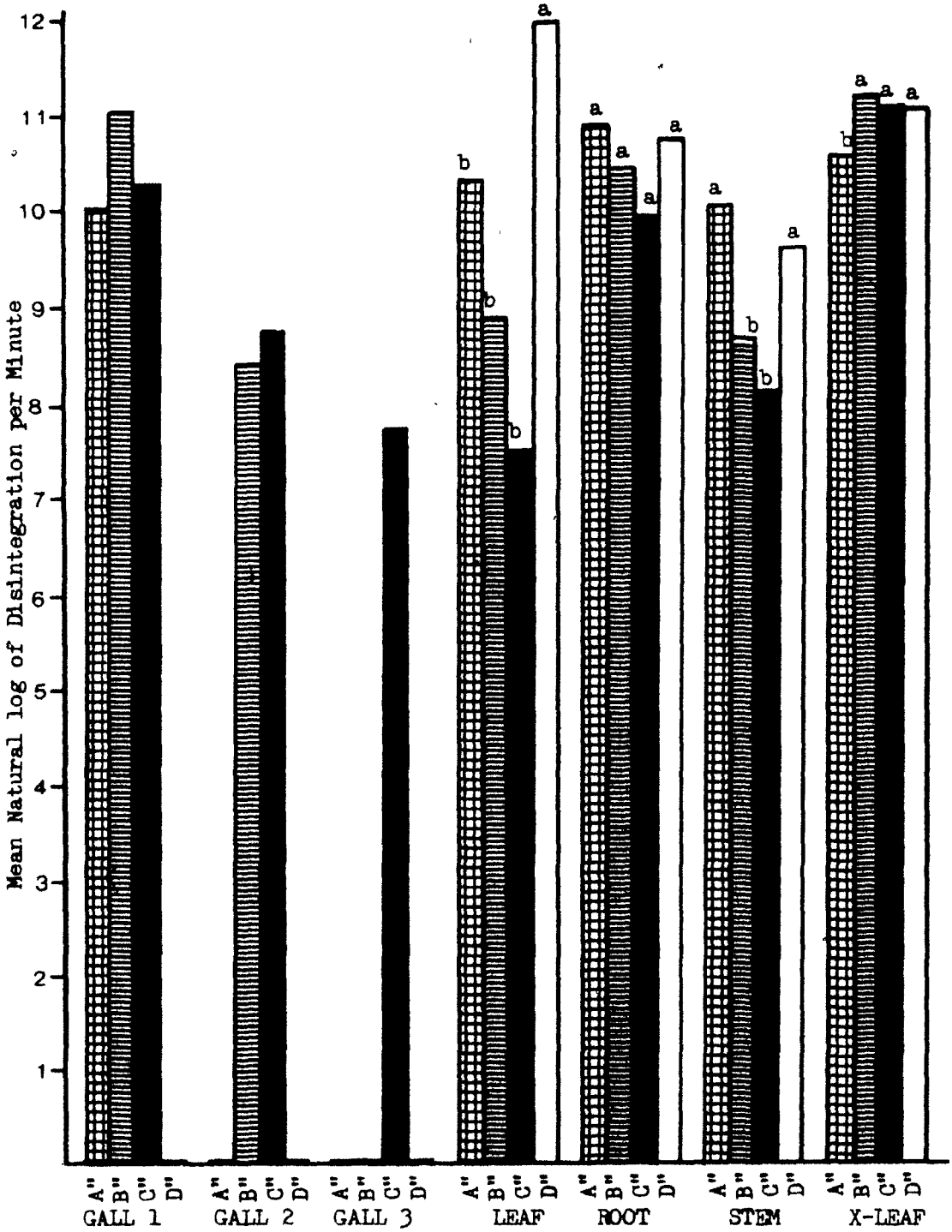
Figure 39. Comparing the distribution of total radioactivity using means of the square root of disintegrations per minute recovered in the plant part (Table 12), in galled and nongalled Russian knapweed plants from which only presentation leaf (X-LEAF) photosynthesised in $^{14}CO_2$ chamber. Bars topped with same letter are not significantly different at the 5% level using a Student pair 't' test, when galled and nongalled plant parts are compared.



A" - Plants with one gall in the meristem region.
B" - Plants with two galls, one gall in the meristem region and another on a leaf.
C" - Plants with three galls, one gall in the meristem region, second gall on a leaf below meristem, third gall on a leaf below second gall.
D" - Plants with no galls (control).

Figure 39

Figure 40. Comparing the distribution of total radioactivity using means of the natural log of disintegrations per minute recovered in the plant part (Table 12), in galled and nongalled Russian knapweed plants from which only presentation leaf (X-LEAF) photosynthesised in $^{14}CO_2$ chamber. Bars topped with same letter are not significantly different at the 5% level using a Student pair 't' test, when galled and nongalled plant parts are compared.



A" - Plants with one gall in the meristem region.
B" - Plants with two galls, one gall in the meristem region and another on a leaf.
C" - Plants with three galls, one gall in the meristem region, second gall on a leaf below meristem, third gall on a leaf below second gall.
D" - Plants with no galls (control).

Figure 40

Table 13. Correlation coefficient of Russian knapweed parameters. (Experiment 3)

Russian knapweed parameters		Correlation with counts per minute (r)	Correlation with disintegration per minute (r)
Plants with 1 gall	Fresh weight/Dry weight ratio of the gall in the meristem region	0.75466*	0.56973
		0.0116 **	0.0856
Plants with 2 galls	Fresh weight/Dry weight ratio of the gall in the meristem region	-0.12519	-0.22661
		0.7304	0.5290
	Fresh weight/Dry weight ratio of the gall on leaf	0.72796*	0.76752***
		0.0170	0.00092
Plants with 3 galls	Fresh weight/Dry weight ratio of gall in the meristem region	0.79919***	0.82387***
		0.0055	0.0034
	Fresh weight/Dry weight ratio of the gall on leaf below the meristem	0.81593***	0.83342***
		0.0040	0.0027
	Fresh weight/Dry weight ratio of the gall on leaf below the second gall	0.87356***	0.86990***
		0.0010	0.0011

* Significant at 5 percent level.
*** Significant at 1 percent level
** Probability level

EXPERIMENT 4

The autoradiography obtained for the whole plants which were left in contact with the X-Ray film (Figures 41 and 42) shows that the radioactivity in the nongalled plants is translocated mainly to the leaves, whereas in the galled plants, most of the translocation is into the galls of the plant. But when sections of the galled and nongalled plant parts were used for autoradiography (Figures 43, 44 and 45), the stem of the nongalled plant shows the accumulation of radioactivity and in galled plants, beside the galls, the root and stem also show the properties of 'physiological sink'. This response is slightly variable or it could be an artifact. But, one thing which is clearly represented by the autoradiographs is that the galls accumulate most of the radioactivity which is translocated towards them from the different parts of the plant.

Figure 41. Autoradiograph of nongalled Russian knapweed plant showing the distribution of C^{14}. Only the presentation leaf (X-LEAF) of the plant was allowed to photosynthesize in $^{14}CO_2$ chamber. The plant was left in contact with the film for 7 days. The plant was outlined and lighter areas correspond to locations of greater radioactivity.

Figure 42. Autoradiograph of galled Russian knapweed plant showing the distribution of C^{14}. Only the presentation leaf (X-LEAF) of the plant was allowed to photosynthesize in $^{14}CO_2$ chamber. The plant was left in contact with the film for 7 days. The plant was outlined and lighter areas correspond to locations of greater radioactivity.

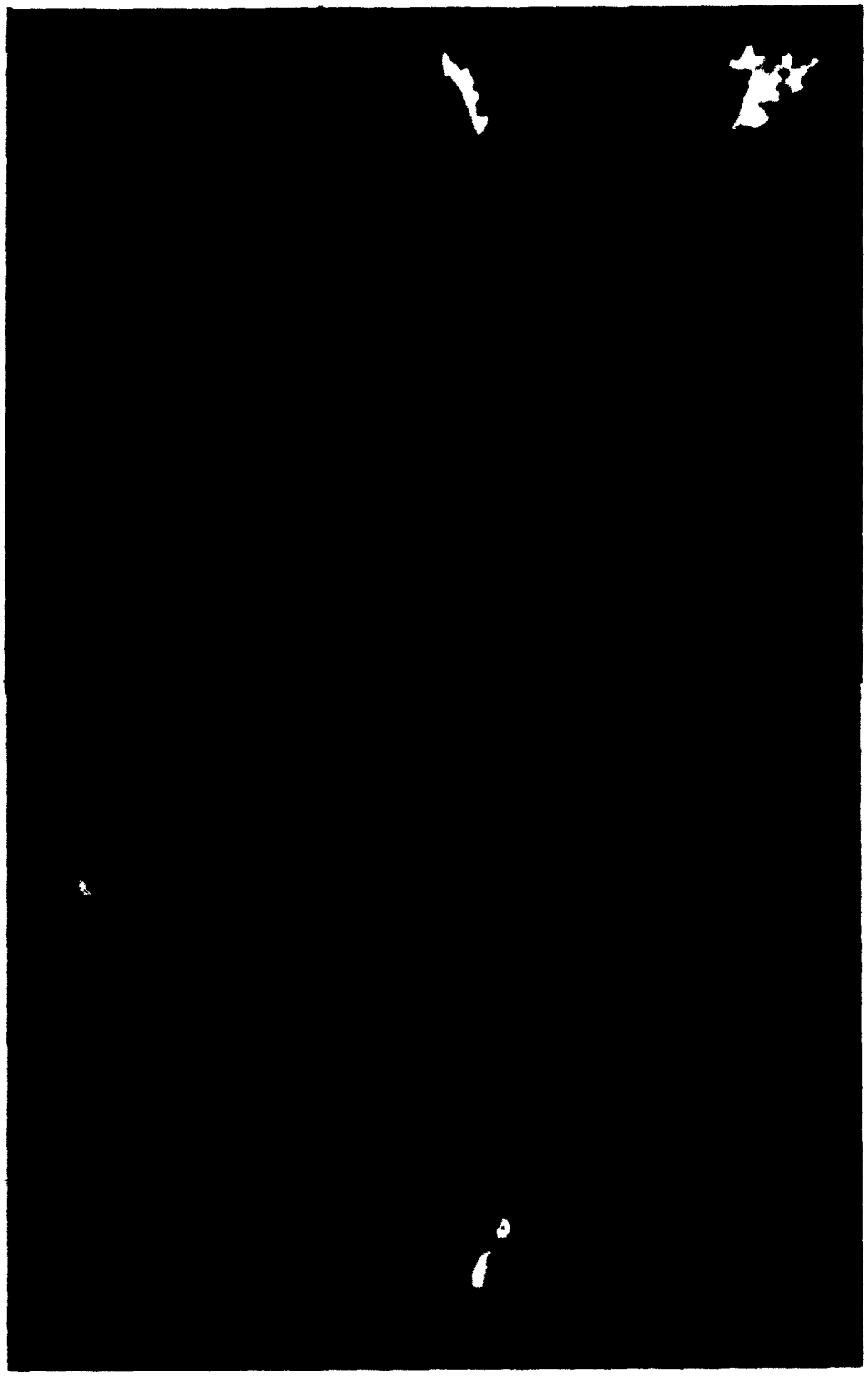
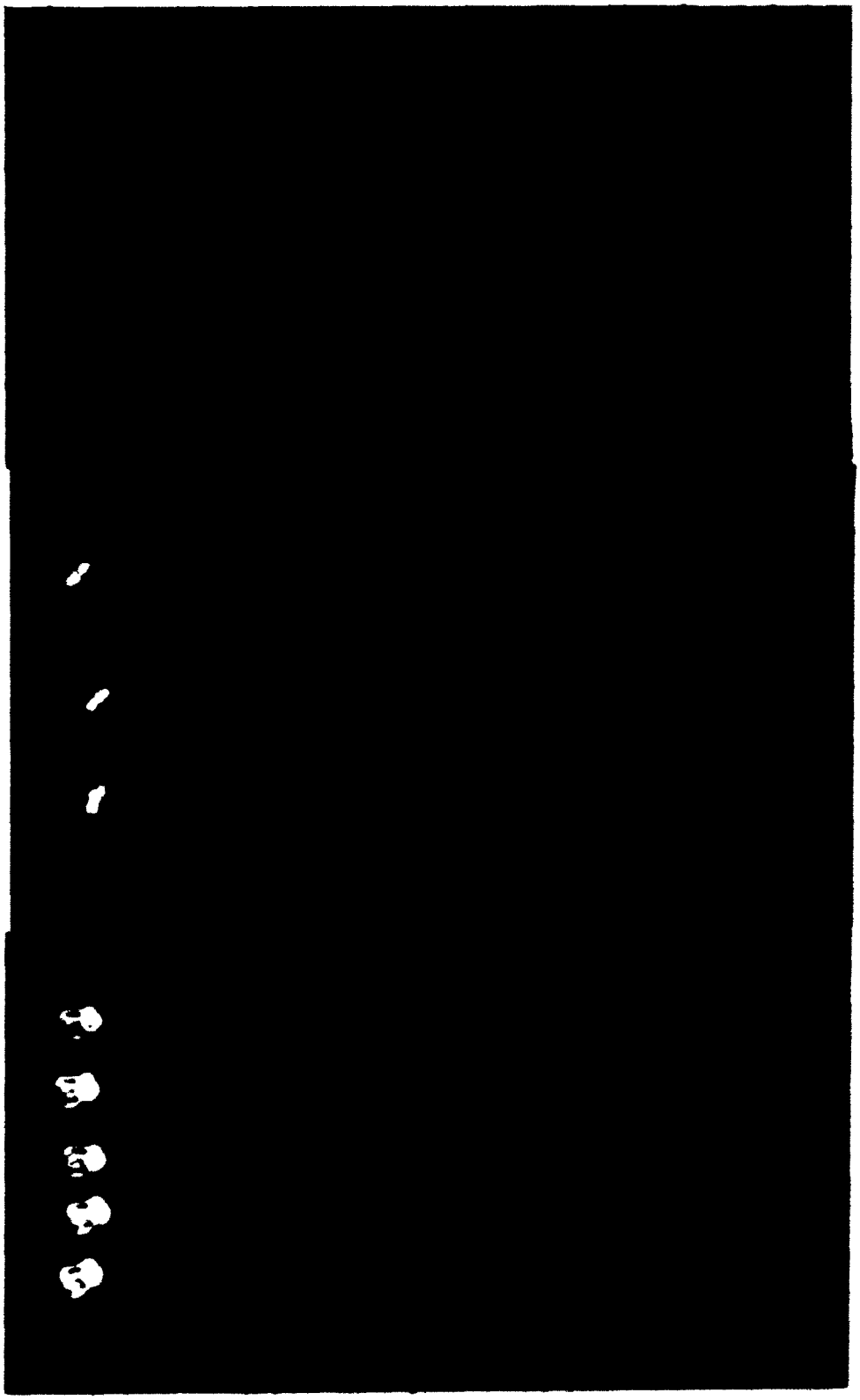


Figure 43. Autoradiographs of nongalled Russian knapweed plant parts (X-LEAF, LEAF, ROOT and STEM) sectioned at 25 . Only the presentation leaf (X-LEAF) was allowed to photosynthesize in $^{14}CO_2$ chamber. The sections were left in contact with the film for 21 days. Lighter areas correspond to location of radioactivity.

Figure 44. Autoradiographs of galled Russian knapweed plant parts (X-LEAF, LEAF, ROOT, STEM and GALL) sectioned at 25 . Only the presentation leaf (X-LEAF) was allowed to photosynthesize in $^{14}CO_2$ chamber. The sections were left in contact with the film for 21 days. Lighter areas correspond to location of radioactivity.

Figure 45. Autoradiographs of galled Russian knapweed plant parts (X-LEAF, LEAF, ROOT, STEM and GALL) sectioned at 25 . Only the presentation leaf (X-LEAF) was allowed to photosynthesize in $^{14}CO_2$ chamber. The sections were left in contact with the film for 21 days. Lighter areas correspond to location of radioactivity.


GALL
LEAF
ROOT
STEM
X-LEAF

III.D. DISCUSSION

When ever it is stated in the text that the radioactive substance which was injected in the plant or fed to the leaves became localized, it implies that either the original substance or its products or both became localized. The results indicate that the galls on Russian knapweed formed by the nematode showed properties attributable to a physiological sink, namely, a movement of labelled assimilates into it, the consequent, accumulation of radioactivity within it. Assimilates can be drawn to the gall from all parts of the plant through the phloem.

When a correlation was done between counts per minute recorded in 10 mg dry weight gall samples or disintegration per minute recovered in the galls with fresh weight/dry weight ratio of the gall (Tables 10 and 13), it was observed to be positive. Little radioactivity entered the galls which were brown and dry with a low fresh weight/dry weight ratio compared to the galls which were green and fleshy with a high fresh weight/dry weight ratio (Table I, II and III of Appendix).

Mani (1964) and Maresquelle and Meyer (1965) have suggested that the growth of the gall ceases if the parasite within is killed. It is likely that the parasite continually secretes a substance or a mixture of substances which stimulate the sink-like activity of the gall tissue. This is an example of a high degree of physiological adaptation exhibited by the parasite which in this case is able to modify the functions of the plant organ to its advantage. During the life cycle of Paranguina picridis the second stage larvae go into a state of quiescence (cryptobiosis). This state is induced by dehydration as the plant matures (Cooper and Van Gundy, 1971; Watson, 1975). The fresh weight/dry weight ratio of the

galls was lower when the galls were brown and dry, and little radioactivity was translocated towards them as opposed to the galls with a higher fresh weight/dry weight ratio with a higher translocation of radioactivity. This indicates that when the nematodes undergo into a state of quiescence, the galls stop functioning as a physiological sink. Shaw and Samborskii (1956) using rust and Jankiewicz et al. (1969) using insects have demonstrated the same type of results.

At the cellular level there is little doubt that the nematode induced galls act as strong sinks. Whether this transport is 'hormone directed' is not clear (Patrick, 1976). 'Sink strength', the potential capacity of tissues to accumulate metabolites, can be defined as the product of 'sink activity' (the potential rate of metabolic uptake per unit weight and time measured in counts per minute for 10 mg dry weight samples) and 'sink size' (dry weight). The sink may itself be able to regulate transport of metabolites to it (Patrick, 1976). 'Matthew effect' appears to operate in source-sink situations (Evans, 1976), that is, large sinks have an advantage with respect to small sinks in attracting solutes. Thus by creating an abnormally large sink (at cellular level), the nematode mobilises plant cell machinery to enhance transport to that sink without the need to resort to elaborate mechanism to control cell formation (Jones, 1981). There is evidence that the plant endoparasitic nematodes feed on these modified cells of the gall and the form of modified cell, giant cell, syncytia or nurse cell is controlled by the nematode (Jones and Dropkin, 1975). The nematode-induced transfer cell has a distinct pattern of growth which can be closely correlated with the physiological age of the nematode (Bird, 1962). It is essential for successful obligate parasitism that substrates be supplied to the invaded cells at a rate high enough to maintain their organization

and metabolism in the presence of the parasite, which cannot exist on dead tissue. Whether or not the parasite obtains an adequate supply of substrates for continued growth must depend on the relative rates of utilization and supply (transport) of substrates. Since the nematode P. picridis has a high biotic potential (Watson, 1975), it indicates a substantial nutrient supply to the galls.

In experiment 1, when the radioactive Fructose D [$^{14}C(U)$] was injected into the stem of the galled and nongalled Russian knapweed plants, the stem (Stem.X) and roots did not show a significant difference when a student pair 't' test was performed comparing the galled and non-galled plants, whereas the leaves of the galled and nongalled showed a significant difference at the 5 percent level. The radioactivity was injected into the phloem of the stem, which was the translocation pathway and it was therefore easily translocated to the roots which were natural sink for the plant. The highest amount of radioactivity translocated was into the leaves (Table 7, Figures 26, 27 and 28). The reason for this high recovery of radioactivity in the leaves, since the leaves were at a developmental stage, they also had some 'sink' properties and in their case, the 'sink size' was quite large. The 'sink activity' of the galled and nongalled plant parts is represented by Table 6 and Figures 23, 24, and 25 in counts per minute for 10 mg dry weight samples, and the galls show the highest amount of activity recorded. The 'sink activity' for the stem (Stem.X) and roots were not significantly different for galled and nongalled plants but for the leaves the difference was significant. So the reason for the high 'sink strength' for the leaves was probably the 'sink size', because leaves do not have a high 'sink activity'.

In experiment 2, where the whole plants were put in the $^{14}CO_2$ chamber, the amount of radioactivity recovered in the leaves was highest, and the difference in the galled and nongalled plants was not significant. The leaves retain most of the radioactivity. In plants with one gall, the radioactivity translocated to the roots was second highest, but in plants with two galls, the galls accumulate more radioactivity than the roots and had a higher 'sink strength'. The stem of the galled and non-galled plants had the least amount of radioactivity translocated towards them and when a student pair 't' test was performed comparing galled and nongalled plants on the mean percent disintegration per minute basis, the difference was found to be not significant (Table 9 and Figure 32). When the square root and natural log transformations were applied to the data and galled and nongalled plant parts were compared, the leaves of plants with two galls were significantly different at the 5 percent level with leaves of nongalled plants, and also the roots and stem of galled and nongalled plants were significantly different at a 5 percent level from each other. Only the leaves of plants with one gall were not significantly different from the nongalled plant (Table 9, Figures 33 and 34) indicating that in plants with one gall, the translocation of the assimilates into the gall was from roots and stem and as the number of galls increased on the plant, the assimilates were derived towards the galls from all the parts of the plant.

The 'sink activity' of the leaves was expected to be high in experiment 2 in galled and nongalled plants because the whole plants were photosynthesised in the $^{14}CO_2$ chamber and most of the radioactivity was retained by the leaves, giving a value which indicated high 'sink activity' (Table 8, Figure 29). When a student pair 't' test was performed and

comparisons were made between galled and nongalled plant parts, the leaves did not show any significant difference, whereas roots and stem of the galled and nongalled plants were significantly different at the 5 percent level. A similar type of result was obtained when square root and natural log transformations were applied to the data, with the only exception that in data when natural log transformation was applied, the root and stem of plants with one gall did not show a significant difference when compared with nongalled plants (Table 8, Figures 30 and 31). This indicates that as the number of galls increased, the 'sink activity' of other natural sinks of the plant like roots and stem, in this case, decreased.

In experiment 3, when only one leaf, also referred to as 'presentation leaf' (X.Leaf) was put in the $^{14}CO_2$ chamber, 55 to 80 percent of the radioactivity was translocated from it and only 20 to 45 percent was retained, and the difference between the galled and nongalled 'presentation leaf' was not significant (Table 12 and Figure 38). In nongalled plants, almost 50 percent of the photosynthates were translocated to the leaves and the high 'sink strength' was due to the large 'sink size'. In the galled plants as the number of galls increased due to the presence of the gall former, the 'sink size' of the leaves in the galled plant decreases, which resulted in a lower 'sink strength' and a comparison between leaves of the galled and nongalled plants using a student pair 't' test showed that the difference was significant at the 5 percent level.

When the roots and stem of the galled and nongalled Russian knapweed plants were compared, the difference was found to be not significant, but the high radioactivity recovered in the roots indicating a

high 'sink strength' (Table 12 and Figure 38) was due to the large 'sink size' (Table III of the Appendix).

When square root and natural log transformations were applied to the data of disintegration per minute recovered in the different parts of galled and nongalled Russian knapweed plants (Table 12 and Figures 39 and 40), the results obtained show that in plants with one gall, more radioactivity was translocated from the 'presentation leaf' (X.Leaf) to the roots and stem which were the natural sink for the plant. As the numbers of galls increased on the plant (i.e. 2 to 3 galls per plant) the 'sink strength' of the stem in galled plants decreased, probably due to the decrease in 'sink size' (Table III of Appendix) and the radioactivity was translocated towards the galls. Similar results were obtained for the leaves of galled plants and the difference between galled and nongalled plants was significant at the 5 percent level when compared using a student pair 't' test.

In experiment 3, the 'sink activity' indicated by the counts per minute for 10 mg dry weight samples for the 'presentation leaf' (X.Leaf) was expected to be the highest (Table 11 and Figure 35), because it was from this leaf that the translocation of the radioactivity took place. The significant difference at the 5 percent level between the 'presentation leaf' of galled and nongalled plants indicates that either the 'sink activity' or the photosynthetic capabilities of the leaves had decreased in the galled plants.

The 'sink activity' of the leaves in galled plants was decreased when compared to the leaves of nongalled plants and as the number of galls increased on the galled plants, the roots and stem of the galled plants also showed a decrease in the 'sink activity' which was significant at the 5 percent level. When square root and natural log transformations

were applied to the data (Table 11 and Figures 36 and 37) the decrease in the roots and stem of all galled plants and the leaves of plants with one gall was not significantly different when compared to the non-galled plants. This indicated that the translocation of the assimilates towards the galls was mainly from the leaves of the galled plants.

The reason why the radioactivity recovered in roots in experiment 1 (Table 7 and Figure 26) and roots and stem of plants with one gall, and roots of plants with two galls in experiment 3 (Table 12 and Figure 38) had a higher accumulation of radioactivity than the nongalled plant parts the difference not being significant), can be attributed to the fact that these experiments were performed at the time when the natural sink for assimilates was provided by the stem and roots.

The translocation of the radioactivity into the galls was primarily from the leaves. The galls accumulate nutrients in competition with other physiological sinks within the plant which were roots and stem and such a competition was expected not to be markedly different here, since leaves usually export assimilates preferentially to the nearest sink (Quinland, 1965; Wardlaw, 1968). The galls were also known to have an increased rate of water loss which can cause accumulation of photosynthates. This had been demonstrated by Shaw and Samborski (1956) for obligate parasites.

The galls in the meristem region accumulate more radioactivity than the galls on leaf (Tables 9 and 11 and Figures 32, 33, 34, 38, 39 and 40). This phenomenon can be due to the fact that there was more meristematic activity in the galls which were in the meristem region and since the meristem of the plant was also a physiological sink, therefore the galls in this region had a higher 'sink strength'.

The total amount of radioactivity recovered in the plants was higher in the nongalled plant than in plants with galls. In experiment 1, when Fructose D [^{14}C(U)] - with an activity of 0.185 MBq was injected into the stem of the plants, about 0.044 MBq was recovered in the galled plants compared to about 0.133 MBq in the nongalled plants. The reason why most of the radioactivity was lost in the galled plants was due to the fact that the presence of the gall former alters the growth of the host plant. The galled plants become stunted, with fewer leaves, and the stem becomes thin when compared to the nongalled plants of the same age. Since the stems were thinner in the galled plants than the nongalled ones, even though they were of the same age and were grown under the same environmental conditions, it was difficult to inject the Fructose D [^{14}C(U)] - with the microsyringe in the stem of the galled plants and most of the radioactivity was lost in the process.

In experiment 2, when the whole plant was put in the $^{14}CO_2$ chamber, about 0.096 MBq of radioactivity was recovered from plants with one gall, about 0.041 MBq from plants with two galls and about 0.115 MBq from the nongalled plants. Since the whole plants were put in the $^{14}CO_2$ chamber, the number of leaves in the nongalled plants was more than the galled plants and as the number of galls increased, the photosynthetic surface decreased. That is why the nongalled plants photosynthesized more $^{14}CO_2$ and the radioactivity recovered in them was higher than in the galled plants.

In experiment 3, when only one leaf of approximately the same size for both galled and nongalled plants, was put in the $^{14}CO_2$ chamber, the total radioactivity recovered from the plants with one gall was about 0.052 MBq; about 0.044 MBq from the plants with two galls, and about

0.033 MBq from the plants with three galls and 0.063 MBq from the non-galled plants. This decrease in the radioactivity recovered in the galled plants can be explained by the decrease in the photosynthetic rate of the galled plants. The percent decrease in the photosynthetic rate of galled plants was determined to be 20 to 50%.

The macroscopic distribution of a radioactive tracer absorbed in plant tissue can be observed by autoradiographic techniques. The tissue was placed against a photographic plate, for a suitable period, after which the plate was developed and the image compared with the tissue structure. The resolution of an autoradiograph is not solely determined by the grain size of the emulsion used, but depends also on the specimen thickness, the separation between specimen and emulsion, and the β-particle range (Thaine and Walters, 1955). In order to obtain an absolute estimate of the specific activity of the sample from the results of such as count, several factors such as self absorption in the source, back-scattering in the emulsion, statistical uncertainties and observational errors must be taken into account. In this experiment, the autoradiographic techniques were used to get a visual indication that the galls formed by P. picridis have 'physiological sink' properties. However, for the purpose of this work the techniques seemed sufficiently accurate in spite of the limitations.

The differential accumulation of radioactivity at the site of parasitic infection following translocation from a noninfected leaf resulting from feeding labelled chemicals or carbon-14 dioxide had been reported by a number of workers (Baldacci et al., 1958; Garraway and Pelletier, 1966; Jankiewicz et al., 1969; Shaw et al., 1954; Shaw and Samborski, 1956; Sydow and Durbin, 1962; Yarwood and Jacobson, 1955). The

distribution of radioactivity following a given migration period gives no direct information about the mechanisms of translocation. Attempts have been made to explain this phenomenon on the basis of a 'physiological sink', which is assumed to result from increased metabolic activity at infection sites (Jankiewicz et al., 1969; Shaw and Samborski, 1956). The accumulation of the radioactivity is the result of an 'active' translocation, induced by the parasite, of substrates from non-infected host tissue to parasitized host tissue.

These experiments indicate that the galls induced by P. picridis on Russian knapweed act as powerful 'physiological sinks' and their uptake of carbon compound increases when there was maximum growth and activity of the galled tissue. That is why the galls in the meristem region accumulate more radioactivity than the galls formed on leaf. The organic nutrients required by these nematodes, at least in part, are from the products of current photosynthesis and would thus presumably be translocated to the nematode galls through the phloem.

On the basis of this hypothesis one would expect to find symptoms of starvation in uninfected organs. The presence of the nematode galls would ultimately exhaust the root reserves of the plant after some time, probably resulting in the death of the plant.

IV. SUMMARY, CONCLUSION AND FUTURE SUGGESTIONS

This study represents the first attempts to determine if viable P. picridis larvae, which form galls on the aerial parts of the Russian knapweed, could be mass produced using tissue culture techniques.

Experiments with Russian knapweed cultures on B5 medium supplemented with different levels of BA and NAA showed no nematode penetration. A modification of the B5 medium, with high concentrations of macronutrients, vitamins, sugar and low concentration of FeEDTA supported nematode penetration. Penetration of the nematodes also took place in cultures when GA_3 was added in the medium in combination with BA and NAA. Cultures with more vascular differentiation had higher nematode penetration. The nematodes in the cultures were present either intercellularly or were contained in a cavity which had necrosis along its walls. The host response to nematodes in cultures in this study was somewhat similar to the nonhost plants observed by Watson (1975). There was no syncytia or giant cell formation and no nematode reproduction occurred.

The effect of the galls formed by P. picridis on the aerial parts of Russian knapweed was examined. Using radioactive tracers, the distribution of C^{14} in galled and nongalled plants was investigated and it was concluded that the galls formed by P. picridis act as powerful 'physiological sinks' attracting 30 to 60% of the photosynthates produced in the plant. Galls in the meristem region attract more photosynthates than galls on the leaves and galls with a higher fresh weight/dry weight ratio had a higher accumulation of radioactivity than galls with low fresh weight/dry weight ratio.

Further studies that should be conducted would involve:

a. higher levels of GA_3 in combination with tryptophan.

b. higher concentration of macronutrients, vitamins, sugar, and low concentration of FeEDTA.

c. Incubation of cultures at lower temperatures to prolong the life of the cultures.

d. subculturing of the dual cultures by transferring them on fresh media with same or different hormonal ratios.

e. sprinkling the medium with sterile sand to reduce the surface tension of the agar.

f. substitution of the agar by other substrates such as perlite or vermiculite would provide suitable environment for nematode penetration.

g. selecting the right stage of nematode development, because the ability of the nematodes to function as gall formers varies considerably depending on its physiological age and the time over which its metabolic activities are being measured. (Bird and Loveys, 1975).

The nematode has been approved for release in North America and has been released on Russian knapweed in Alberta, Saskatchewan and Quebec. The lack of movement in or on the soil suggests that it would have to be spread thoroughly over a knapweed stand in such a manner as a granular herbicide (Watson and Harris in press). The nematode requires an overwintering incubation period in the soil, followed by a moist spring, before it will infect Russian knapweed. A large amount of inoculum would be required if the nematode is mass released on Russian knapweed infestations in North America. Techniques should, therefore be worked out to obtain large quantities of infective 2nd stage larvae.

Inoculation procedures should also be determined to find whether the 2nd stage larvae are to be sprayed in a water suspension or if galls are obtained, whether they could be broadcasted as a granular herbicide. Further studies, involving environmental conditions, nutrition of the host tissue in combination with the hormonal factors should, therefore, be conducted to establish a means for mass culturing P. picridis in laboratory using tissue culture techniques.

REFERENCES

Abramova, K.A., Panasyuk, T.D. and Nikanorova, E.A. 1977. The persistence of Torodon 22K (picloram-potassium) in soil and its residual effects on crop plants. (In Russian) Khimiya v Sel'skom Khozyaistve, 15(3): 55-58. Abstract, Weed Abstr. 27: 2827).

Agadzhanyan, A.A. 1968. The biology of Russian knapweed (Acroptilon picris C.A.M.) under irrigated conditions in the Ararat Plain of Armenia and its chemical control. (In Russian) Biol. Zh. Armenii, 21(2): 58-63. (Abstract - Weed Abstr. 18: 2042).

Agadzhanyan, S.Kh. and Agadzhanyan, A.A. 1967. The distribution of the damage caused by Russian knapweed (Acroptilon picris C.A.M.) in Armenia and its control. (In Russian) Biol. Zh. Armenii, 20(10): 15-21. Abstract - Weed Abstr. 18: 2036).

Agriculture Canada. 1967. Seeds Act and Regulations. Queen's Printer, Ottawa. 50p.

Alley, H.P. 1975. Research in weed science. Research Journal, Wyoming Agricultural Experiment Station No. 91R. (En) Wyoming Agric. Exp. Sta., Laramie, 82071, USA. 91p.

Alley, H.P. and Chamberlain, E.W. 1964. Four year evaluation of soil sterilants for control of Russian knapweed (Centaurea repens). Res. Prog. Rep. Western Weed Control Conf., Wyoming Agric. Exp. Sta., Laramie, 82071, USA. 6-8.

Alley, H.P. and Humburg, N.E. 1977. Research in weed science. Research Journal, Wyoming Agricultural Experiment Station, No. 112, (En) Wyoming Agric. Exp. Sta., Laramie, 82071, USA. 62p.

Alley, H.P. and Humburg, N.E. 1978. Perennial weed control. Research Journal, Wyoming Agricultural Experiment Station, No. 119 (En), Wyoming Agric. Exp. Sta., Laramie, 82071, USA. 62-67.

Amerson, H.V. and Mott, R.L. 1982. Phytopathology and tissue culture alliances. In Forestry Sciences. Tissue culture in Forestry. (eds.) J.M. Bonga and D.J. Durzan. Dr. W. Junk Publishers, The Hague. Boston, Mass, USA. 208-230.

Anonymous. 1952. Sequestrene. Providence, R.I., Alrose Chemical Company. 54p.

Bailey, I.W. 1943. Some misleading terminologies in the literature of 'plant tissue culture.' Science, 93: 539.

Baldacci, E., Betto, E., Foa, R. and Volpi, A. 1958. Absorption and transfer of phosphorus in healthy and diseased plants. Proc. Second Internatl. Conf. Peaceful Uses Atomic Energy, 24: 37-41.

Barker, K.R. and Darling, H.M. 1965. Reproduction of Aphlenchus avenae on plant tissues in culture. Nematologica 11: 162-166.

Barz, W. , Reinhand, E. and Zenk, M.H. eds., 1976. Plant tissue culture and its biotechnological application. First Internatl. Congr. Medicinal Plant Research. Station B, Univ. Munich, Germany. 6-10 Sept. 1976. Springer-Verlag Berlin Heidelberg. New York. 419 p.

Berezovskii, M. Ya. 1974. Residues of herbicides in plants and soil. (In Russian) Doklady Vsesoyuznoi Akademii Sel'skokhozyaistvennykh Nauk imeni V.I. Lenina No.6, 16-18. (Abstract - Weed Abstr. 24: 1137).

Berezovskii, M. Ya. and Krumzdorov, A.M. 1972. Picloram residues in soils and plants. In Russian) Doklady TSKhA. No. 180, II, 151-156. (Abstract - Weed Abstr. 24: 516).

Berezovskii, M. Ya. and Raskin, M.S. 1971. Some biological characteristics of Russian knapweed. (In Russian) Doklady TSKhA. No. 168, 179-183. (Abstract - Weed Abstr. 22: 881).

Berlyn, G.P. and Miksche, J.P. 1976. Botanical microtechnique and cytochemistry. The Iowa State Univ. Press. Ames, Iowa. 326p.

Biddulph, O. and Cory, R. 1957. An analysis of translocation in the phloem of the bean plant using THO, P^{32}, and C^{14}. Plant Physiol. 32(6): 608-619.

Bingefors, S. and Bingefors, S. 1976. Rearing stem nematode inoculum for plant breeding purposes. Swedish Journal of Agric. Res. 6: 13-17.

Bingefors, S. and Eriksson, K.B. 1963. Rearing stem nematode inoculum on tissue culture. Preliminary report. Lantbrugshogsk. Ann. 29: 107-118.

Bird, A.F. 1962. The inducement of giant cells by Meloidogyne javanica. Nematologica 8: 1-10.

Bird, A. F. and Loveys, B.R. 1975. The incorporation of photosynthates by Meloidogyne javanica. J. Nematol. 7: 111-113.

Bornman, C.H. 1974. Cytodifferentiation in tissue culture. In Tissue culture and Plant Science (ed.) H.E.Street. Proc. III Int. Cong. of Plant Tissue and Cell Culture. England. Acad. Press, London, New York. 43-70.

Braun, A.C. and Lipetz, J. 1966. The use of tissue culture in phytopathology. In Cell and Tissue in Culture (ed.) E.N. Willmer. vol.3. Acad. Press, London, New York. p 691-722.

Brewer, J.H. 1968. Mercurials - Inorganic and organic. In Disinfection, Sterilization and Preservation (eds.) C.A. Lawrence and S.S.Block. Lea and Febiger, Philadelphia. p.348-371.

Brown, J.A.A. 1974. Test tube reproduction of Heterodera avenae on resistant and susceptible wheats. Nematologica 20: 192-203.

Butcher, D.N. 1977. Plant tumor cells. In Plant tissue and cell culture ed.) H.E. Street, Univ. of California Press, Berkeley, LA. 429-461.

Butcher, D.N. and Ingram, D.S. 1976. Plant tissue culture studies in Biology. No.65. Edward Arnold Ltd. 67p.

Byars, L.P. 1914. Preliminary notes on the cultivation of the plant parasitic nematode Heterodera radicicola. Phytopath. 4: 323-326.

Cairns, E.J. 1975. Methods in Nematology: A review. In Nematology fundamentals and recent advances with emphasis on plant parasitic and soil forms (eds.) J.N. Sasser and W.R.Jenkins. Univ. North Carolina Press, North Carolina. 33-84.

Canada Weed Committee. 1969. Common and botanical names of weeds in Canada. Can.Dep. Agric. Pub. 1397. 67p.

Chen, T., Kilpatrick, R.A. and Rich, A.E. 1961. Sterile culture techniques as tools in plant nematology research (Phytopathological notes). Phytopath. 5: 799-800.

Chernishov, J.D. 1967. Root exudates of Russian knapweed and their effects on the growth of cultivated plants. (In Russian) Stepove Zemlerob., 1: 102-6. (Abstract - Weed Abstr. 18: 1278).

Chernyshev, I.D. 1978. The influence of herbicides on the viability of Russian knapweed seeds. (In Russian) Khimiya v Sel'skom Khozyaistve, 16(5): 24-26. (Abstract - Weed Abstr. 28: 3180).

Chitwood, D.J. and Krusberg, L.R. 1977. Pectolytic enzymes in three populations of D. dipsaci. J. Nematol. 9(3): 187-192.

Christie, J.R. and Crossman, L. 1936. Notes on the strawberry strains of the bud and leaf nematode, Aphlenchoides fragariae, I. Proc. Helminth. Soc. Wash. 3(2): 69-72.

Cook, T.M. 1923. The Origin and structure of plant galls. Science 57 (1462): 6-14.

Cooper, A.F. and Van Gundy, S.D. 1971. Senescence, quiescence and cryptobiosis. In Plant parasitic nematodes (eds.) B.M.Zuckerman, W.F. Mai and R.A.Rohde. vol.II. Academic Press, N.Y. 297-318.

Crosse, J.E. and Pitcher, R.S. 1952. Studies in the relationship of eelworms and bacteria to certain plant diseases. I. The etiology of strawberry cauliflower disease. Ann. Appl. Biol. 39: 475-484.

Crosse, J.E. and Pitcher, R.S. 1953. A preliminary note on methods for obtaining bacteria-free eelworms. Ann. Rept. East Malling Res. Sta. (England) 326: 138-40.

Darling, H.M., Faulkner, L.R. and Wallendal, P. 1957. Culturing the potato rot nematode. Phytopath. 47: 7 (Abst.)

Dasgupta, D.R., Nand, S. and Seshadri, A.R. 1970. Culturing, embryology and life history studies on the lance nematode, Hoplolaimus indicus. Nematologica 16: 235-248.

Davies, D.R. and Hopwood, D.A. 1980. The plant genome. The John Innes Charity, Norwich.

deFossard, R.A. 1976. Tissue culture for plant propagators. Armidale (Australia): Univ. of New England.

De Mott, H.E. 1965. Observations on the utilization of the hexose monophosphate pathway in nematode infected roots of tomato. Diss. Abst. 27: 4257.

Deubert, K.H. and Rohde, R.A. 1971. Nematode enzymes. In Plant parasitic nematodes. (eds.) B.M. Zuckerman, W.F. Mai, R.A. Rohde. vol.II. Academic Press, N.Y., London. 73-90.

Dodds, J.H. and Roberts, L.W. 1982. Experiments in plant tissue culture. Cambridge Univ. Press, Cambridge. 1-178.

Dolliver, J.S., Hildebrandt, A.C. and Riker, A.J. 1962. Studies of reproduction of Aphelenchoides ritzemabosi on plant tissues in culture. Nematologica 7: 294-300.

Dougherty, E.C. 1975. Cultivation of Aschelminths. In Nematology fundamentals and recent advances with emphasis on plant parasitic and soil forms. (eds.) J.N. Sasser and W.R. Jenkins. Univ. North Carolina, North Carolina, 296-318.

Dropkin, V.H. 1969. Cellular responses of plants to nematode infections. Ann. Rev. Phytopath. 7: 101-122.

Dropkin, V.H. and Boone, W.R. 1966. Analysis of host-parasite relationships of root-knot nematodes by single larvae inoculations of excise tomato roots. Nematologica 12: 225-236.

Engler, A. 1964. Syllabus der Pflanzenfamiliens. II. Band Gebriider Borntraeger, Berlin. 666p.

Eriksson, K.B. 1980. Nematode inoculum propagated in monoxenic plant tissue cultures: Nematode bank. Bull. OEPP (Organ. Eur. Mediterr. Prot. Plant) 10(3): 371-378.

Esau, K. 1977. Anatomy of seed plants (2nd ed.) N.Y., Wiley. 550p.

Evans, L.T. 1976. Transport and distribution in plants. In Transport and Transfer Processes in Plants. (eds.) I.F. Wardlaw and J.B. Passioura. Academic Press, N.Y. 1-13.

Evstratova, R.I., Sheichenko, V.I. and Rybalko, K.S. 1973. The structure of acroptilon - a sesquiterpene lactone from Acroptilon repens In Russian, translated) Kh. Prirod. Soedin. 2: 161-167.

Faulkner, L.R. and Darling, H.M. 1961. Pathological histology, hosts and culture of the potato rot nematode. Phytopathology 51: 778-786.

Faulkner, L.R., Bower, D.B., Evans, D.W. and Elgin, J.H. 1974. Mass culturing of Ditylenchus dipsaci to yield large quantities of of inoculum. Jour. Nematol. 6: 126-129.

Feder, W.A. and Feldmesser, J. 1955. Progress report on studies on the reproduction of the burrowing nematode, Radopholus similis (Cobb) Thorne, on citrus seedlings growing in petri dishes. Plant Dis. Rep. 39: 395-396.

Feder, W.A. and Feldmesser, J. 1957. Observations on the absence of an internal microflora of surface-sterilized Radopholus similis. Phytopath. 47: 11.

Feder, W.A., Hutchins, P.C. and Whidden, R. 1962. Aseptic growth of Radopholus similis on okra root callus tissue. Proc. Fla. State Soc. 75: 74-75.

Fenwick, D.W. 1956. The production of sterile viable larvae of the potato root eelworm, Heterodera rostochiensis. Nematologica 1: 331-336.

Fisyunov, A.V., Mordovets, A.A. and Golovin, V. 1977. The use of Banvel-D (dicamba-dimethylamine) against Russian knapweed in the crop rotation. (In Russian) Khimiya v Sel'skom Khozyaistve 15(2) 63-68. (Abstract - Weed Abstr. 27: 2823.)

Fletcher, R.A. and Renney, A.J. 1963. A growth inhibitor found in Centaurea spp. Can. Jour. Plant Sci., 43(4): 475-481.

Flore du Canada. 1974. Bulletin de terminologie 156. Secrétariat d'état, bureau des traductions, Ottawa, Ont. 634p.

Fukuda, H. and Komamine, A. 1980. Establishment of an experimental system for the study of tracheary element differentiation from single cells isolated from mesophyll of Zinnia elegans. Plant Physiol. 65: 57-60.

Gamborg, L.O. and Wetter, L.R. 1975. Plant tissue culture methods. National Research Council of Canada, Saskatoon, Saskatchewan, S7N 0W9, NRCC 14383. 109p.

Garraway, M.O. and Pelletier, R.L. 1966. Distribution of C^{14} in potato plant in relation to leaf infection by P. infestans. Phytopath. 56: 1184-1189.

Gautheret, R.J. 1939. Sur la possibilité de réaliser la culture indéfinie des tissu de tubercules de carotte. C.R. Acad. Sci. (Paris) 208: 118-121.

Gautheret, R.J. 1955. The nutrition of plant tissue cultures. Ann. Rev. Plant Physiol. 6: 433-484.

Gautheret, R.J. 195[illegible]. La Culture des Tissue Vegetaux: Tecniques et Realisations. Masson et Cie, Paris, [illegible]3p.

Giebel, [illegible]. 197[illegible]. Biochemical mechanisms of plant resistance to nematodes: A review. Jour. Nematol. [illegible]: 17[illegible]-184.

Gommers, F.[illegible] and Dropkin, V.H. 197[illegible]. Quantitative histochemistry of nematode-induced transfer cells. Phytopath. 67: [illegible].

Goodey, [illegible].B. 19[illegible]4. The structure of the leaf galls of Plantago lanceolata L. induced by Anguillulina dipsaci (Kuhn) Gerv. & v. Ben. Jour. Helminth., vol.XVII, No.4, 183-190.

Goodey, [illegible].B. 19[illegible]. The galls caused by Anguillulina balsamophila (Thorne) Goodey, on the leaves of Wyethia amplexicaulis Nutt. and Balsamorhiza sagittata Nutt. Jour. Helminth., vol.XXII, No.[illegible], 109-11[illegible].

Goodey, T. 19[illegible]4. Anguillulina cecidoplastes n.sp., a nematode causing galls on the grass, Andropogon pertusus Willd. Jour. Helminth., vol. XII, No.4, 225-236.

Goodey, T. 1935. The pathology and aetiology of plant lesions caused by parasitic nematodes. Imp. Bur. Agr. Parasitol., St.Albans, England. 34p.

Goodey, T. 1938. Observations on Anguillulina millefolii (Löw 1874) Goodey, 1932, from galls on the leaves of yarrow, Achillea millefolium L. Jour. Helminth., vol.XVI, No.2, 93-108.

Groh, H. 1940. Turkestan alfalfa as a medium of weed introduction. Sci. Agric. 21: 36-43.

Gruzdev, G.S. and Popov, V.G. 1974. Effectiveness of picloram for the control of Russian knapweed. (In Russian) Izvestiya Timiryazevskoi Sel'skokhozyaistvennoi Akademii, No.5, 139-146. (Abstract - Weed Abstr. 24: 1502).

Haberlandt, G. 1902. Kulturversuche mit isolierten. Pflanzenzellen. Sber. Akad. Wiss. Wien, 111: 69-92.

Harris, P. 1973. The selection of effective agents for the biological control of weeds. Can. Ent. 105: 1495-1503.

Hastings, R.J. and Boscher, J.E. 1938. A study of the pathogenicity of the meadow nematode and associated fungus, Cylindrocarpon radicicola. Can. Jour. Res., Sect.C, 16(6): 225-229.

Higgins, R.E. 1967. Loss caused by noxious weeds in potatoes. Down to Earth, 22(4): 2-24.

Hoagland, D.R. and Aron, D.I. 1938. The water-culture method for growing plants without soil. California Agric. Exp. Sta. Circ. 347: 4-32.

Hollings, M. 1965. Disease control through virus-free stock. Ann. Rev. Phytopathol. 3: 367-396.

Hopkins, W.L. 1961. Chemical roadside weed control. Proc. 13th Ann. California Weed Conf. (Agric. Chem. Industries Inc., Washburn Agric. Serv., Davis, California), 15-22.

Ingram, D.S. 1976. Growth of biotrophic parasites in tissue culture. In Encyclopedia of Plant Physiology: Physiological Plant Pathology (eds.) R. Heitefuss and P.H. Williams. Springer-Verlag., Berlin, 743-759.

Ingram, D.S. 1977. Application in plant pathology. In Plant tissue and cell culture. ed. H.E. Street. Univ. of California Press. Berkeley, LA: 463-500.

Ingram, D.S. 1978. Tissue culture methods in plant pathology. In Tissue culture methods by plant pathologists. eds. D.S. Ingram and J.B. Helgeson. Blackwell Sci. Pub., Boston. [illegible].

Ingram, D.S. and Helgeson, J.P. eds. 1980. Tissue culture methods for plant pathologists. Blackwell Sci. Pub., Boston. [illegible].

Inserra, R.N. and Barker, K.R. 1977. Rearing migratory endoparasitic nematodes in citrus callus and roots produced from citrus leaves. Jour. Nemato., 9: 241-245.

Ivannikov, A.I., Kazenas, V.L., Marikovskii, P.I., Tyrtyaev, S.S., Shamsutdinova, Z.S. and Yakushkin, V.T. 1976. Biological control of weeds in Kazakhstan. In Russian) Biologicheskii metod bor'by s sornyakami v Kazakh. Alma Ata, Kazakh SSR: Nauka. 105p. (Abstract - Weed Abstr. 27: 3082.

Ivanova, T.S. 1960. Experimenting with a biological method for controlling Russian knapweed. (In Russian) Izv. Akad. Nauk. tadzhik, SSR (Otdel. biol. Nauk.) 2(23): 51-53. Abstract - Weed Abstr. 18: 858).

Jankiewicz, L.S., Plich, H. and Antoszewski, R. 1969. Preliminary studies on the translocation of C^{14}-labelled assimilates and $^{32}PO^{3-}$ towards the galls evoked by Cynips diplolepis) Quercusfolii L. on oak leaves. Marcellia, 36: 163-174.

Jeffs, R.A. and Northcote, D.H. 1967. The influence of IAA and sugar on the pattern of induced differentiation in plant tissue cultures. Jour. Cell Sci., 2: 77-88.

Jensen, W.A. 1962. Botanical Histochemistry: Principles and Practice. W.H. Freeman and Company, San Francisco. 480p.

Johnson, R.N. and Viglierchio, D.R. 1969a. Sugarbeet nematode (H.schachtii) reared on axenic Beta vulgaris root explants. I. Selected environmental factors affecting penetration. Nematologica, 15: 129-143.

Johnson, R.N. and Viglierchio, D.R. 1969b. Sugarbeet nematode reared on axenic Beta vulgaris root explants. II. Selected environmental and nutritional factors affecting development and sex-ratio. Nematologica, 15: 144-152.

Jones, I.B. and Evans, J.O. 1973. Control of Russian knapweed and field bindweed and dicamba, 2,4-D and their combinations, with and without DMSO. In Proceedings of the Western Society of Weed Science, 26: 39-43.

Jones, M.G.K. 1980. The interaction of plant parasitic nematodes with excised root and tissue cultures. In Tissue culture methods for plant pathologists (eds.) D.S. Ingram and J.P. Helgeson. Blackwell Sci. Pub., Boston. 161-166.

Jones, M.G.K. 1981. Host cell responses to endoparasitic nematode attack: structure and function of giant cells and syncytia. Annals of Applied Biology, 27: 353-372.

Jones, M.G.K. and Dropkin, V.H. 1975. Cellular alterations induced in soybean roots by three endoparasitic nematodes. Physiol. Plant Path., 5: 119-124.

Jones, M.G.K. and Northcote, D.H. 1972a. Multinucleate transfer cells induced in Coleus roots by root-knot nematode. Protoplasma, 75: 381-395.

Jones, M.G.K. and Northcote, D.H. 1972b. Nematode induced syncytium - a multinucleate transfer cell. Jour. Cell Sci., 10: 789-809.

Kasimova, G.A. 1978. A record of the knapweed nematode Paranguina picridis (Kirjanova, 1944) on Russian knapweed in Azerbaidzhan. (In Russian) Doklady Akademii Nauk Azerbaidzhanskoi SSR, 34(1): 51-53. (Abstract - Weed Abstr. 28: 766).

Khera, S. and Zuckerman, B.M. 1962. Studies on the culturing of certain ectoparasitic nematodes on plant callus tissue. Nematologica, 8: 272-274.

Khodorovskii, Yu.M. 1969. Increasing the effectiveness of spring wheat chemical weeding by irrigation. (In Russian) Vest. sel'.-khoz. Nauki, Alma-Ata, (5), 71-7. (Abstract - Weed Abstr. 19: 1911).

Kidrishev, T.K. and Styazhkovoi, V.V. 1980. Herbicides for controlling Russian knapweed in Tselinograd Province. (In Russian) Khimiya v Sel'skom Khozyaistve, 18(11): 24-26. (Abstract - Weed Abstr. 30: 1809).

Kirjanova, E.S. and Ivanova, T.S. 1969. New species of Paranguina Kirjanova, 1955 (Nematode: Tylenchidae) in Tadzhikistan. (In Russian). Ushchel's Kondara (Akad. Nauk. tadzhik. SSR) 2: 200-217. (Translation - Translation Bureau, Canada Dept. Secretary of State).

Kostoff, D. and Kendall, J. 1930. Cytology of nematode galls on Nicotiana roots. Zentr. Bakteriol. II. Abt. 81: 86-91.

Kovalev, O.V. 1973. The use of a biological method of controlling weedy plants. Primencie, Bio. Met. V. Bor. S. Som. Rast. Moscow, USSR. (In Russian). Obz. inform. No.749, 32p. (Translation - Translation Bureau, Canada Dept. Secretary of State, No. 619707).

Kovalev, O.V., Danilov, L.G. and Ivanova, T.S. 1973. Methods of controlling Russian knapweed. (In Russian) Opisanie Izorbriteniia Kavtorakomu Svedelet'stuv Byull. 38, 2p.

Krikorian, A.D. 1982. Cloning higher plants from aseptically cultured tissues and cells. Biol. Rev., 57: 151-218.

Krikorian, A.K. and Berquam, D.L. 1969. Plant cell and tissue cultures: the role of Haberlandt. Bot. Rev., 35: 59-88.

Krumzdorov, A.M. 1974. The residual effect of dicamba on crop plants. (In Russian) Khimiya v Sel'skom Khozyaistve 12(8): 604-606. (Abstract - Weed Abstr. 24: 356).

Krumzdorov, A.M. 1975. The effectiveness dicamba against Russian knapweed and its rate inactivation in relation to times of treatment. (In Russian) Izvestiy Timiryazevskoi Sel'skokhozyaistvennoi Akademii N. 3: 163-168. (Abstract - Weed Abstr. 25: 773).

Krumzdorov, A.M. 1976. The effect of picloram and dicamba on Russian knapweed, and their residual effects on maize. (In Russian) Khimiya v Sel'skom Khozyaistve 14(7): 39-43. (Abstract - Weed Abstr. 26: 2253).

Krusberg, L.R. 1960. Hydrolytic and respiratory enzymes of species of Ditylenchus and Pratylenchus. Phytopath., 50: 9-22.

Krusberg, L.R. 1961. Studies on the culturing and parasitism of plant parasitic nematodes, in particular D. dipsaci and Aphlenchoides ritzemabosi on alfalfa tissue. Nematologica, 6: 181-200.

Krusberg, L.R. 1967. Pectinases in D. dipsaci. Nematologica, 13: 443.

Krusberg, L.R. and Babineau, D.E. 1979. Application of Plant Tissue Culture to plant nematology. In Plant Cell and tissue culture principles and application. (eds.) W.R. Sharp et al. Ohio State Univ. Press, Columbus. 401-419.

Krusberg, L.R. and Blickenstaff, M.L. 1964. Influence of plant growth regulating substances on reproduction of D. dipsaci, Pratylenchus penetrans and P. zeae on alfalfa tissue cultures. Nematologica, 10 145-150.

Kruse, P.F. and Patterson, M.K. (eds.) 1973. Tissue culture methods and application. Academic Press, N.Y. 868p.

Kulescha, Z. and Gautheret, R.J. 1949. Recherches sur l'action du tryptophane sur la proliferation des cultures de tissus de quelques végétaux. Compt. rend. soc. biol., 143: 460-463.

Kursanov. A.L., Chailakhian, M.K., Bavlinova, O.A., Turkina, M.V., and Brovchenko, M.I. 1958. Translocation of sugars in grafted plants. Fiziol. Rastenii. (Transl.) 5(1): 1-12.

Küster, E. 1911. 'Die Gallen der Pflanzen.' Liepzig.

Ladonin, V.F. 1968. The effect of Acroptilon picris Fisch. (Centaurea repens L.) on crop plants. (In Russian) Bot. Zh., 53(9): 1305-8. (Abstract - Weed Abstr. 18: 818).

Lapage, G. 1933. Cultivation of parasitic nematodes. Nature, April 22, 1933. 583-584.

Lewis, S.A. and McClure, M.A. 1975. Free amino acids in roots of infected cotton seedlings resistant and susceptible to Meloidogyne incognita Jour. Nematol. 7: 9-15.

Libbert, E., Erdmann, N. and Schiewer, U. 1970. Auxin biosynthese. Biol. Rundsch., 8: 369-390.

Loo, S.W. 1945. Cultivation of excised stem-tips of asparagus in vitro. Am. Jour. Bot. 32: 13-17.

Lownsbery, B.F. and Lownsbery, J.W. 1956. A procedure for testing the sterility of large number of nematodes after treatment with various sterilants. Pl. Dis. Rep., 40(11): 989-990.

Lownsbery, B.F., Huang, C.S. and Johnson, R.N. 1967. Tissue culture and maintenance of the root-lesion nematode Pratylenchus vulnus. Nematologica, 13: 390-394.

Mani, M.S. 1964. The ecology of plant galls. Dr. W. Junk Publishers, the Hague, 434p.

Maheshwari, R. 1969. Application of plant tissue and cell culture in the study of physiology of parasitism. Proc. Indian Acad. Sci. Sect. B, 59: 152-172.

Maresquelle, H.J. and Meyer, J. 1965. Physiologie et morphologie des galles d'origine animale (zoocécides) Encyclopedia of Plant Physiology. Springer-Verlag, Berlin.

McClure, M.A. and Viglierchio, D.R. 1966a. Penetration of M. incognita in relation to growth and nutrition of sterile, excised cucumber roots. Nematologica, 12: 237-247.

McClure, M.A. and Viglierchio, D.R. 1966b. The influence of host nutrition and intensity on the sex-ratio and development of M. incognita in sterile agar cultures of excised cucumber roots. Nematologica, 12 248-258.

Merchant, D.J., Kahn, R.H. and Murphy Jr., W.H. 1960. Handbook of cell and organ culture. Burgen Publishing Co. 188p.

Metcalf, H. 1903. Cultural studies of a nematode associated with plant decay. Trans. Amer. Micros. Soc., 24: 89-102.

Meyer, J. 1969. Problèmes actuels de cecidologie. Bull. Soc. Bot. Fr., 116: 445-481.

Miller, C.E. 1963. A method of obtaining Meloidogyne sp. in aseptic cultures. Phytopathology, 53: 350-351.

Miller, J.H. 1963. Perennial herbacious weeds. Res. Progr. Rep. Western Weed Control Conf., 1-4.

Miller, P.M. and Sanda, D.S. 1977. Effects of hydrolytic enzymes on plant parasitic nematodes. Jour. Nematol., 9(3): 192-197.

Moore, R.J. 1969. How weedy thistles came to Canada. Greenhouse Garden Grass, 8: 1-2.

Moore, R.J. and Frankton, C. 1974. The thistles of Canada. Can. Dep. Agric. Monogr. No.10, Information Canada, Ottawa, Ont. 111p.

Mordovets, A.A. and Golovin, V.V. 1974. The residual effect of herbicides used against Russian knapweed on the principal field crops. (In Russian) Khimiya y Sel'skom Khozyaistve, 12(8): 602-603. (Abstract - Weed Abstr. 24: 864).

Mordovets, A.A. and Golovin, V.V. 1976. Control of Acroptilon repens (= Centaurea repens) under irrigated conditions. (In Russian) Zashchita Rastenii, No.12, 48-49. (Abstract - Weed Abstr. 27: 3557).

Mordovets, A.A. and Nazarenko, G.I. 1971. The effectiveness of Tordon 101 against Russian knapweed. (In Russian) Khimiya v Sel'skom Khozyaistve, 9(8): 600-601. (Abstract - Weed Abstr. 21: 3030).

Mordovets, A.A., Chernyshev, I.D., Nazarenko, G.I. and Golovin, V.V. 1974. Testing Tordon. (In Russian) Kukuruza, 19(4): 31-32. (Abstract - Weed Abstr. 24: 587).

Morel, G. 1960. Producing virus-free cymbidium. Am. Orchid Soc. Bull., 29: 495-497.

Morel, G. 1975. Meristem culture techniques for the long-term storage of cultivated plants. In Crop genetic resources for today and tomorrow. (eds.) O.H. Frankel and J.G. Hawkes, Cambridge Univ. Press, 327-332.

Morgan, G.T. and McAllan, J.W. 1962. Hydrolytic enzymes in plant-parasitic nematodes. Nematologica, 8: 209-215.

Morgan, T.H. 1901. Regeneration, London: Macmillan.

Moriarty, F. 1964. The monoxenic culture of beet eelworm (Heterodera schachtii Schm.) on excised roots of sugarbeet (Beta vulgaris L.) Parasitology, 54: 289-293.

Mountain, W.B. 1954. Studies of nematodes in relation to brown rot of tobacco in Ontario. Can. Jour. Bot., 32: 737-759.

Mountain, W.B. 1955. A method of culturing plant parasitic nematodes under sterile conditions. Proc. Helminth. Soc. Wash., 22(1): 49-52.

Mountain, W.B. and Patrick, Z.A. 1959. The peach replant problem in Ontario. VII. The pathogenicity of P. penetrans. Can. Jour. Bot., 37: 459-470.

Munch, E. 1930. Stoffbewegungen in der Pflanse. Gustav Fischer. Jena, Germany, 234p.

Murashige, T. 1974. Plant propagation through tissue culture. Ann. Rev. Plant Physiol., 25: 135-166.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Planterum, 15: 473-497.

Myers, R.F. 1965. Amylase, Cellulase, Invertase and Pectinase in several free living mycophagus and plant parasitic nematodes. Nematologica, 11: 441-448.

Myers, R.F., Feder, W.A. and Hutchins, P.C. 1965. The rearing of Radopholus similis on grape fruit, okra and alfalfa root callus tissues. Proc. Helminth. Soc., Wash., 32: 94-95.

Narayanaswamy, S. 1977. Regeneration of plants from tissue cultures. In Plant cell, tissue and organ culture. (eds.) J. Reinert and Y.P.S. Bajaj. Springer-Verlag, Berlin. 179-206.

Nickell, L.G. and Burkholder, P.R. 1950. Atypical growth of plants. II. Growth in vitro of virus tumours of Rumex in relation to temperature, pH, and various sources of nitrogen, carbon and sulfur. Am. Jour. Bot., 37: 538-547.

Nobécourt, P. 1939. Sur la perennite et l'augmentation de volume des cultures de tissue vegetaux. C.R. Soc. Biol. (Paris), 130: 1270-1271.

O'Brian, T.P. and McCully, M.E. 1981. The study of plant structure. Principles and selected methods. Termarcarphi Pty. Ltd., Melbourne, Australia.

Ojima, K. and Ohira, K. 1978. Nutritional requirements of callus and cell suspension cultures. In Frontiers of plant tissue culture. (ed.) T.A.Thorpe, Calgary: International Association for Plant Tissue Culture, 265-275.

Owens, R.G. and Rubinstein, J.G. 1966. Metabolic changes induced by root-knot nematodes in host tissues. Contrib. Boyce Thompson Inst., 23: 199-213.

Owens, R.G. and Specht, H.N. 1966. Biochemical alterations induced in host tissue by root-knot nematodes. Contrib. Boyce Thompson Inst., 23: 181-198.

Paracer, S.M. and Zuckerman, B.M. 1967. Monoxenic culturing of Dolichodorus heterocephalus on corn root callus. Nematologica, 13: 478.

Pate, J.S. and Gunning, B.E.S. 1972. Transfer cells. Ann. Rev. Plant Physiol., 23: 173-196.

Patrick, J.W. 1976. Hormone directed transport of metabolites. In Transport and Transfer Processes in Plants. eds.) I.F. Wardlaw and J.B. Passioura, Academic Press, N.Y., 433-444.

Paul, J. 1975. Cells and tissue culture, 5th edition, Edinburgh: Churchill Livingstone Ltd., London. 484p.

Peacock, F.C. 1961. A note on the attractiveness of roots to plant parasitic nematodes. Nematologica, 5: 85-86.

Polychronopoulos, A.G. and Townsbery, B.F. 1968. Effect of Heterodera schachtii on sugarbeet seedlings under monoxenic conditions. Nematologica, 14: 626-634.

Popov, V.G., Raskin, M.S. and Kruzdev, G.S. 1973. The noxious effects of Russian knapweed Acroptilon repens L.) DC.) in field plant associations. In Russian) Izvestiya Timiryazevskoi Sel'skokhozyaistvennoi Akademii, No.2, 221-223. Abstract - Weed Abstr. 22: 2371).

Pierik, R.L.M. (ed.) 1979. In vitro culture of higher plants. Wageningen.

Pimentel, D. 1961. Animal population regulation by genetic feed-back mechanism. Am. Midl. Nat., 95: 65-79.

Pitcher, R.S. and Crosse, J.E. 1958. Studies in the relationship of eelworms and bacteria to certain plant diseases. II. Further analysis of the strawberry cauliflower disease complex. Nematologica, 3: 244-256.

Prasad, S.K. and Webster, J.M. 1967. Effect of temperature on the rate of development of Nacobbus serendipiticus in excised tomato roots. Nematologica, 13: 85-90.

Pristupa, N.A. 1959. The transport form of carbohydrates in pumpkin plants. Fiziol. Rastenii Akad. Nauk. SSSR., 4: 243-53.

Quinland, J.D. 1965. The pattern of distribution of ^{14}C in apple root stock following assimilation of $^{14}CO_2$ by single leaf. East Malling Res. Sta. Ann. Rep. 1964, AA8. 117-118.

Raskin, M.S., Chernyshev, I.D., Khaskin, I.D., Labadze, V.D. and Yunin, P.F. 1978. The herbicidal activity of esters of methoxydichlorobenzoic acid. (In Russian) Khimiya v Sel'skom Khozyaistve, 16(11): 52-55. (Abstract - Weed Abstr. 28: 2843).

Rebois, R.V., Madden, P.A. and Eldridge, B.J. 1975. Some ultrastructural changes induced in resistant and susceptible soybean roots following infection by Rotylenchulus reniformis. Jour. Nematol., 7(2): 122-139.

Reinert, J. and Bajaj, Y.P.S. eds. 1977. Plant cell, tissue and organ culture. Springer-Verlag, N.Y. 803p.

Renney, A.J. 1958. Basic studies. 5 reports. Res. Rep. W. Section. Nat. Weed Comm., Canada 122-128.

Renney, A.J. 1954. Centaurea spp. infestations in British Columbia. Proc. Joint Meet. 10th N. Cent. and 10th West. Canada Weed Control Conf., 18-19.

Renney, A.J. 1977. Allelopathy of knapweeds. Proc. Knapweed Symp. B.C. 13p.

Renney, A.J. and Dent, W.J. 1958. Growth inhibition caused by Russian knapweed Centaurea repens L.). Research Rept. Nat. Weed Comm. West. Sec., 122-123.

Reversat, G. 1975. Monoxenic cultures of H. oryzae on rice. Ann. Zool-Ecol. Anim. 7: 81-89.

Riedel, R.M. and Foster, J.G. 1970. Monoxenic culture of D. dipsaci and P. penetrans with modified Krusberg's and White's media. Plant Dis. Rept., 54: 251-254.

Riedel, R.M., Foster, J.G., and Mai, W.F. 1973. A simplified medium for monoxenic culture of P. penetrans and D. dipsaci. Jour. Nematol., 5: 71-72.

Riker, A.J. and Gutsche, A.E. 1948. The growth of sunflower tissue in vitro on synthetic media with various organic and inorganic sources of nitrogen. Am. Jour. Bot., 35: 227-237.

Roberts, L.W. 1976. Cytodifferentiation in plants. Xylogenesis as a model system. Cambridge Univ. Press.

Rogers, C.F. 1928. Canada thistle and Russian knapweed and their control. Colo. Exp. Sta. Bul. 348, 42p.

Romberger, J.A. and Tabor, C.A. 1971. The Picea abies shoot apical meristem culture. I. Agar and autoclaving effects. Am. Jour. Bot., 58: 131-140.

Rothblat, G.H. and Cristofalo, V.J. (eds.) 1972. Growth nutrition and metabolism of cells in culture. Vol.II. Academic Press, N.Y., 372-406.

Salisbury, F.B. and Ross, C. 1969. Plant Physiology. Wadsworth Publishing Company, Inc., Belmont, California. 747p.

Sandstedt, R. and Schuster, M.L. 1966a. Excised tobacco pith bioassays for root-knot nematode-produced plant growth substances. Physiol. Plant, 19: 99-104.

Sandstedt, R. and Schuster, M.L. 1966b. Role of auxins in root-knot nematode-induced growth on excised tobacco stem segments. Physiol. Plant, 19: 460-467.

Sayre, R.M. [illegible]. Plant tissue culture as a tool in the study of the physiology of root-knot nematode Meloidogyne incognita Chit. Ph.D. Thesis. University of Nebraska, Lincoln, USA.

Schneider, E.A. and Wightman, F. 1974. Metabolism of auxin in higher plants. Ann. Rev. Plant Physiol., 25: 487-513.

Schneider, E.A. and Wightman, F. 1978. Auxins. In Phytohormones and related compounds - A comprehensive treatise. The biochemistry of phytohormones and related compounds. eds. D.S. Letham, P.B. Goodwin and T.J.V. Higgins. Vol.I. Amsterdam, Elsevier. 29-105.

Schroeder, P.H. 1963. Reproduction of P. penetrans on corn roots cultured on various nutrient media. Phytopathology, 53: 888-889 abstr.)

Schroeder, P.H. and Jenkins, W.R. 1963. Reproduction of Pratylenchus penetrans on root tissues grown on three media. Nematologica, 9: 327-331.

Schuster, M.L. and Sullivan, T. 1960. Species differentiation of nematodes through host reaction in tissue culture. I. Comparisons of Meloidogyne hapla, M. incognita and Nacobbus batatiformis. Phytopathology, 50: 874-876.

Schwann, Th. 1939. Mikroskopische Untersuchungen über die Überreinstimmung in der Struktur und dem Wachstume der Tiere und Pflanzen. Leipzig: W. Engleman, Nr. 179, Oswalds Klassiker der exakten Wissenschaften, 1910.

Seaman, F.C. 1982. Sesquiterpene lactones as taxonomic characters in Asteraceae. Bot. Rev., 48(2): 121-595.

Selleck, G.W. 1964. A competition study of Cardaria spp. and Centaurea repens. Proc. 7th Br. Weed Control Conf., 569-576.

Sembdner, G., Gross, D., Liebisch, H.W. and Schneider, G. 1980. Biosynthesis and metabolism of plant hormones. In Hormonal regulation of Development. I. (ed.) J. MacMillan. Springer-Verlag, Berlin. Heidelberg, New York. 281-444.

Shabde, M.N. and Murashige, T. 1977. Hormonal requirements of excised Dianthus caryophyllus L. shoot apical meristem in vitro. Am. Jour. Bot., 64: 443-448.

Shabde, M.N. and Murashige, T. 1979. Organ culture. In Nicotiana. Procedures for experimental use. (ed.) R.D. Durbin, Washington, D.C., USDA Tech. Bull. No. 1586, 40-51.

Sharp, W.R. and Larsen, P. [illegible]. Plant cell and tissue culture: Current applications and potential. In Plant Cell and Tissue Culture Principles and Applications. eds. W.R. Sharp, P.O. Larsen, E.F. Paddock and V. Raghavan. Ohio State Univ. Press., Columbus. 115-120.

Shaw, M. and Samborski, D.J. 1956. The physiology of host-parasite relation. I. The accumulation of radioactive substances at infections of facultative and obligate parasites including TMV. Can. Jour. Bot., 34: 389-407.

Shaw, M., Brown, A.A. and Jones, R. [illegible]. Uptake of radioactive carbon and phosphorus by parasitized leaves. Nature, 173(4408): 768-769.

Skinner, J.A., Orr, C.C. and Robinson, A.F. 1980. Histopathgenesis of the galls induced by N. phyllobia in S. elaeagnifolium Cav. J. Nematol., 12(2): 141-150

Skoog, F. and Miller, C.O. 1957. Chemical regulation of growth and organ formation in plant tissues cultured in vitro. Symp. Soc. Exp. Biol., 11: 118-130.

Smith, R.H. and Murashige, T. 1970. In vitro development of the isolated shoot apical meristem of angiosperms. Am. Jour. Bot., 57: 562-568.

Street, H.E. (ed.) 1974. Tissue culture and plant science. Academic Press, N.Y. 502p.

Street, H.E. 1977a. Applications of cell suspension cultures. In Plant cell, tissue and organ culture. (eds.) J. Reinert and Y.P.S. Bajaj, Springer-Verlag: Berlin. 649-667.

Street, H.E. (ed.) 1977b. Plant tissue and cell culture. 2nd ed. Bot. Monographs. vol.II. Univ. of California Press, Berkeley, Los Angeles. 614p.

Street, H.E., Henshaw, G.G. and Buiatti, M.C. 1965. The culture of isolated plant cells. Chem. Ind., 23: 23-33.

Swanson, C.A. and El-Shishiny, E.D.H. 1958. Translocation of sugars on Concord grapes. Plant Physiol., 33(1): 33-37.

Sydow, B.von, and Durbin, R.D. 1962. Distribution of C^{14} containing metabolites in wheat leaves infected with stem rust. Phytopath., 52: 169-170.

Tamura, H. and Mamiya, Y. 1975. Reproduction of Bursaphlenchus lignicolus on alfalfa callus tissue. Nematologica, 21: 449-454.

Thaine, R. and Walters, M.C. 1955. Experiments on the application of autoradiographic techniques to the study of problems in plant physiology. Aust. Jour. Biol. Sci., 8: 354-368.

Thomas, [illegible] and Davey, M.[illegible] [illegible]. From single cells to plants. Wykeham Pub. Ltd., London. [illegible]p.

Thorpe, T.A. ed. 1978. Frontiers of plant tissue culture. Univ. of Calgary Offset Printing Services. The Bookstore, Univ. of Calgary, Alberta, Canada. 550p.

Tiner, J.D. 1960. Cultures of the plant parasitic nematode genus *Pratylenchus* on sterile excised roots. I. Their establishment and maintenance. Exp. Parasitol., 9: 121-126.

Tiner, J.D. 1961a. Laboratory cultures of the plant parasitic nematode genus *Pratylenchus*: A new instrument and a new nematocide test. Jour. Parasitol., 45: 25 (abstr.)

Tiner, J.D. 1961b. Cultures of the plant-parasitic nematode genus *Pratylenchus* on sterile excised roots. II. A trap for collection of axenic nematodes and quantitative initiation of experiments. Exp. Parasitol., 11: 231-240.

Townshend, J.L. 1963. The pathogenicity of *Pratylenchus penetrans* to celery. Can. Jour. Plant Sci., 43: 70-74.

Townshend, J.L. 1974. Monoxenic culture of *Pratylenchus projectus*. Nematologica, 20: 264-266.

Tracey, M.V. 1958. Cellulase and Chitinase in plant nematodes. Nematologica, 3: 179-183.

Viglierchio, D.R. 1971. Nematodes and other pathogens in auxin-related plant-growth disorders. Bot. Rev., 37(1): 1-21.

Viglierchio, D.R., Siddiqui, I.A. and Croll, N.A. 1973. Culturing and population studies of *D. dipsaci* under monoxenic conditions. Jour. Agric. Sci., 42(6): 177-214.

Warden, R.L. 1964. Tordon for control of field bindweed and Canada thistle in the North Central United States. Down to Earth, 20: 6-10.

Wardlaw, I.A. 1968. The control and pattern of movement of carbohydrates in plants. Bot. Rev., 34: 79-105.

Watson, A.K. 1975. The potential of a nematode, *Paranguina picridis*, Kirjanova and Ivanova, as a biological control agent of Russian knapweed (*Acroptilon repens* (L.) D.C.) Ph.D. Thesis, Univ. Saskatchewan, Saskatoon, Canada. 136 p.

Watson, A.K. 1977. The biological control of Russian knapweed with a nematode, pp.221-223. *In* T.E. Freeman (ed.) Proc. IV Int. Symp. Biol. Control Weeds, Univ. Florida, Gainesville, 1976. 299p.

Watson, A.K. 1980. The biology of Canadian weeds. 43. Acroptilon (Centaurea) repens L.) DC. Canadian Jour. Plant Sci., 60(3): 993-1004.

Watson, A.K. and Harris, P. Acroptilon repens (L.), Russian knapweed (Asteraceae). In press.

Watson, A.K. and Shorthouse, J.D. 1977. Gall formation on Cirsium arvense by Ditylenchus dipsaci. Jour. Nematol., 11: 19-21.

Webster, J.M. 1966. Production of oat callus and its susceptibility to plant parasitic nematode. Nature, 212: 1472.

Webster, J.M. 1967a. Some effects of 2,4-D herbicides on nematode infested cereals. Plant Path., 16: 23-26.

Webster, J.M. 1967b. The influence of plant-growth substances and their inhibitors on the host-parasite relationships of Aphlenchoides ritzemabosi in culture. Nematologica, 13: 256-262.

Webster, J.M. and Lowe, D. 1966. The effect of the synthetic plant-growth substance, 2,4-D, on the host-parasite relationships of some plant-parasitic nematodes in monoxenic callus cultures. Parasitology, 56: 313-322.

Weinstein, P.P. and Jones, M.F. 1956. The in vitro cultivation of Nippostrongylus muris to the adult stage. Jour. Parasitol., 42(3): 215-236.

Weiser, C.L., Wang, C.H. and Blaney, L.T. 1962. A simple apparatus for feeding $C^{14}O_2$ to photosynthetically active plants at a constant predetermined rate. Proc. Amer. Soc. Hort. Sci., 80: 661-665.

Westphal, E. 1977. Morphogenise ultrastructure et étiologie de quelques galles d'Eriophyes (Acariens). Marcellia, 39: 193-375.

Wetmore, R.H. and Rier, J.P. 1963. Experimental induction of vascular tissues in callus of angiosperms. Am. Jour. Bot., 50: 418-430.

Wetmore, R.H. and Sorokin, S. 1955. On differentiation of xylem. J. Arnold Arbor., 36: 305-324.

White, P.R. 1933. Plant tissue cultures: results of preliminary experiments on the culturing of isolated stem-tips of Stellaria media. Protoplasma, 19: 97-116.

White, P.R. 1934. Potentially unlimited growth of excised tomato root tips in a liquid medium. Plant Physiol., 9: 585-600.

White, P.R. 1939. Potentially unlimited growth of excised plant callus in an artificial medium. Am. Jour. Bot., 26: 59-64.

White, P.R. 1943. A handbook of plant tissue culture. Jacques Cattell Press, Tempe, Arizona.

White, P.R. 1951. Nutritional requirements of isolated plant tissues and organs. Ann. Rev. Plant Physiol., 2: 231-244.

White, P.R. 1963. The cultivation of animal and plant cells. 2nd ed. Ronald Press, N.Y. 228p.

White, P.R. 1968. Tissue culture in service of phytopathology. Indian Phytopathol., 21: 14-27.

White, P.R. and Braun, A.C. 1942. A cancerous neoplasm of plants. Autonomous bacteria-free crown-gall tissue. Cancer Res., 2: 597-617.

White, P.R. and Grove, A.R. 1965. Proceedings of International Conference on Plant Tissue Culture. McCutchan Pub. Co., Berkeley, California. 553p.

Widdowson, E., Doncaster, C.C. and Fenwick, D.W. 1958. Observations on the development of Heterodera rostochiensis Woll. in sterile root cultures. Nematologica, 3: 308-314.

Willmer, E.N. (ed.) 1966. Cells and tissue in culture: Methods, Biology and Physiology. Vol.3. Academic Press, N.Y. 826p.

Wood, H.N., Braun, A.C., Brandes, H. and Kende, H. 1969. Studies on the distribution and properties of a new class division-propagating substances from higher plant species. Proc. Nat. Acad. Sci. (USA), 62(2): 349-356.

Yarwood, C.E. and Jacobson, L. 1955. Accumulation of chemicals in diseased areas of leaves. Phytopathol., 45: 43-48.

Yeoman, M.M. and Forsche, E. 1980. Cell proliferation and growth in callus cultures. In Perspectives in Plant Cell and Tissue Culture, (ed.) I.K. Vasil, International Review of Cytology Supplement IIA. 1-21.

Yeoman, M.M. and MacLeod, A.J. 1977. Tissue (callus) culture techniques. In Plant tissue and cell culture. (ed.) H.E. Street. Bot. Monographs, vol.II, Univ. of California Press, Berkley and Los Angeles, 31-59.

Young, S., Brown, W.W. and Klinger, B. 1970. Nigropallidal encephalomalacia in horses fed Russian knapweed (Centaurea repens L.). Am. Jour. Vet. Res., 31: 1393-1404.

Zeroni, M. and Hall, M.A. 1980. Molecular effects of hormone treatment on tissue. In Hormonal regulation of development, I. (ed.) J. MacMillen, Encyclopedia of Plant Physiology (vol.9). Springer-Verlag, Berlin, Heidelberg, New York. 511-586.

Zimmermann, M.H. 1960. Transport in the phloem. Ann. Rev. Plant Physiol., 11: 167-190.

Zuckerman, B.M. 1971. Gnotobiology. In Plant Parasitic Nematodes. (eds.) B.M. Zuckerman, W.F. Mai and R.A. Rohde. vol.II. Academic Press, N.Y. 159-184.

Zweigelt, F. 1942. Beitrage zur Kenntnis der Blattlausgallen. Biol. Gen., 16(4): 554-572.

APPENDICES

APPENDIX A. MODIFIED HOAGLAND'S SOLUTION

Compound	Stock solution	Amount/L
KH_2PO_4	138.000 g/L	1 ml
$MgSO_4$	246.225 g/L	2 ml
$CaCl_2$	147.000 g/L	5 ml
K_2SO_4	85.125 g/L	5 ml
NH_4NO_3	60.000 g/L	0.5 ml
Micronutrients		1 ml
H_3BO_3	2.860 g/L	
$MnCl_2.4H_2O$	1.810 g/L	
$ZnSO_4.7H_2O$	0.220 g/L	
$CuSO_4.5H_2O$	0.080 g/L	
$NaMoO_4.2H_2O$	0.026 g/L	
FeEDTA		25 mg

(Hoagland and Aron, 1938).

APPENDIX B. MS MEDIUM

		Stock Solution	Amount/L
Micronutrients (store in freezer)		mg/100 ml	1 ml
Manganous sulfate	$MnSO_4.4H_2O$	2230	
Boric acid	H_3BO_3	620	
Zinc sulfate	$ZnSO_4.7H_2O$	860	
Sodium molybdate	$Na_2MoO_4.2H_2O$	25	
Copper sulfate	$CuSO_4.5H_2O$	2.5	
Cobalt chloride	$CoCl_2.6H_2O$	2.5	
Vitamins (store in freezer)		mg/100 ml	1 ml
Nicotinic acid		100	
Thiamine.HCl		1000	
Pyridoxine.HCl		100	
Myo-Inositol		10000	
Calcium chloride	$CaCl_2.2H_2O$	15 g/100 ml	2.9 ml
Potassium iodide (store in amber bottle in freezer)	KI	75 mg/100 ml	1 ml
Macronutrients (store in refrigerator)		g/l	100 ml
Ammonium nitrate	NH_4NO_3	16.5	
Potassium nitrate	KNO_3	19.0	
Magnesium sulfate	$MgSO_4.7H_2O$	3.7	
Potassium sulfate	KH_2PO_4	1.7	
FeEDTA		20 to 40 mg/L	25 mg
Sugar		3%	15 g
Agar		0.6 to 0.8%	8 g

Final pH adjusted to 5.8 with 0.2N KOH or 0.2N HCl.

(Murashige and Skoog, 1962)

APPENDIX C. B5 MEDIUM AND ITS MODIFICATIONS

		STOCK SOLUTION	B5 AMOUNT/L	B5mod. AMOUNT/L	B5½mod. AMOUNT/L
Micronutrients (store in freezer)		mg/100 ml	1 ml	1 ml	1 ml
Manganous sulfate	$MnSO_4.4H_2O$	1000			
Boric acid	H_3BO_3	300			
Zinc sulfate	$ZnSO_4.7H_2O$	200			
Sodium molybdate	$Na_2MoO_4.2H_2O$	25			
Copper sulfate	$CuSO_4.5H_2O$	2.5			
Cobalt chloride	$CoCl_2.6H_2O$	2.5			
Vitamins (store in freezer)		mg/100 ml	1 ml	2 ml	0.5 ml
Nicotinic acid		100			
Thiamine.HCl		1000			
Pyridoxine.HCl		100			
Myo-Inositol		10000			
Calcium chloride	$CaCl_2.2H_2O$	15g/100 ml	1 ml	3 ml	0.5 ml
Potassium iodide (amber bottle, in freezer)	KI	75 mg/100 ml	1 ml	2 ml	0.5 ml
Macronutrients (store in refrigerator)		g/L	100 ml	150 ml	50 ml
Sodium phosphate dibasic	$NaH_2PO_4.H_2O$	1.5			
Potassium nitrate	KNO_3	25			
Ammonium sulfate	$(NH_4)_2SO_4$	1.34			
Magnesium sulfate	$MgSO_4.7H_2O$	2.5			
FeEDTA	20 to 40 mg/L		30 mg	15 mg	15 mg
Sucrose	3%		15 g	30 g	7.5 mg
Agar	0.6 to 0.8%		8 g	8 g	8 g

Final pH adjusted to 5.5 with 0.2N KOH or 0.2N HCl

(Gamborg and Wetter, 1975)

APPENDIX D. FAA (formalin-acetic-alcohol) 'Pempel's mixture'

Glacial acetic acid	80 ml
Formaldehyde (37-40%)	130 ml
Ethanol (95%)	300 ml
Water	600 ml

APPENDIX E. PROCEDURE FOR DEHYDRATION AND EMBEDDING IN PARAPLAST

TBA (Tertiary Butyl Alcohol) dehydration series

Steps	Solutions	Time
1	Transfer fixed material to H_2O	2 hr
2	30% ethanol	1 hr
3	50% ethanol	1 hr
4	70% ethanol	1 hr
5	90% ethanol	1 hr
6	95% ethanol	1 hr
7	Absolute alcohol:TBA (1:1)	1 hr
8	Absolute alcohol:TBA (1:3)	1 hr
9	100% TBA	1 hr
10	Parafin oil:TBA (1:1)	1 day
11	Pour off 1/3, add paraplast (60°C)	2 hr
12	Embedding in paraplast	

(Berlyn and Miksche, 1976)

APPENDIX F. STAINING WITH SAFRANIN - FAST GREEN

Steps	Solutions	Time
1	Xylene	5 min
2	Xylene	5 min
3	100% ethyl alcohol	2 min
4	70% ethyl alcohol	2 min
5	50% ethyl alcohol and safranin (53°C) 1.0%	10 min
6	70% ethyl alcohol	2 min
7	95% ethyl alcohol and fast green FCF 0.5%	2-10 sec
8	100% ethyl alcohol	2 min
9	100% ethyl alcohol	2 min
10	carbol-xylene	5 sec
11	xylene	10 min
12	xylene	10 min

(Berlyn and Miksche, 1976)

APPENDIX G. SAMPLE PREPARATION FOR LIQUID SCINTILLATION COUNTING

1. 10 mg of sample (air dried) is dissolved in 0.5 ml of 1:2 Perchloric acid 60% and H_2O_2 30%, by incubating for 1 hr at 40°C in an incubator.
2. Let the vials cool down.
3. Add 15 ml of scintillation fluid and shake well until a clear solution obtained.

   The scintillation fluid contains:

   PPO ——— 12 gm

   Toluene ——— 2000 ml

   Ethylene glycol monoethyl ether ——— 1000 ml
4. The samples are placed in a liquid scintillation counter, calibrating it for counting ^{14}C.

Table I. Fresh and dry weights of different parts of galled and nongalled Russian knapweed plants injected with Fructose D [^{14}C(U)] -; the number in parentheses indicates the visual condition of the gall on a scale of 1 to 4 and the number outside is the fresh weight/dry weight ratio of the gall (Experiment 1).

Group* (Type of plant)	Replicate	(Fresh weight/Dry weight in gm) GALL		LEAF		ROOT		STEM.X	
		Galled	Nongalled	Galled	Nongalled	Galled	Nongalled	Galled	Nongalled
A	1	(3)** 0.088/0.108 = 4.89	-	3.168/0.440	1.987/0.288	2.284/0.692	2.261/0.646	0.390/0.089	0.312/0.182
	2	(3) 0.069/0.015 = 4.60	-	1.400/0.200	1.408/0.200	1.404/0.360	1.824/0.521	0.383/0.088	0.607/0.164
	3	(3) 0.058/0.102 = 4.83	-	1.642/0.225	1.171/0.165	2.092/0.510	2.557/0.752	0.275/0.058	0.318/0.082
B	1	(2) 0.069/0.014 = 4.93	-	1.850/0.250	1.108/0.142	1.426/0.432	2.050/0.554	0.530/0.123	0.068/0.015
	2	(2) 0.090/0.016 = 5.62	-	1.590/0.220	1.785/0.248	0.861/0.287	2.176/0.640	0.301/0.075	0.536/0.134
	3	(1) 0.101/0.015 = 6.77	-	1.368/0.180	0.728/0.095	0.851/0.230	1.765/0.464	0.200/0.040	0.180/0.045
C	1	(1) 0.101/0.020 = 6.05	-	1.820/0.241	0.787/0.100	0.968/0.269	2.615/0.688	0.417/0.098	0.050/0.010
	2	(1) 0.109/0.016 = 6.81	-	1.993/0.258	1.147/0.149	0.764/0.241	0.823/0.235	0.374/0.090	0.536/0.134
	3	(1) 0.096/0.013 = 7.38	-	1.617/0.210	1.152/0.144	1.628/0.524	0.786/0.202	0.277/0.068	0.225/0.055

* Group A - Plants with lobed leaf margin and lyrate leaf (gall on leaf in galled plants)
B - Plants with entire leaf margin and spatulate leaf (gall on petiole in galled plants)
C - Plants with entire leaf margin and spatulate leaf (gall on leaf in galled plants)

** Visual condition of gall: 1 = green and fleshy
2 = green and non-fleshy
3 = brown and non-fleshy
4 = grown and dry

Table II. Fresh and dry weights of different parts of galled and nongalled Russian knapweed plants which photosynthesised in $^{14}CO_2$ chamber. The number in parentheses indicates the visual condition of the gall on a scale of 1 to 4 and the numbers outside are the fresh weight/dry weight ratio of the gall (Experiment 2).

Group* (Type of Replicate plant)		(Fresh weight/Dry weight in gm)				
		GALL 1	GALL 2	LEAF	ROOT	STEM
A'	1	(1)** 0.127/0.015 = 8.47	-	1.707/0.250	1.781/0.450	0.101/0.127
	2	(2) 0.068/0.101 = 6.80	-	0.753/0.101	1.217/0.307	0.131/0.030
	3	(1) 0.102/0.011 = 9.27	-	1.211/0.169	1.186/0.320	0.081/0.021
	4	(2) 0.071/0.010 = 7.10	-	0.643/0.090	1.072/0.230	0.099/0.025
	5	(1) 0.099/0.012 - 8.25	-	0.981/0.111	1.254/0.343	0.176/0.041
B'	1	(1) 0.113/0.011 = 10.27	(3) 0.061/0.010 = 6.10	0.307/0.041	1.091/0.148	0.161/0.041
	2	(2) 0.076/0.010 = 7.60	(2) 0.083/0.011 = 7.54	0.183/0.030	1.139/0.162	0.120/0.030
	3	(1) 0.121/0.014 = 8.64	(4) 0.046/0.011 = 4.18	0.225/0.038	0.596/0.131	0.143/0.033
	4	(1) 0.129/0.012 = 10.75	(2) 0.106/0.013 = 8.15	0.191/0.025	0.961/0.099	0.247/0.059
	5	(2) 0.081/0.010 = 8.10	(3) 0.072/0.011 = 6.54	0.381/0.071	1.007/0.199	0.200/0.048
C'	1	-	-	1.612/0.210	2.101/0.519	0.197/0.040
	2	-	-	1.199/0.168	1.191/0.327	0.212/0.043
	3	-	-	1.688/0.211	2.176/0.567	0.271/0.059
	4	-	-	2.333/0.316	1.166/0.315	0.242/0.059
	5	-	-	1.591/0.199	1.872/0.500	0.136/0.035

* Group (Plants with entire leaf margin and spatulate leaf)
A' - Plants with one gall in the meristem region.
B' - Plants with two galls, one gall in the meristem region and another gall on a leaf.
C' - Plants with no galls (control).

** Visual condition of gall: 1 = green and fleshy; 2 = green and non-fleshy; 3 = brown and non-fleshy; 4 = brown and dry.

Table III. Fresh and dry weights of different parts of galled and nongalled Russian knapweed plants from which only presentation leaf (X.LEAF) photosynthesized in $^{14}CO_2$ chamber. The number in parentheses indicates the visual condition of the gall on a scale of 1 to 4 and the numbers outside are fresh weights/dry weights ratio of the galls (Experiment 3)

Group* (Type of Replicate Plant)		Fresh weight/dry weight in gm						
		GALL 1	GALL 2	GALL 3	LEAF	ROOT	STEM	X.LEAF
A"	1	(1) 0.160/0.026 = 6.15	-	-	1.262/0.171	2.306/0.630	0.298/0.079	0.100/0.013
	2	(1) 0.089/0.013 = 6.85	-	-	1.777/0.250	1.763/0.471	0.224/0.057	0.089/0.012
	3	(1) 0.120/0.020 = 6.00	-	-	0.924/0.137	2.275/0.651	0.519/0.134	0.144/0.015
	4	(1) 0.127/0.017 = 7.47	-	-	1.591/0.227	1.157/0.333	0.280/0.069	0.081/0.011
	5	(1) 0.083/0.015 = 5.53	-	-	1.771/0.170	0.841/0.247	0.167/0.042	0.101/0.013
	6	(1) 0.070/0.011 = 6.36	-	-	0.795/0.107	1.924/0.540	0.200/0.053	0.970/0.012
	7	(1) 0.100/0.013 = 7.69	-	-	1.390/0.192	1.800/0.487	0.460/0.107	0.088/0.011
	8	(4) 0.051/0.010 = 3.10	-	-	1.080/0.132	1.231/0.356	0.210/0.050	0.083/0.011
	9	(3) 0.050/0.011 = 4.54	-	-	1.616/0.194	1.156/0.380	0.159/0.042	0.109/0.013
	10	(3) 0.045/0.010 = 4.50	-	-	0.620/0.088	0.987/0.321	0.219/0.055	0.090/0.012
B"	1	(1) 0.100/0.011 = 9.09	(1) 0.337/0.045 = 7.50	-	0.232/0.030	1.064/0.276	0.074/0.012	0.121/0.013
	2	(1) 0.097/0.010 = 9.70	(1) 0.125/0.017 = 7.37	-	0.525/0.069	0.927/0.250	0.119/0.025	0.100/0.011
	3	(1) 0.107/0.010 =10.70	(3) 0.114/0.019 = 6.00	-	0.555/0.071	0.717/0.217	0.080/0.017	0.088/0.010
	4	(1) 0.127/0.013 = 9.78	(4) 0.120/0.027 = 4.44	-	0.650/0.088	1.222/0.315	0.082/0.019	0.132/0.014
	5	(1) 0.120/0.012 =10.00	(3) 0.115/0.018 = 6.39	-	0.307/0.040	0.499/0.121	0.087/0.020	0.091/0.010
	6	(1) 0.130/0.015 = 8.67	(4) 0.040/0.010 = 4.00	-	0.290/0.037	2.010/0.527	0.109/0.022	0.121/0.013
	7	(1) 0.110/0.012 = 9.17	(3) 0.060/0.010 = 6.00	-	0.579/0.077	1.009/0.287	0.107/0.024	0.099/0.011
	8	(1) 0.101/0.010 =10.10	(4) 0.041/0.010 = 4.10	-	0.300/0.049	0.815/0.265	0.096/0.017	0.146/0.015
	9	(1) 0.131/0.013 =10.08	(4) 0.061/0.011 = 5.51	-	0.410/0.060	0.968/0.321	0.141/0.027	0.111/0.013
	10	(1) 0.089/0.010 = 8.90	(3) 0.076/0.013 = 5.85	-	0.271/0.051	1.311/0.311	0.195/0.031	0.137/0.014
C"	1	(1) 0.105/0.010 =10.50	(1) 0.120/0.010 =12.00	(4) 0.057/0.010 = 5.70	0.125/0.018	0.428/0.117	0.144/0.029	0.081/0.010
	2	(1) 0.123/0.012 =10.08	(4) 0.041/0.010 = 4.10	(1) 0.113/0.010 =11.30	0.065/0.010	0.692/0.204	0.084/0.017	0.100/0.021
	3	(2) 0.133/0.017 = 7.82	(2) 0.130/0.012 =10.83	(4) 0.057/0.011 = 5.18	0.130/0.020	0.651/0.195	0.091/0.012	0.089/0.011
	4	(3) 0.097/0.015 = 6.47	(2) 0.135/0.015 = 9.00	(1) 0.100/0.010 =10.00	0.075/0.011	0.560/0.162	0.100/0.020	0.093/0.010
	5	(1) 0.099/0.010 = 9.90	(2) 0.071/0.010 = 7.10	(4) 0.067/0.012 = 5.58	0.147/0.017	0.321/0.097	0.141/0.033	0.097/0.011
	6	(1) 0.111/0.010 =11.10	(2) 0.120/0.014 = 8.57	(1) 0.121/0.010 =12.10	0.080/0.012	0.717/0.209	0.127/0.025	0.081/0.010
	7	(3) 0.076/0.010 = 7.60	(2) 0.072/0.011 = 6.54	(1) 0.117/0.010 =11.70	0.071/0.017	0.310/0.099	0.091/0.017	0.079/0.010
	8	(2) 0.096/0.012 = 8.00	(2) 0.073/0.010 = 7.30	(4) 0.066/0.011 = 6.00	0.191/0.031	0.622/0.181	0.137/0.026	0.087/0.011

(Continued)

Table III. (Continued)

Group* (Type of plant)	Replicate	GALL 1	GALL 2	GALL 3	LEAF	ROOT	STEM	X.LEAF
C"	9	(2) 0.091/0.011 = 8.27	(2) 0.091/0.012 = 7.58	(4) 0.049/0.010 = 4.90	0.187/0.027	0.417/0.127	0.101/0.018	0.081/0.010
	10	(4) 0.046/0.010 = 4.60	(1) 0.125/0.010 =12.50	(4) 0.061/0.011 = 5.50	0.114/0.022	0.587/0.176	0.076/0.013	0.096/0.012
D"	1	-	-	-	1.165/0.182	1.184/0.309	0.167/0.044	0.208/0.033
	2	-	-	-	0.616/0.220	1.100/0.258	0.161/0.036	0.201/0.026
	3	-	-	-	1.296/0.186	1.380/0.340	0.110/0.025	0.157/0.021
	4	-	-	-	1.697/0.243	1.720/0.452	0.153/0.036	0.173/0.027
	5	-	-	-	2.331/0.316	1.166/0.315	0.242/0.058	0.191/0.024
	6	-	-	-	1.981/0.260	0.919/0.250	0.183/0.041	0.189/0.024
	7	-	-	-	1.117/0.152	0.784/0.210	0.166/0.040	0.146/0.018
	8	-	-	-	1.259/0.171	1.112/0.300	0.192/0.046	0.197/0.025
	9	-	-	-	1.382/0.185	1.003/0.271	0.177/0.043	0.174/0.020
	10	-	-	-	1.414/0.196	2.417/0.622	0.171/0.040	0.181/0.021

*Group (Plants with entire leaf margin and spatulate leaf)

A" - Plants with one gall in the meristem region
B" - Plants with two galls, one gall in the meristem region and another gall on a leaf
C" - Plants with three galls, one gall in the meristem region, second gall on a leaf below meristem, third gall on a leaf below the second gall
D" - Plants with no galls (control).

**Visual condition of the gall: 1 - green and fleshy
2 - green and non-fleshy
3 - brown and non-fleshy
4 - brown and dry.

Table IV. Formula for determining the counter's efficiency from Carbon-14 Standard Quench Curve plotted from samples with known amount of radioactivity.

Observations	Efficiency (X)	Automatic External Standard (Y)	Counts per Minute (CPM)
1	0.89	560576	45376
2	0.83	389084	42282
3	0.78	283219	39953
4	0.68	177529	34782
5	0.60	132221	30616
6	0.48	81242	24693

The regression equation $Y = \hat{\beta}o + \hat{\beta}_1 X$

where Y = dependent variable

X = independent variable

$\hat{\beta}o$ = Y- intercept = -507355.74744898

$\hat{\beta}_1$ = slope of the line = 1095775.93537415

To determine the efficiency for particular plant sample from above equation

$$\text{Efficiency} = \frac{\text{Automatic External Standard (for plant sample)} + 507355.74744898}{1095775.93537415}$$

Table V. Radioactivity (corrected for background) in different parts of galled and nongalled Russian knapweed plants injected with Fructose D[^{14}C(U)] - (Experiment 1)

Group* (Type of plant)	Replicate	Counts per minute (CPM) in 10 mg dry weight samples							
		GALL		LEAF		ROOT		STEM.X	
		Galled	Nongalled	Galled	Nongalled	Galled	Nongalled	Galled	Nongalled
A	1	8281	-	5333	3048	121	223	6637	55462
	2	2169	-	174	13001	587	912	1652	6137
	3	1054	-	177	19952	153	195	181	31189
B	1	10210	-	1123	28184	170	786	6039	26977
	2	10447	-	8054	9516	69	740	7015	4897
	3	46773	-	1811	40517	580	783	8709	6012
C	1	94841	-	2243	45919	262	774	13092	2655
	2	35727	-	3147	17179	1324	764	8511	14223
	3	87297	-	636	31677	2202	982	8260	18749

* Group A - Plants with lobed leaf margin and lyrate leaf (gall on leaf in galled plants)
B - Plants with entire leaf margin and spatulate leaf (gall on petiole in galled plants)
C - Plants with entire leaf margin and spatulate leaf (gall on leaf in galled plants)

Table VI. Radioactivity (corrected for background) in different parts of galled and nongalled Russian knapweed plants which photosynthesised in the $^{14}CO_2$ chamber (Experiment 2)

Group* (Type of plant)	Replicate	Counts per minute (CPM) in 10 mg dry weight samples				
		X.GALL 1	X.GALL 2	X.LEAF	X.ROOT	X.STEM
A'	1	20073	-	16074	322	1516
	2	11058	-	33386	457	2040
	3	11746	-	4937	717	2080
	4	9698	-	41935	1146	270
	5	27197	-	31092	147	290
B'	1	24336	2172	31998	861	157
	2	6434	2161	28572	2170	2013
	3	26514	799	46238	88	1128
	4	28998	9917	6850	216	1976
	5	7114	1869	19572	43	288
C'	1	-	-	20942	1121	3715
	2	-	-	6881	4798	2617
	3	-	-	20627	983	2766
	4	-	-	9987	3952	3211
	5	-	-	12653	827	2186

* Group (Plants with entire leaf margin and spatulate leaf)

A' - Plants with one gall in meristem region
B' - Plants with one gall in meristem region and another gall on leaf
C' - Plants with no galls (control)

Table VII. Radioactivity (corrected for background) in different parts of galled and nongalled Russian knapweed plants from which only presentation leaf (X.LEAF) photosynthesised in $^{14}CO_2$ chamber. (Experiment 3).

Group* (Type of plant)	Replicate	Counts per minute (CPM) in 10 mg dry weight samples						
		GALL 1	GALL 2	GALL 3	LEAF	ROOT	STEM	X.LEAF
A"	1	33298	-	-	32620	682	2659	26463
	2	36454	-	-	1949	355	3964	18977
	3	12240	-	-	410	1900	1480	3690
	4	26775	-	-	1150	578	916	25944
	5	20073	-	-	16074	322	1516	15673
	6	17844	-	-	791	2124	5980	22333
	7	17979	-	-	139	490	4831	18514
	8	647	-	-	1350	4696	6179	58109
	9	1905	-	-	1835	195	4424	17542
	10	1586	-	-	349	1135	157	33751
B"	1	56684	12922	-	1125	617	2361	84053
	2	70136	26682	-	295	183	1628	95477
	3	32583	1259	-	183	3204	9819	104415
	4	52603	181	-	174	703	2482	25950
	5	11897	2862	-	7749	1119	589	18714
	6	26775	726	-	1151	578	916	27944
	7	57397	3308	-	135	1587	619	19989
	8	25842	287	-	7244	963	3519	27311
	9	27784	923	-	116	155	973	37295
	10	16977	1995	-	4571	1286	1713	19831
C"	1	70224	36606	177	192	5594	8514	99097
	2	24736	179	15214	196	880	452	42689
	3	11396	5125	889	958	1423	896	57056
	4	5740	7408	3436	1161	1150	1980	22735
	5	50565	1269	175	382	2229	1712	35934
	6	35663	3209	11736	3556	447	358	4222
	7	7117	1197	6785	2872	627	196	42983
	8	17761	1876	612	1616	917	980	37586

(Continued)

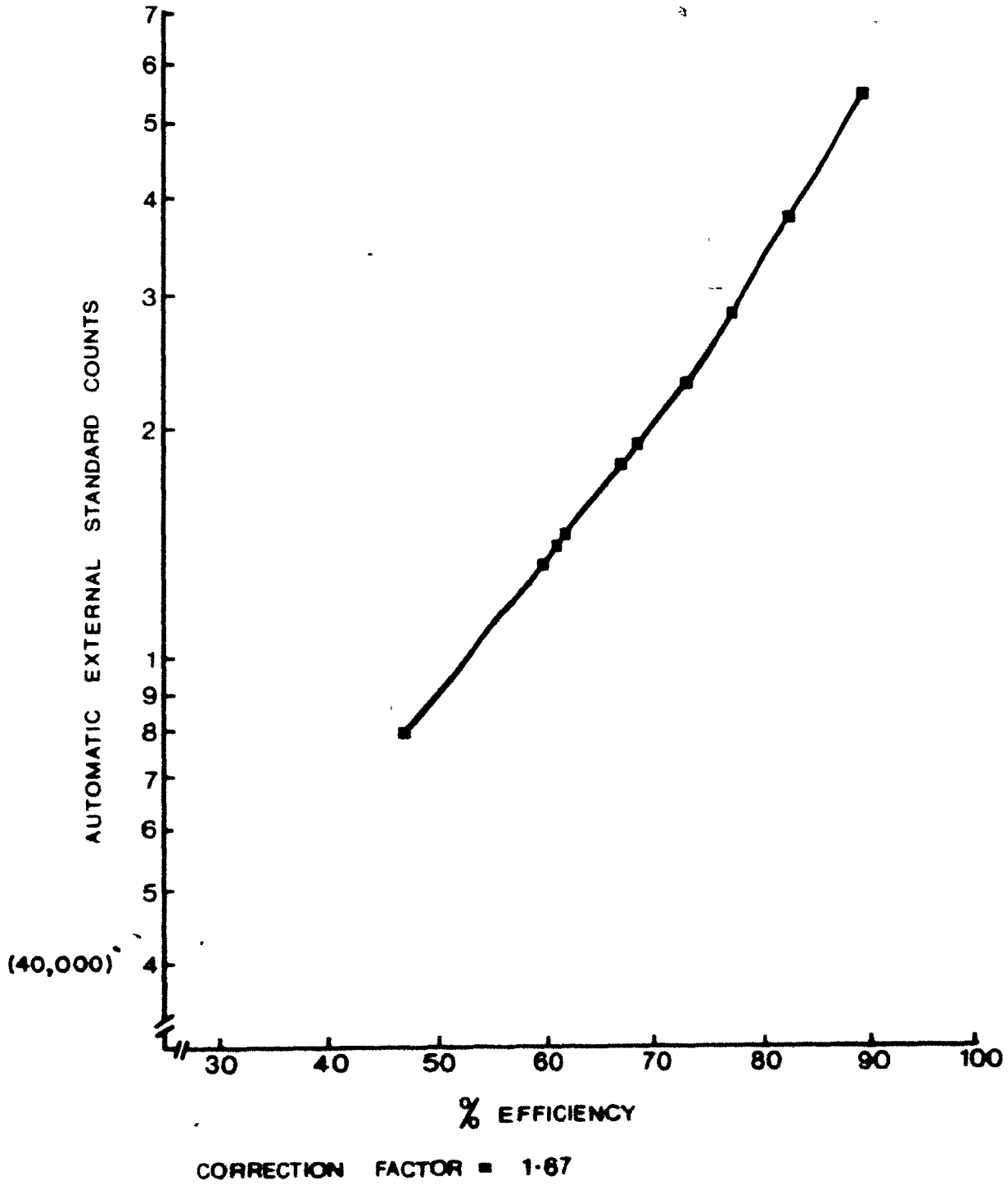
Table VII (Continued)

Group* (Type of plant	Replicate	GALL 1	GALL 2	GALL 3	LEAF	ROOT	STEM	X. LEAF
C"	9	21611	2718	333	217	313	1129	78651
	10	981	37751	716	166	87	683	68349
D"	1	-	-	-	11139	1142	2518	27350
	2	-	-	-	11127	1158	2223	22712
	3	-	-	-	2978	2896	5327	12588
	4	-	-	-	1164	388	3163	34168
	5	-	-	-	5935	127	2619	28878
	6	-	-	-	1119	1062	1190	12701
	7	-	-	-	5405	3024	3037	18494
	8	-	-	-	6155	392	3188	35061
	9	-	-	-	6720	429	1257	41683
	10	-	-	-	9697	768	1390	21823

*Group (Plants with entire leaf margin and spatulate leaf)

A" - Plants with one gall in the meristem region
B" - Plants with two galls, one gall in the meristem region and another gall on a leaf
C" - Plants with three galls, one gall in the meristem region, second gall on a leaf below meristem, third gall on a leaf below second gall
D" - Plants with no galls (control)

Appendix Figure I. CARBON-14 Standard Quench Curve.



CORRECTION FACTOR = 1·67