Chapter 1. LITERATURE REVIEW

1.1 LITERATURE REVIEW ON OROBANCHACEAE

1.1.1 INTRODUCTION

Distributed world wide, parasitic angiosperms cause significant crop losses in areas of heavy infestation. Although precise numbers are not available, it has been estimated that roughly 1% of the flowering plants, about 3,000 species in total, are parasitic (Sauerborn 1991). Parasitic plants are capable of forming a close connection with the vascular system of their host plant, and are at least partially dependent on the host for their supply of water, nutrients, and metabolites, but the degree of host dependency varies among parasitic plant species.

Parasitic angiosperms are found throughout the plant kingdom and are generally categorized according to their point of attachment to the host (root or shoot parasite) or the presence or absence of chlorophyll (holoparasite or hemiparasite). About 60% of all parasitic species are root parasites. About 20% of all parasitic species are holoparasites. Holoparasitic plants are obligate parasites that lack chlorophyll and therefore have no ability to assimilate and fix carbon (Stewart and Press 1990). On the other hand, hemiparasites can be either facultative or obligate, and although they contain chlorophyll and have some photosynthetic capacity, they depend upon the host for water and essential minerals. The distinguishing feature of all parasitic plants is the haustorium, which forms a connection between the parasite and the vascular system of the host (Kuijt 1977; Visser and Dörr 1986).

Broomrapes, *Orobanche* spp., are among the major parasitic weeds in legume and vegetable crops grown in the Mediterranean basin and Eastern Europe. Broomrapes are holoparasites that subsist on the roots of host plants from which the parasites derive carbon, water, and nutrients needed for growth (Baccarini and Melandri 1967; Saghir et al. 1973). *Orobanche* spp. parasitism often leads to significant yield reductions and in some cases complete crop failure (Foy et al. 1989).

All *Orobanche* spp. are host-specific to some degree, but some like *Orobanche aegyptiaca* Pers., *Orobanche ramosa* L., and *Orobanche crenata* Forsk. have a wide host range. Economically important crops parasitized by broomrapes include broad (also referred to interchangeably as faba) bean (*Vicia faba* L.), pea (*Pisum sativum* L.), tomato (*Lycopersicon esculentum* Mill.), potato (*Solanum tuberosum* L.), tobacco (*Nicotiana tabacum* L.), eggplant (*Solanum melongena* L.), carrot (*Daucus carota* L.), sunflower (*Helianthus annuus* L.), alfalfa

(*Medicago sativa* L.), red clover (*Trifolium pratense* L.), white clover (*Trifolium repens* L.), lettuce (*Lactuca sativa* L.), and some varieties of vetch (*Vicia* spp.). As it can be seen from the Table 1.1.1., nearly 31% of the economically important crops parasitized by broomrapes are legumes. In addition, almost all of the legumes listed in the Table 1.1.1. could be parasitized by both *Orobanche minor* Sm. and *O. aegyptiaca*.

Legumes (species of family Leguminosae) provide a home for bacteria and energy to fix or gather air nitrogen (N_2). In return, the host receives fixed nitrogen from the nodule and in process produces food and forage protein. The ability of the legume crops to work symbiotically with rhizobia to produce protein is important in world agriculture. Moreover, understanding of the interactions that take place in the symbiosis between legumes and rhizobacteria could aid, perhaps, in understanding the signal exchange between parasitic weed and its legume host. For example, during studies of the root exudates from faba bean for a possible isolation of the natural stimulant, there has often been difficulty to obtain exudates with activity on *O. crenata*. One possible explanation for this, as proposed by Petzoldt (1979), is that production of the stimulant is dependent on the infection of roots with *Rhizobium* nodules and that these nodules on the roots of faba bean are the main site of attachment for *O. crenata*.

Current measures for broomrape control can be used only on a small scale and are economically unacceptable in modern production agriculture. The development of resistant, transgenic crop varieties appears to be one of the key directions for the control of parasitic weeds. Studies on the host-parasite interaction should help in finding effective and affordable control measures; however, basic information regarding physiology and biochemistry of the parasitic weeds is limited. It is possible that a control approach could be determined after a detailed look at the interactions between parasite infestation and *Rhizobium* nodulation in legume crops. Although a general overview of Orobanchaceae is given, *O. minor* and *O. aegyptiaca*, broomrapes commonly parasitizing legumes, will be discussed in more detail than other species of the broomrape family.

1.1.2 TAXONOMY OF OROBANCHACEAE

Parasitism in flowering plants is believed to have evolved independently in various families of the Plant Kingdom (Kuijt 1969). Parasitism is observed in 18 families (Sauerborn 1991) in which only four families present economically important species whose infestations could result in significant damage to agricultural crops and forest cultures: Cuscutaceae, Loranthaceae, Orobanchaceae, and Scrophulariaceae.

A great deal of disagreement still exists in the classification of the family Orobanchaceae.

However, as more morphological, microscopic, biochemical, and anatomical data become available, the consensus on the broomrape taxonomy should improve. As of now, the majority of researchers working with broomrapes follow the three major classifications: (1) Dahlgren's Superorder Lamiiflorae; Scrophulariales; (2) Cronquist's Subclass Asteridae; Scrophulariales; (3) Takhtajan's Subclass Asteridae; Lamianae; Scrophulariales.

The family Orobanchaceae contains approximately 180 species, which are divided into 15 genera; *Aeginetia, Boschniakia, Christisonia, Cistanche, Conopholis, Epifagus, Gleadovia, Kopsiopsis, Mannafettaea, Necranthus, Orobanche, Phacellanthus, Phelypaea, Platypholis, Xylanche* (Watson and Dallwitz 1992). The genus *Orobanche* alone is estimated to contain as many as 150 species (Musselman 1980).

1.1.3 GEOGRAPHIC DISTRIBUTION

Orobanche species are found primarily in the regions with arid to temperate climates, and, although some broomrape species have been found all over the world, the main area of distribution is the Mediterranean basin (Sauerborn 1991; Figure 1.1.1).

One of the major parasites on legumes, *O. minor* is distributed over a range that overlaps that of many other *Orobanche* species in the Mediterranean region; however, parasite infestations have been found as far as eastern and southern Africa, New Zealand, and the United States (Musselman 1994; Figure 1.1.2). Another important legume parasite, *O. aegyptiaca* occurs mainly in northeastern Africa and the Middle East extending to Kazakhstan and Northern India. *Orobanche cernua* Loefl. is found in various regions from the Atlantic coast of Europe to China, and was found in Western and Eastern Australia; *O. ramosa*, one of the most widely distributed species, could be found in the Middle East, central Europe, and northern Africa, with introduced populations in Kentucky, California, Texas, and Central and South America.

Several species of *Orobanche* occur in the United States. However, among them only *O. minor* and *O. ramosa* present a potential threat to local agro-ecosystems. It is suspected that *O. minor* and *O. ramosa* have been introduced to North America along with host crops such as tobacco, clover, etc. In the United States, three separate infestations of *O. ramosa* and several of *O. minor* had been reported (Eplee et al. 1994; Figure 1.1.3). However, for *O. ramosa* most of the infested areas were eradicated. It is believed that *O. minor* was introduced into the United States throughout the past 116 yr, with reported infestations from 32 counties in 12 states and the District of Columbia (Frost and Musselman 1980). Although *O. minor* is not widespread in the United States at this time, it remains a potential threat to agriculture. A cautionary example comes from New Zealand, where after 70 yr of somewhat benign presence *O. minor* suddenly

became widespread in 1955, and now causes significant damage to clover (Evans 1962).

1.1.4 BIOLOGY OF OROBANCHACEAE

1.1.4.1 SEEDS

The life cycle of *Orobanche* spp. is an example of specialized adaptation to the growth habits of the host plants (Figure 1.1.4). The majority of the species in the genus *Orobanche* are annuals and reproduce by seeds which are among the smallest (about 0.35 by 0.25 mm) in the Plant Kingdom (Kadry and Tewfic 1956). Average seed weight is 3 to 6 μg (Parker and Riches 1993). Broomrape seed coat has ridges on the surface which are helpful in dispersal by wind and water (Figure 1.1.5). *Orobanche* species produce seeds in large quantities. For example *O. crenata* (one of the more robust species) can produce up to 500,000 seeds per plant (Cubero and Moreno 1979), while smaller species like *O. ramosa* may produce up to 20,000 seeds per plant (Linke et al. 1989). High seed production combined with the ability to survive in the soil for more than 10 yr (Puzzilli 1983) make these parasitic plants extremely persistent.

1.1.4.2 GERMINATION AND RADICLE DEVELOPMENT

The seeds remain dormant during a period of after-ripening, until they are in the vicinity of a suitable host and the environmental conditions are favorable (Sauerborn 1991). Factors influencing germination include suitable soil temperature, pH, moisture, soil type, availability of nutrients, and presence of germination stimulant in root exudates of host plants.

1.1.4.2.1 Preconditioning

In order to germinate, broomrape seeds must meet two basic requirements. First, seeds must be preconditioned in warm and moist surroundings. Optimum temperatures for preconditioning vary and can be specific for different broomrape species. Kasasian (1973) determined the optimum temperatures for conditioning and germination of *O. crenata* at 18 C, and for *O. ramosa* at 23 C. Temperatures over 28 C were unfavorable, especially during the conditioning phase. Interestingly, light is inhibitory to *O. crenata* seeds (Hiron 1973) and *O. aegyptiaca* (Jain and Foy 1987), whereas not for *O. ramosa* (ter Borg 1986). Preconditioning

could range from several days to a few weeks (Brown 1965). However, at this time the mechanism of the preconditioning period is not fully understood.

1.1.4.2.2 Germination Stimulation and Radicle Formation

The second requirement is the presence of the germination stimulant, a chemical signal from host root exudates that will trigger the germination of preconditioned seeds. Although natural germination stimulants have not yet been identified, substances that have been reported to influence *Orobanche* germination include gibberelic acid (Abdel Halim et al. 1975; El-Ghamrawy et al. 1990), dl-strigol (Stewart and Press 1990), and strigol analogs such as GR-7 and GR-27 (Johnson et al. 1976). Exposure of *O. cumana* and *O. minor* (Foy et al. 1989; Takeuchi et al. 1995) seeds to calcium hypochlorite and brassinosteroids increased germination.

As broomrape seeds germinate, they develop a radicle (Figure 1.1.6), which could be up to 4 mm in length. Radicle emergence can occur within 96 h in the presence of the host or a suitable root exudate (Brown 1965). Elongation of the radicle is achieved by cell division and extension (Parker and Riches 1993). If a broomrape radicle contacts a host root, the radicle can attach to the root surface. The radicle attaches to the host root mainly in the area of root elongation and absorption (Foy et al 1989). On contact with the root, sticky papillae are formed presumably for adherence of the radicle to the root surface (Aber 1984; Losner-Goshen et al. 1994). Anchoring of the radicle on the host root surface is required for parasite penetration.

1.1.4.3 ATTACHMENT TO THE HOST

1.1.4.3.1 Haustorium Development

Upon attachment to the host root, the radicle undergoes rapid cell division, thickens, and forms a haustorium, a multicellular organ that penetrates the host tissue and serves as a physiological bridge between its vascular system and that of the host. Once the connection has been established the parasite begins to draw nutritional resources from the host plant, as broomrapes are totally dependent on the host for all organic carbon (Parker and Riches 1993). The crucial steps in the development of the haustorium are not well understood. Although some research has been done on *Orobanche* haustorium penetration (Aber 1984; Dörr and Kollman 1974, 1975, 1976), the question of whether the haustorium penetration is mechanical or

enzymatic remains unclear.

1.1.4.3.1.1 Mechanical Penetration

Mechanical penetration involves application of pressure on the cells of the host. If penetration occurs between the cells, the middle lamella raptures separating the cells of the host. When the pressure of the parasite penetration is applied directly on the host cell wall, the result could be either cell wall rupture, stretching and thinning of the cell wall, or crushing of the cell wall (Joel and Losner-Goshen 1994). However, recent reports suggest that broomrape penetration involves the excretion of the enzymatic substances that alter the integrity of the host cell wall (Joel and Losner-Goshen 1994; Losner-Goshen et al. 1994; Shomer-Ilan 1994) rather than relying on mechanical pressure alone.

1.1.4.3.1.2 Enzymatic Penetration

Enzymatic penetration appears to be the most likely pathway for broomrape intrusion. The haustorial cells must make their way through a branched and inter-laced network of lignin microfibrils, pectins, cellulose, and hemicellulose cross-linked with ferulic acid esters. Enzymatic penetration is accompanied by the release of enzymes, resulting in the structural changes of the middle lamella and host cell wall, or their degradation (Joel and Losner-Goshen 1994). Enzymes that degrade the cell wall components were obtained from the radicle and haustorium of *O. aegyptiaca* in vitro in the absence of the host (Shomer-Ilan 1994). The extracellular activities of polygalacturonase, carboxymethyl-cellulase, lignin peroxidase, and β-glucosidase have been reported (Shomer-Ilan 1992, 1993, 1994). In the presence of these enzymes, the cell walls of the host become thinner, disintegrate, or disappear in the areas of contact with the intrusive cells of the haustorium, thus reducing the resistance to penetration (Joel and Losner-Goshen 1994). Since the turn-over time for the *Orobanche*-excreted enzymes is short, it has been suggested that a quick penetration allows broomrape to establish the continuity between the vascular system of the host before the induction of the host's defense mechanisms, which might prevent the establishment of the parasite (Shomer-Ilan 1994).

Although haustorial penetration appears to be due to the enzymatic dissolution of the host cell walls, the process should be viewed as a complex, involving both excretion of the enzymatic substances and application of mechanical pressure at the point of radicle attachment and haustorium induction.

1.1.4.3.2 Tubercle Formation

Following the successful attachment and simultaneously with haustorial penetration, the radicle of the parasite that remains outside of the host root tissue develops into a tubercle (Figure 1.1.7). The tubercle enlarges and eventually develops a shoot that emerges above the soil surface and forms the floral spike (Figure 1.1.8). The underground stages of parasite development can last up to 60 d (Foy et al. 1989), while the emergence, flowering, and seeding may last for only 15 to 18 d (Mukumov 1974). After flowering, seed capsules are formed containing seeds that may be dispersed by wind, water, or human activity. Most scientists believe that the majority of the crop damage due to the *Orobanche* parasitization happens before the floral spike of the parasite emerges from the ground. By the time the shoot emerged, 88% of the dry mass of *O. crenata* had been already formed (Singh et al. 1971).

1.1.5 ECONOMIC IMPORTANCE

The growth of parasitic plants occurs at the expense of water, minerals, and organic compounds obtained from the host plant, which consequently leads to a lower dry weight accumulation by the host (Baker et al. 1995). Parasitism is thus able to cause damage and retardation of host growth, which is ultimately detrimental to the crop yield. *O. aegyptiaca*, arguably the most devastating representative of the genus, in combination with *O. crenata* present problems in more than 4 million ha of legume crops in West Asia and Northern Africa (Sauerborn 1991). An additional 2.6 million ha of tomato, potato, eggplant, and tobacco are threatened by a combination of *O. ramosa* and *O. aegyptiaca* (Linke et al. 1989). *O. cernua* affects nearly 6.5 million ha of sunflower fields in Europe and the Near East.

Yield reductions are dependent on the severity and timing of infestation and could range from 5 to 100% (Stewart and Press 1990). In some experiments an average of four *O. crenata* plants per broad bean, also known as faba bean, plant reduced the yield of the crop by half (Mesa-Garcia and Garcia-Torres 1984). The extent of damage also depends on, but is not limited to, the host's age at the time of broomrape attack and environmental conditions including available soil moisture (Jain and Foy 1989). In tobacco, *O. cernua* can reduce the yield by up to 52% depending on the time in the growing season, strength of infestation and availability of soil moisture (Krishnamurthy et al. 1985).

Besides reducing the crop yield, broomrapes contribute to the loss of crop quality by

adding foreign materials. Moreover, since some broomrape varieties could have multiple hosts, the number of alternatives for crop rotation is reduced in the fields infested with parasitic weeds. In addition, the ability of *Orobanche* spp. seeds to survive in the soil for a number of years presents a problem of losing the field to broomrapes for prolonged periods of time.

Due to yield losses caused by *Orobanche* spp., crop production practices have often been modified, but no single method is known at this time that can control *Orobanche* spp. infestations in a cost-effective manner.

1.1.6 MANAGEMENT OF OROBANCHACEAE

Control of *Orobanche* spp. is difficult for a number of reasons. The high seed production of broomrapes, prolonged viability of seeds in the soil (Cubero and Moreno 1979; Linke and Saxena 1991), lack of seed germination in the absence of a suitable host, and location of seeds deep in the soil are just some of the reasons.

Methods attempted for control of *Orobanche* spp. are numerous and varied, including biological, chemical, physical, and cultural practices. Despite some success, most of the methods have limitations with respect to effectiveness, cost, and application efficiency under field conditions (Jacobsohn 1986).

1.1.6.1 MECHANICAL, CULTURAL, AND PHYSICAL METHODS

1.1.6.1.1 Hand Weeding, Deep Inversion Plowing, Fire, and Tillage

Mechanized cultivation has not been feasible, generally, because the parasite's underground development is out of sight and out of reach of mechanical control. Hand pulling of parasite shoots before seed set has been the most commonly used technique and is still practiced where labor is inexpensive (Gharib 1983); however, such weeding only limits reinfestation and can be too late to prevent yield reductions. Although research in India indicated that hand pulling could exterminate *O. ramosa* within 3 yr (Krishnamurthy et al. 1977b), this method remains labor intensive, and time-consuming. Moreover, it can be done only after the parasite's shoot has emerged above the ground and after the major damage to the crop has been done. Moreover, timing of the hand weeding is important, because removing broomrape shoots before they blossom has been observed to result in additional emergence of *Orobanche* shoots

(Sauerborn 1991).

Deep inversion plowing, fire, and tillage have been proposed as means of controlling *Orobanche*. Burying *O. cernua* seeds at the depth of 20 cm resulted in little emergence of the parasite (Krishnamurthy et al. 1985). Although reductions in infestations of 80 to 90% can be achieved, subsequent cultivation can bring buried seeds up to the soil surface and reinfestation may occur (Parker and Riches 1993). Burning plant residue at the end of the growing season could prevent further increase of the seed bank in the soil and reduce carry-over of the seeds, however the use of fire is impossible during crop growth and development (Parker and Riches 1993). Hoeing between the rows also has a poor efficacy due to the close proximity of the parasite to the host plants (Garcia-Torres 1994; Parker and Riches 1993), thus increasing the risk of injury to the host.

1.1.6.1.2 Time of Planting, Trap and Catch Crops

The alteration of the crop sowing date and the use of trap and catch crops have also been suggested to reduce the population of broomrapes in the field. Late plantings have been shown to progressively decrease *O. crenata* infestations in faba bean, pea, vetch and carrot (Mesa-Garcia and Garcia-Torres 1986; Van Hezewijk et al. 1987). The sowing of winter crops in December/January instead of October/November is a traditional control measure employed in the Orobanche-infested fields in the Mediterranean region (Sauerborn 1991). However, late planting consistently results in the decrease of the crop yield (Garcia-Torres et al. 1991) due to shorter growing season, unless early-maturing varieties are used.

The use of trap and catch crops is perhaps one of the best methods currently available to manage broomrape infestations as part of the integrated control practices (Sauerborn 1991). Trap crops and catch crops stimulate the germination of the broomrape seeds. Trap crops cannot be infested; however, catch crops (which can be infested) along with germinating or developing broomrape must be destroyed before the parasite blooms and develops seeds. Several trap crops have been discovered for *O. aegyptiaca*, *O. ramosa*, *O. cernua*, *O. cumana*, and *O. crenata* (Abu-Irmaileh 1984; Kasasian 1971; Krishnamurthy and Chandwani 1975; Krishnamurthy et al. 1977a), however only very few practical examples of trap crops used by farmers have been reported. This method is only partially effective since many parasitic weed seeds remain viable but do not germinate in the lower soil horizons. In addition, small farms often cannot afford to leave the field for a year or two to be sown with false hosts such as trap or catch crops (Garcia-Torres 1994).

1.1.6.1.3 Soil Solarization

Soil solarization is a technique that sterilizes the soil using solar irradiance. The controlling effect of solarization is a soil temperature increase. Solarization efficacy is dependent on the proper timing and duration of the method. During the time of the year with the highest sunlight, the ground is covered with transparent polyethylene sheets and is left for at least 50 d (Sauerborn et al. 1989). Solarization for 10 d during the hottest time of the year and for 50 d during a milder season resulted in stimulation and conditioning of the *Orobanche* seed bank and as a result more broomrape shoots emerged instead of being killed (Sauerborn 1991). Most recently, clear plastic has been replaced by black plastic, which appeared to be effective under the hottest air temperature conditions (Abu-Irmaileh 1991). Moreover, moist soil is essential for better heat conductivity and for keeping parasitic seeds in amore susceptible, imbibed state (Parker and Riches 1993). Soil solarization appears to be an effective method for broomrape control with reports claiming *O. aegyptiaca, O. crenata*, and *O. ramosa* infestations reductions by 90 to 100% (Braun et al. 1987; Jacobsohn et al. 1980; Sauerborn and Saxena 1987). However, although many weeds could be controlled by short-term solarization, there are some species that tolerate the treatment (Sauerborn and Saxena 1987). Additionally, as the soil during the treatment is heated up at a depth of no more than 10 to 15 cm, seeds that survived at the lower soil horizons could be brought up by operations requiring soil disturbance and immediately become troublesome in the planted crop (Parker and Riches 1993). Moreover, soil solarization remains an expensive method, mainly due to the cost of the plastic and equipment.

1.1.6.1.4 Effects of Soil Fertility

Severe infestations of broomrape have been associated with low soil fertility. Farmers in Jordan have claimed that the addition of manure to soil reduced the infestation in their fields (Abu-Irmaileh 1979). However, it also has been observed that while high levels of nitrogen reduced *O. ramosa* infestations in tomato and tobacco, it reduced the tomato crop yield as well (Abu-Irmaileh 1981). On the contrary, when nitrogen was applied with potassium and phosphorus, broomrape was effectively controlled and there was a significant increase in tomato yield (Abu-Irmaileh 1981). Such results suggest that further research is needed to better understand the effects of fertilizers on broomrape parasitism. Moreover, use of high levels of nitrogen is impractical due to the high cost of the application and possibility of groundwater contamination.

1.1.6.1.5 Resistant Varieties

Using cultivars resistant or tolerant to *Orobanche* infestations appears to be the most economical way to fight these weeds. Several *Orobanche*–resistant and economically important crops, such as sunflower (Antonova 1978; Dörr et al. 1994; Wegmann et al. 1991), faba bean (Cubero 1991; Zaitoin and ter Borg 1994), vetch (Gil et al. 1984, 1987; Goldwasser et al. 1997; Linke et al. 1993), tomato (Avdeev and Shcherbinin 1976; Kaswari and Abu-Irmaileh 1989), tobacco (Cubero 1986, 1991), cucurbits, tobacco (Cubero 1986, 1991), eggplant (Dalela and Mathur 1971) have been reported. However, the loss of resistance has been observed and reported in previously resistant sunflower cultivars (Antonova 1994). The loss of resistance is probably due to the existence of very diverse and complex *Orobanche* populations, which shift to more aggressive biotypes in order to adapt to the newly introduced cultivars (Garcia-Torres 1994). In addition, in some legumes broomrape parasitism has been associated with rhizobacterial nodulation. Bacterial nodulation was proposed to be a mechanism by which *O. crenata* could by-pass host root rhizodermis and gain infestation, whereas in the absence of simultaneous bacterial and parasite development no infestation on the faba bean was observed despite heavy broomrape germination (Petzoldt 1979). Nevertheless, development of resistant or tolerant cultivars appears to be one of the most promising directions in forming an integrated management program to control *Orobanche* infestations.

1.1.6.2 BIOLOGICAL METHODS

Biological control of broomrape has also been attempted by means of the insect *Phytomiza orobanchia* Kalt. (Hammad et al. 1967), which feeds on the inflorescences of the parasite (Mihajlovic 1986). The fungus *Fusarium solani* Mart. (Panchenko 1981) and the more aggressive *Fusarium oxysporum* Schlect. (Foy et. al. 1989) were found to be the most suitable pathogens for biocontrol of broomrapes. Although some success has been achieved via biological control, this method alone could not provide complete control of the parasite (Girling et al. 1979). Development and use of biological control practices is difficult due to complex interactions existing in biomes. Since fields usually contain a large variety of weeds that needs to be controlled at the same time, it is often difficult to maintain the selectivity of biological control.

1.1.6.3 CHEMICAL METHODS

Chemical methods offer more hope for *Orobanche* control. Research efforts in chemical control have been divided between chemicals that are applied to broomrapes either directly or indirectly. Direct attack may involve preplant treatments of the soil to destroy the seed reserve, preventing germination. Chemicals may be used to stimulate the growth of the parasite in noncrop situations so that broomrapes are killed due to lack of nutrients in the absence of the host plant. Direct attack also can be used in postemergence situations to inhibit further development and to prevent future seed formation. Indirect control could be achieved by treating host plants just prior to or after attachment of the parasite. Systemic herbicides such as glyphosate proved to be most effective in this case (Foy et al. 1989; Hershenhorn et al. 1998a, 1998b; Jacobsohn and Kelman 1980; Kukula et al. 1985; Schmitt et al. 1979). Herbicide application may also be aimed at control of the ability of the host plant to exude nutrients and other stimulants for broomrape seed germination.

1.1.6.3.1 Germination Stimulants

In order to survive, broomrapes must attach to the host plant within a few days of germination. Therefore, broomrape germination in the absence of a suitable host is potentially suicidal (Eplee 1975), and any stimulation of the broomrape germination has a potential as a control method. Germination stimulants such as ethylene, strigol analogs (GR5, GR7, GR24, and GR28) have been shown to have an effect on the *O. ramosa*, and *O. crenata* (Johnson et al. 1976; Saghir 1986; Zahran 1982). However, reports coming from the laboratory as well as the field tests are often inconsistent.

1.1.6.3.2 Preplant and Preemergence Herbicides

Until the 1980s no soil-applied herbicide had been accepted as an effective tool in *Orobanche* control. Although some compounds such as trifluralin (Table 1.1.2.), and diphenamid (Puzzilli 1972, 1973, 1974, 1976) were shown to have some selectivity, none of them appeared to be reliable enough to become effective field treatments (Foy et al. 1989). Latest research has shown that imazethapyr has a good selectivity against *O. crenata* in faba bean, lentil, chickpea, and pea (Garcia-Torres and Lopes-Granados 1991; Jacobsohn and Eldar 1992). Related to imazethapyr, imazaquin and imazapyr appeared less successful in legumes than

imazethapyr but imazapyr was effective in sunflower (Parker and Riches 1993). Chlorsulfuron was reported to control *O. ramosa* in both faba bean and sunflower (Parker and Riches 1993). In vitro studies have shown significant reduction of parasite radicle elongation when *Orobanche* seeds were treated during preconditioning and germination stages with sulfonylurea herbicides (Hershenhorn et al. 1998a, 1998b); however, these studies await field testing.

1.1.6.3.3 Soil Fumigation

One of the most reliable and effective method of *Orobanche* spp. control available, to date, is soil sterilization prior to planting by fumigation with methyl bromide (Foy et al. 1989). Methyl bromide is especially effective (Wihelm et al. 1959) and is routinely used in large-scale farms in Israel (Parker and Riches 1993). This chemical controls nearly 100% of the parasite seed bank in the soil; however, it also destroys other microfauna of the soil. The application of methyl bromide is extremely hazardous to human health, expensive, requires special equipment and technology, and can be used only in very special cases and/or on high value crops (Foy et al 1989).

1.1.6.3.4 Postemergence Herbicides

Although more than 200 herbicides have been tested for *Orobanche* control, most of them appeared to be ineffective. Currently, the best results have been achieved with glyphosate (Jacobsohn and Kelman 1980; Kukula et al. 1985; Schmitt et al. 1979), a postemergence, systemic herbicide that translocates in the xylem as well as in the phloem and accumulates in the root parasite. Unfortunately, only a few crops have any level of glyphosate tolerance. Moreover, if the number of parasite attachments on the host is high, the total amount of the herbicide applied to the plant is going to be divided among the attachments and could be less then sufficient to destroy the parasite. In addition, the glyphosate application date is difficult to determine, since it has to be early in the season before *Orobanche* shoots emerge from the ground, making it difficult to determine the economic threshold for application.

In addition to glyphosate, the imidazolinone herbicides have proved to be effective in a variety of crops when applied postemergence. Imazaquin has given better control of broomrapes than glyphosate when applied to faba bean (Linke 1992) and tobacco (Vasilakakis et al. 1988). The range of crops in which herbicides could be applied is likely to increase with further research in genetic engineering of herbicide-resistance. Some of these crops are already available and are

widely marketed.

1.1.6.3.5 Genetically Engineered Herbicide-Resistant Crops

Most recently, the search for crops tolerant and/or resistant to *Orobanche* spp. infestations as well as studies of the resistant mechanisms and breeding of the tolerant/resistant varieties has become increasingly popular. Reports indicate that purple vetch (*Vicia atropurpurea* Desf.), as opposed to common vetch (*Vicia sativa* L.), exhibits resistance to *Orobanche aegyptiaca* (Goldwasser et al. 1997). Earlier, crop resistance was reported in sunflower (Dörr et al. 1994) and faba bean (Zaitoun and ter Borg 1994).

Introduction of herbicide-resistant crops for broomrape controls were first mentioned by Joel (1992). Isolation of the gene(s) responsible for host resistance/tolerance to herbicides and application of transgenic crops are of major interest to farmers as well as scientists.

Perhaps discovery, selection, or breeding of crop varieties resistant to both herbicide and parasite could assist in the reduction of broomrape populations. Development of transgenic crops such as tomato, soybean, and so on could allow foliar application of a translocatable herbicide or plant growth regulator which would not be toxic to the host but prevent the development and/or attachment of the parasite.

1.2 LITERATURE REVIEW ON RHIZOBIACEAE

1.2.1 INTRODUCTION

Acquisition and assimilation of nitrogen is second in importance after photosynthesis for plant growth and development (Vance 1997). The ability of a plant to acquire nitrogen is greatly enhanced by endophytic symbioses with a variety of microorganisms. In such symbioses the host plant provides microorganisms (endophytes) with photosynthates while receiving reduced nitrogen from the microsymbionts. One of the most studied of plant-bacteria associations is that which involves legumes (species of the family Leguminosae) and diazotrophic (nitrogen-fixing) soil bacteria belonging to the family Rhizobiaceae. Leguminous crops obtain nitrogen, the major growth-limiting factor for most plants, from a vast supply of gaseous N_2 in the air, by hosting symbiotic bacteria in nodules on their roots. The communication between bacteria and host plants, and the nodules that result from this interaction, form one of the most intriguing examples of specialized plant development.

Root nodules are structures produced on the roots of most legume plants following inoculation with rhizobacteria. The infecting bacteria fix atmospheric nitrogen and make it available to the host plant in a reduced form. In turn, the host plant provides bacteria with a source of energy and a place to live (the nodule). It is considered in many cases a condition of symbiosis because it is assumed that the plant benefits more from the fixed nitrogen than it loses sugars and other nutrients to the bacteria (Somasegaran and Hoben 1994).

Even though symbiosis is regarded as beneficial to both the host plant and bacteria, nodulation has been correlated with infestations by parasitic weeds (Parker and Riches 1993; Petzoldt 1979). Nevertheless, in recent years very little attention has been devoted to interactions between broomrape parasitism and *Rhizobium* nodulation in legumes. While an increase in parasitic weed infestation on nodulating legumes has been reported (Parker and Riches 1993; Petzoldt 1979), these discoveries have not been confirmed by research. Perhaps study of the interactions between broomrapes and their legume hosts could provide some insight into the physiology and anatomy of the parasitism, knowledge of which remains limited at this time.

1.2.2 CHARACTERISTICS OF THE RHIZOBACTERIA

Rhizobium spp. and their allies (*Azorhizobium*, *Bradyrhizobium*, and *Sinorhizobium*) are Gram-negative, nitrogen-fixing bacteria that cause formation of nodules on roots of legume

plants. Rhizobia (fast growing *Rhizobium* spp. and slow-growing *Bradyrhizobium* spp.) are medium-sized, rod-shaped cells, 0.5 to 0.9 μm in width, and 1.2 to 3.0 μm in length. Unlike many other soil microorganisms, rhizobia do not form endospores. *Rhizobium* spp. grow well in the presence of O_2, utilizing relatively simple carbohydrates and amino compounds. They are mobile by a single polar flagellum or two to six flagella (Somasegaran and Hoben 1994). Rhizobia multiply by simple cell division, with regeneration times ranging from 2 to 4 h for "fast-growing" species and 6 to 8 h for "slow-growers". Most rhizobia species produce white- and beige-colored colonies.

Rhizobia are facultative, unicellular, free-living soil bacteria. They are found mainly on the root surface (rhizoplane) and its adjacent soil area (rhizosphere). The presence and size of rhizobial populations in soil can be affected by the presence of host plants, soil pH, soil moisture, and soil temperature. Optimal growth temperature for most *Rhizobium* spp. ranges from 25 to 30°C with environmental pH in the 6.0 to 7.0 range. Excess soil moisture can have a negative effect on *Rhizobium* spp., whereas members of the genus *Bradyrhizobium* have been found to remain viable even after a year-long storage in purified water at ambient temperatures (Somasegaran and Hoben 1994). The number of bacteria in soil can range from undetectable to one million rhizobia per gram of soil.

The current classification system of rhizobacteria, a genetically diverse and physiologically heterogeneous group of bacteria, is based on the ability of rhizobia to nodulate particular members of the family Leguminosae. This reflects the high specificity of the rhizobia-legume association. The system arranges various legumes in cross-inoculation groups, while pairing a particular group with its rhizobial symbiont. The family Rhizobiaceae is subdivided into the following genera: *Rhizobium*, *Bradyrhizobium*, *Agrobacterium*, and *Phyllobacterium*, among which only the first two genera are known to be capable of symbiotic nitrogen fixation on legumes. The system has no value in a taxonomic sense, but serves a practical purpose. However, as with any taxonomic arrangement, with new findings proposing new genera and species the classification becomes increasingly complex. One should always keep in mind that taxonomy does not necessarily represents the true relationship of species in nature but is an attempt to organize current knowledge in a logical framework.

1.2.3 NODULATION

Root nodules are highly organized, hyperplastic tissue masses derived from cortical cells of the root (Hirsch 1992; Vance et al. 1988). Major steps involved in the infection and development of a soybean [*Glycine max* (L.) Merr.] nodule are summarized Figure 1.2.1.

Nodulation involves a complex signal exchange between host plant and inoculating bacteria, followed by the physical contact and root hair deformation. Upon root hair curling, bacteria initiate formation of the infection thread, which grows toward the nodule primordium where bacteria are released in the primordium cells and form symbiosomes. In symbiosomes, bacteria form bacteroids and proceed to fix nitrogen.

1.2.3.1 INFECTION

1.2.3.1.1 Signal Exchange Between Legume and Rhizobacteria

The nodulation process begins with a complex signal exchange process between the host legume and bacteria. The plant root exudes a chemical signal, usually a phenolic compound such as flavone, flavanone, or isoflavanone (Figure 1.2.2.), which induces a gene expression (*nod* genes) in the bacteria (Peters et al. 1986). *Nod* genes encode proteins, which catalyze the synthesis of specific lipo-chito-oligosaccharides, Nod factors (Long 1996). In turn, Nod factors released by the rhizobacteria induce cell division in the root cortex, resulting in new cells forming a nodule primordium. The signal exchange is followed by the physical contact between rhizobacteria and the root hair surface (Franssen et al. 1992; Hirsch 1992). Upon contact with the host root, the bacteria penetrate the root and move in the root cortex toward the nodule primordium. In red clover, alfalfa, and pea, where rhizobacteria form elongate-cylindrical nodules with indeterminate apical meristematic activity (Figure 1.2.3a.), the nodule primordium is formed in the inner cortex. In this type, nodule-fixed nitrogen is transported as amides. In other legumes, such as soybean and common bean (*Phaseolus vulgaris* L.), the nodule primordium is formed in the outer cortex, resulting in spherical nodules and determinate internal meristematic activity (Figure 1.2.3b.). In these nodules fixed nitrogen is transported as ureids (Vance 1997).

1.2.3.1.2 Penetration of the Host Legume

Penetration of the host root by bacteria can be either intracellularly or intercellularly (Pawlowski and Bisseling 1996). In intracellular infection, after attachment of bacteria to the root hair tips, the tips curl tightly, trapping rhizobia in the curls (Kijn 1992). Inside the “trap” bacteria induce the hydrolysis of an adjacent portion of the root hair cell wall (Callaham and

Torey 1981; van Spronsen et al. 1994), which leads to invagination of the plasma membrane and deposition of new cell wall material (Brewin 1991; Kijn 1992). Curling of the root hair is followed by formation of the infection thread, a tubular structure that ramifies through root cortex (Figure 1.2.4) and provides the passage for infecting bacteria to enter the root (Rolfe and Gresshoff 1988). An infection thread begins to grow toward a nodule primordium by traversing cortical cells (Berry and Sunell 1990) through radially aligned cytoplasmic bridges (van Brussel et al. 1992). In a manner similar to the root hair and pollen tube growth, the infection thread grows at its tip (Dart 1974). After reaching the nodule primordium, bacteria become enclosed in a membrane of plant origin, the peribacteroid membrane (PBM), and are released into primordium cells (Udvardi and Day 1997).

1.2.3.1.3 Symbiosome Formation

In response to chemical signals that are poorly understood, the infection thread disintegrates, while bacteria are taken into the cells via endocytosis of the plasma membrane, where along with PBM and the space between the membrane and bacteria, they converge to form a symbiosome (Bassett et al. 1977; Bergersen and Briggs 1958; Roth and Stacey 1989; Roth et al. 1988). In the symbiosomes, rhizobia differentiate into bacteroids and begin to fix nitrogen (Newcomb 1981). Simultaneously, the PBM undergoes differentiation and proliferation filling the infected cell with symbiosomes which number in the mature legume nodule could exceed several thousands (Bergersen 1982). In legume-rhizobia interactions the PBM represents the physical interface between the symbionts, and is essential for stable symbiosis. PBM degradation leads to symbiosis arrest and senescence (Werner et al. 1985). Since the infection thread appears to determine the speed and direction of bacterial penetration through the cortical cells of the root, the intracellular infection route permits the plant to have more stringent control over the microsymbiont during the infection process (Pawlowski and Bisseling 1996).

Lateral or adventitious roots emerging from the main root and/or stem form gaps in the root epidermis. These gaps can also be used by bacteria for root penetration, called intercellular infection (Chandler et al. 1982; James et al. 1992). This type of infection pathway is generally used for formation of stem nodules on *Sesbania* spp. (Tsien et al. 1983) and is not common for major legume crops. Rhizobia entering a root via an intercellular route penetrate the middle lamella between intact epidermal cells (Miller and Baker 1985; Ndoye et al. 1994). However, in some cases the intercellular infection may be followed by the collapse of cortical cells, showing characteristics of a pathogen attack by bacteria (Chandler et al. 1982).

1.2.3.2 NODULE INITIATION

1.2.3.2.1 Cortical Cell Divisions

Nodule formation begins with the activation of specific cells in the root cortex and occurs simultaneously with the infection process. However, in legumes bacterial infection is not a prerequisite for initiation of cell divisions, and purified rhizobial Nod factors were found to mitotically reactivate root cortical cells (van Brussel et al. 1992; Long 1996). Although the mechanism of Nod factor initiation of mitotic activity in root cells is not fully understood, Nod factors appear to have an effect on the phytohormone balance in the root cortex, most likely an increase in levels of cytokinin (Cooper and Long 1994), and could replace natural plant growth regulators (Long 1996; Spaink et al. 1993; Truchet et al. 1991).

During nodulation initiation, the expression of the early nodulin genes such as *ENOD12* (Scheres et al. 1990), *ENOD40* (Kouchi and Hata 1993; Matvienko et al. 1994; Yang et al. 1993), *Gm93* (Kouchi and Hata 1993), and *MtPRP4* (Wilson et al. 1994) is induced in primordia, resulting in tissue differentiation between nodule primordia and meristems of root or shoot. Recent studies report that in addition to the cells of epidermis and cortex, pericycle cells also react to Nod factors. For example, *ENOD40* gene is activated in the pericycle region opposite to the dividing cortical cells (Kouchi and Hata 1993; Matvienko et al. 1994; Yang et al. 1993). Furthermore, only certain cortical cells respond to Nod factors. As mentioned above, in tropical legumes, such as soybean, only outer cortical cells are mitotically reactivated. In temperate legumes, such as pea, vetch, and alfalfa it is the inner cortical cells that are reactivated, and only those that are located opposite the protoxylem poles (Kijn 1992; Libbenga and Bogers 1974), in a manner similar to lateral roots (Figure 1.2.5.). Unfortunately, the mechanisms controlling susceptibility of cortical cells in either case remain unknown. However, based on the experiments with compounds which mimic Nod factors mitogenic activity, a suggestion has been made that *ENOD40* expression in the pericycle of legume roots can cause the cytokinin/auxin ratio of the cortical cells to shift, and induce cell division (Mylona et al. 1995).

1.2.3.2.2 Regulation of the Nodule Formation

Nodule formation in legumes is highly regulated. Once a certain number of nodules has been formed, the nodulation process is inhibited (Dobritsa and Novik 1992). In addition, several

experiments with supernodulating mutants that do not restrict excess nodulation indicated that the aboveground biomass of the plants was reduced in growth (Hansen et al. 1990). Therefore, an assumption can be made that if a plant loses the ability to control the number of nodules formed, the symbiotic relationship can become detrimental.

Furthermore, nodule induction is restricted to the zone of root hair elongation which migrates with the growing root tip (Bhuvaneswari et al. 1981), and once the nodule is formed, the formation of other nodules in the zone of hair elongation and on the opposite side of the root is suppressed (Dobritsa and Novik 1992; Pawlowski and Bisseling 1996), but not eliminated.

1.2.3.3 NODULE STRUCTURE AND FUNCTION

When rhizobia are released into the cell from the infection thread, the nodule primordia begin to differentiate to form nitrogen-fixing nodules. Some of the early stages of this process involve the development of nitrogen-fixing bacteroids and proliferation of the PBM until the infected cells are filled with symbiosomes. Depending on the nature of the plant-bacteria symbiosis, one or more bacteroids may be found in each symbiosome. Bacteroids have frequent lobes, and express a complement of genes not expressed in the free-living bacteria (Layzell and Atkins 1997). The entire infected cell undergoes changes during symbiosome differentiation and becomes generally much larger with a more specialized C and N metabolism than normal uninfected cell (Day and Copeland 1991; Streeter 1995; Vance and Heichel 1991). The cytoplasm of the infected cells is filled with leghemoglobin, an O_2-binding protein which facilitates the transport of O_2 from the cell surface to the O_2-consuming symbiosomes in the center of the infected cell. Often in the sliced nodule, the presence of the deep-red colored leghemoglobin helps in distinguishing between true functional (effective) nodules which are maroon in color and non-functional (ineffective, senescent) nodules that have a whitish to pale-green appearance.

Nodules vary widely in shape, size, color, texture, and location. Nodules produced by the strain of rhizobacteria on one host legume may not resemble the nodules formed on other host in the same cross-inoculation group, suggesting that the shape and location of nodules is largely determined by the host plant. In addition, size, color and distribution of nodules is related to the status and nitrogen-gathering efficiency of rhizobia-legume association (FAO 1984). Two types of nodules are formed on legumes: determinate and indeterminate (Figure 1.2.6.). Both nodule types develop peripheral vascular bundles and a central tissue containing infected and uninfected cells (Figure 1.2.7.). In addition, in its vascular tissue, the nodule cortex contains phloem and xylem which are continuous with similar tissues of the host. In indeterminate nodules,

continuous cell divisions in apical meristems constantly produce new cells. These newly formed cells are added to the four different tissue regions in the nodule. Perhaps a developmental gradient from the meristem to the senescent zone is present and acts along the nodule axis (Vasse et al. 1990). On the contrary, in determinate nodules meristem activity ceases early in nodule development and new cells are formed by the division of infected cells. As a result cells in the central tissue are similar at any stage of development and, for the most part, already contain bacteria in them (Newcomb 1981; Rolfe and Shine 1984).

The legume nodule is a unique structure. Although it has a peripheral vascular system and thus has a stem-like organization, the origins for this type of morphology are still a subject of debate. It has been suggested that the structural complexity of legume nodules are a result of a specific and unique developmental program in leguminous plants (Pawlowski and Bisseling 1996). In fact, some legumes were found to produce rhizobia-free nodules spontaneously (Truchet et al. 1989), which are assumed to function as carbon storage organs (Pawlowski and Bisseling 1996).

It has been assumed that, in the context of the whole plant, nodules act as sinks for carbon assimilates (Hawker 1985) and sources of nitrogen (Mylona et al. 1995). Symbiotic nitrogen fixation takes place in the bacteroids where the bacterial enzyme nitrogenase catalyzes the following reaction:

$$N_2 + 8H^+ + 8e^- + 16\text{Mg-ATP} \rightarrow 2NH_3 + H_2 + 16\text{Mg-ADP} + 16P_i$$

Ammonia produced in the process is released by the bacteroid into the cytoplasm of the infected cell from where “sink” forces of the plant drive it out and redirect into other parts of the plant (Udvardi and Day 1997).

1.3 CONCLUSIONS

Many species of the family Leguminosae, as well as many other broadleaf crops, are susceptible to broomrape (*Orobanche* spp.) parasitism. Losses in crop yields and crop quality suggest that the studies on the host plant - bacteria - parasitic weed relations are necessary. Literature reports indicated a correlation between parasitic weeds infestations and rhizobacterial nodulation in legumes (Parker and Riches 1993). One possible explanation for this, as proposed by Petzoldt (1979), is that production of the stimulant is dependent on the infection of roots with *Rhizobium* nodules and that these nodules on the roots of a legume are the main site of broomrape attachment. In addition, infestations were observed to be more intense in aerobic conditions (Cubero 1973) when rhizobia are most active. However, these reports have not been

confirmed by other scientists. Perhaps, there are similarities in broomrape parasitism and rhizobacterial nodulation or one might have an effect on the other; however, the basic information regarding possible interactions is limited.

1.4 OBJECTIVES

The objectives of this project were to investigate the possibility of interaction between *Orobanche spp.* attack and *Rhizobium* nodulation in legumes, to study the anatomy of the *Orobanche minor* and *Orobanche aegyptiaca* connections to the host legume, and to study the effects of addition of the rhizobacteria on the *O. minor* and *O. aegyptiaca* development and growth.

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Table 1.1.1. Economically important species and crops parasitized by *Orobanche* spp.

		Orobanche				
Family	Crop	*aeg.* [a]	*mut.*	*cre.*	*cer.*	*min*
Solanaceae	Tomato (*Lycopersicon esculentum* Mill.)	+ + + [b]	+ +		+ + +	
	Eggplant (*Solanum melongena* L.)	+ + +	+ +		+ +	
	Bell pepper (*Capsicum frutescens* L.)	+				
	Potato (*Solanum tuberosum* L.)	+ + +	+ + +			
	Tobacco (*Nicotiana tabacum* L.)	+ + +	+ + +		+ +	+ +
Fabaceae	Broad bean (*Vicia faba* L. var. *major* L.)	+ +		+ + +		+ +
	Pea (*Pisum sativum* L.)			+ + +		
	Vetch (*Vicia sativa* L.)	+ + +		+ +		
	Chickpea (*Cicer arietinum* L.)	+ +		+ +		
	Berseem clover (*Trifolium alexandrinum* L.)	+ +				
	Peanut (*Arachis hypogaea* L.)	+ + +	+ +			
	Alfalfa (*Medicago sativa* L.)					+ +
	Birdsfoot trefoil (*Lotus corniculatus* L.)					+ +
	Red clover (*Trifolium pratense* L.)					+ +
	White clover (*Trifolium repens* L.)					+ +
	Subterranean clover (*Trifolium subterraneum* L.)					+ +
	Lentil (*Lens culinaris* Medic.)	+ + +		+ +		+
Apiaceae	Carrot (*Daucus carota* L.)	+ + +		+ + +		
	Celery [*Apium graveolens* L. var. *dulce* (Mill.)	+ +		+ +		
	Parsley [*Petroselinum crispum* (Mill.) Nym.]	+ +		+ +		
	Fennel (*Foeniculum vulgare* Mill.)	+ +		+ +		
	Angelica (*Angelica archangelica* L.)	+ +		+ +		
	Chervil [*Anthriscus cerefolium* (L.) Hoffm.]	+		+		
Brassicaceae	Cole (*Brassica oleracea* L.)	+ +				
	Horseradish (*Armoracia lapathifolia* Gilib.)	+ +		+ +		
	White mustard (*Sinapis alba* L.)	+ +				
	Turnip (*Brassica rapa* L.)	+ + +				
Asteraceae	Sunflower (*Helianthus annuus* L.)	+ +	+ +	+	+ + +	+ +
	Safflower (*Carthamus tinctorius* L.)	+ +				+ +
	Lettuce (*Lactuca sativa* L.)	+ +	+ +			+ +
	Aster (*Asater* spp.)	+ +				
	Gazania (*Gazania longiscapa* DC.)			+ +		
	Chamomile (*Matricaria chamomilla* L.)	+ +				
	Niger seed (*Guizotia abyssinica* L. F. Cass.)					+ +
Cucurbitaceae	Cucumber (*Cucumis sativus* L.)	+ +				
	Squash (*Cucurbita pepo* L.)	+				
	Muskmelon (*Cucumis melo* L.)	+ +				
Lamiaceae	Sage (*Salvia sclarea* L.)	+ +		+ +		
	Sweet basil (*Ocimum basilicum* L.)	+ +				
Geraniaceae	Geranium (*Pelargonium graveolens* L.)			+ +		
Verbenaceae	European vervain (*Verbena officinalis* L.)			+ +		

[a] *aeg. – O. aegyptiaca; mut. – O. mutelii; cre. – O. crenata; cer.- O. cernua; min. – O. minor.*
[b] + = rare; + + = common; + + + = very common, severe crop damage. (Foy et al. 1989).

Table 1.1.2. Common and chemical names of compounds listed in chapter 1.

Common name	Chemical name
Chlorsulfuron	2-chloro-*N*-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl1]benzenesulfonamide
Diphenamid	*N*,*N*-dimethyl-α-phenyl-benzeneacetamide
Glyphosate	*N*-(phosphonomethyl)glycine
Imazaquin	2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-3-quinolinecarboxylic acid
Imazapyr	(±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-3-pyridinecarboxylic acid
Imazethapyr	2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid
Methyl bromide	bromomethane
Trifluralin	2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)benzenamine

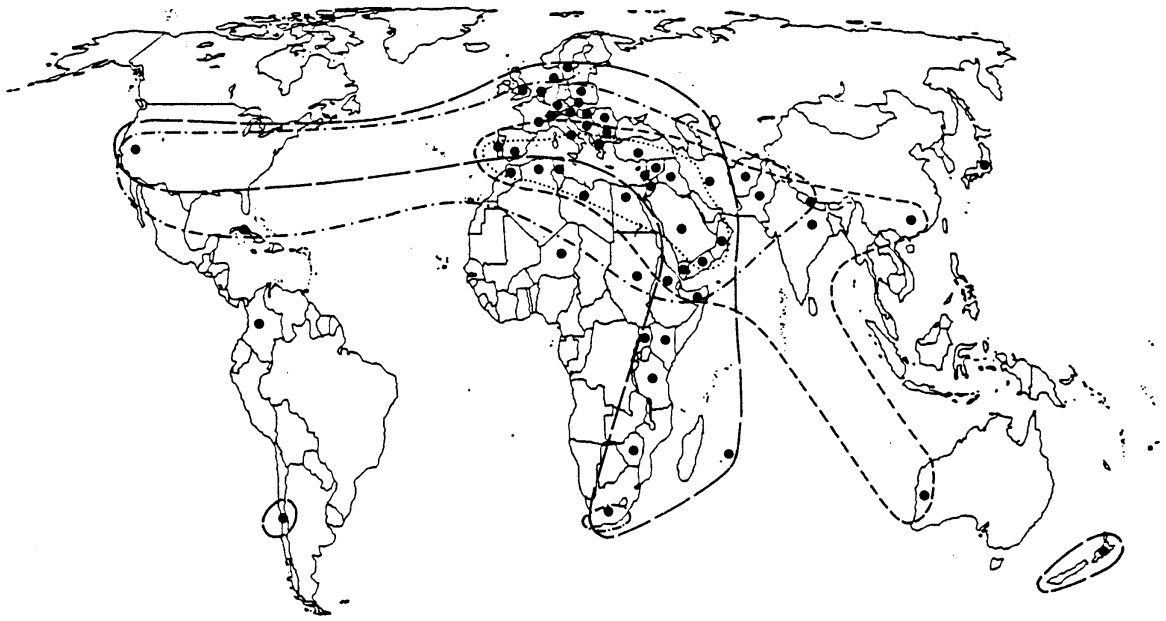

Figure 1.1.1. Worldwide distribution of important *Orobanche* species.

· countries with reported *Orobanche* occurrences

-------- *Orobanche cernua/cumana* __ __ __ *Orobanche minor*

..... *Orobanche crenata* _.._.._.._ *Orobanche ramosa/aegyptiaca*

(Sauerborn 1991).

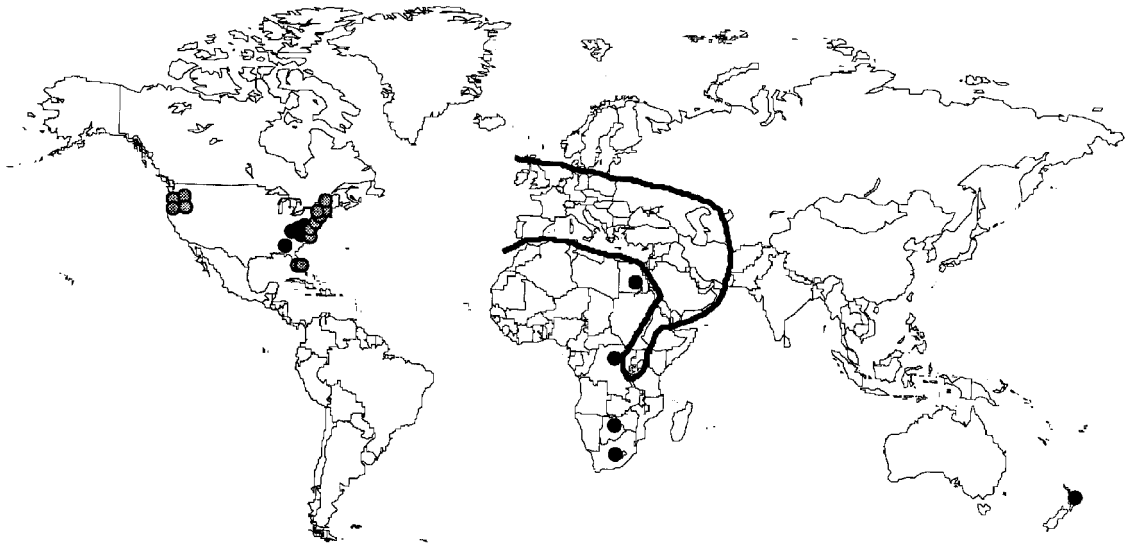

Figure 1.1.2. Small broomrape (*Orobanche minor* Sm.) general range of distribution. Black dots mark introductions; patterned dots mark introductions extirpated. (Musselman 1994).

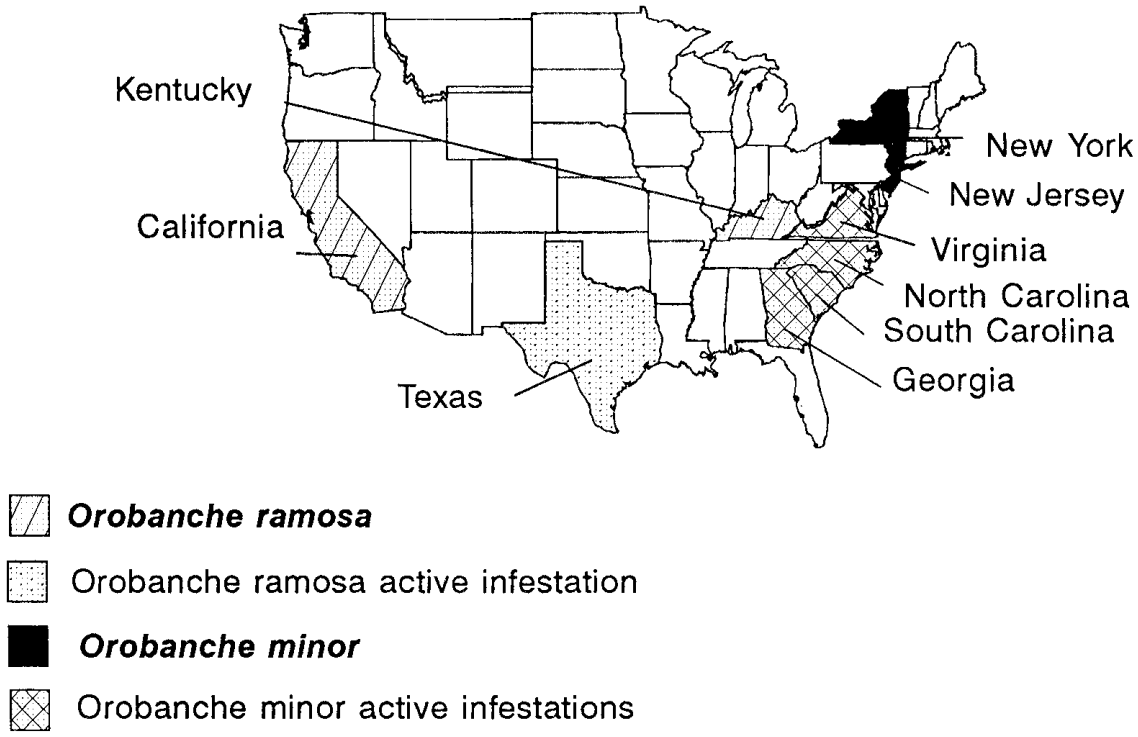

Figure 1.1.3. *Orobanche* infestations in the United States. (Eplee et al. 1994).

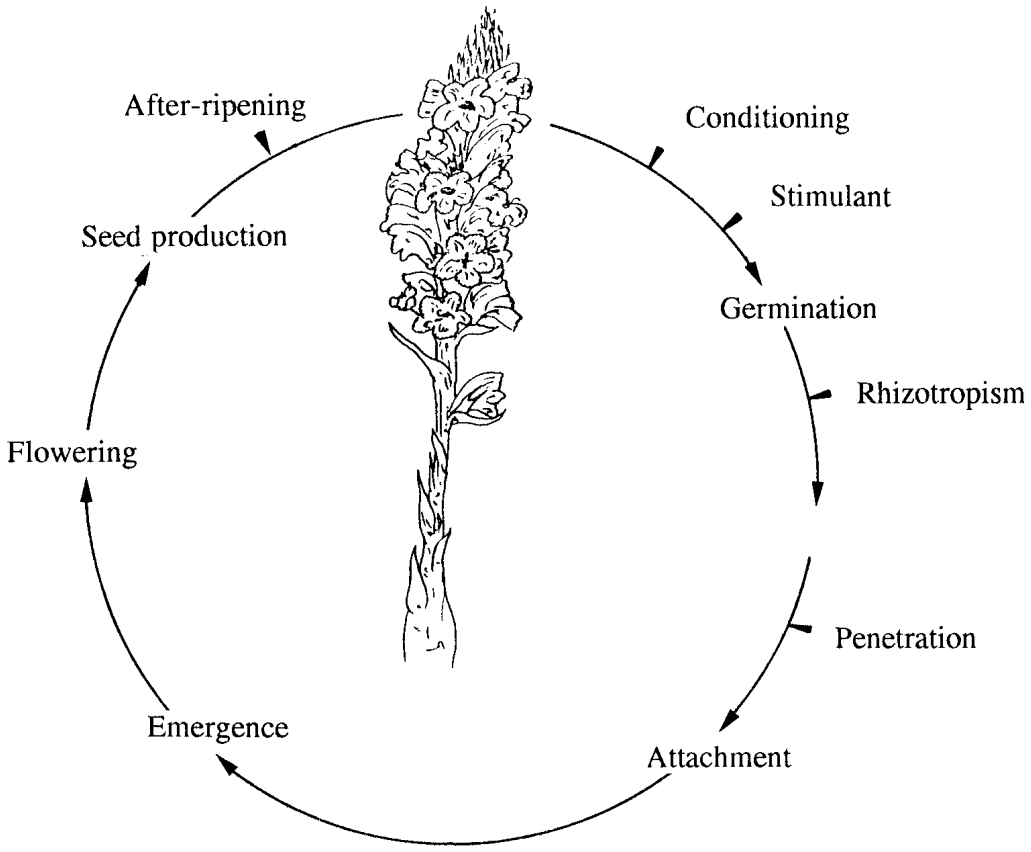

Figure 1.1.4. Life cycle of parasitic angiosperms. (Sauerborn 1991).

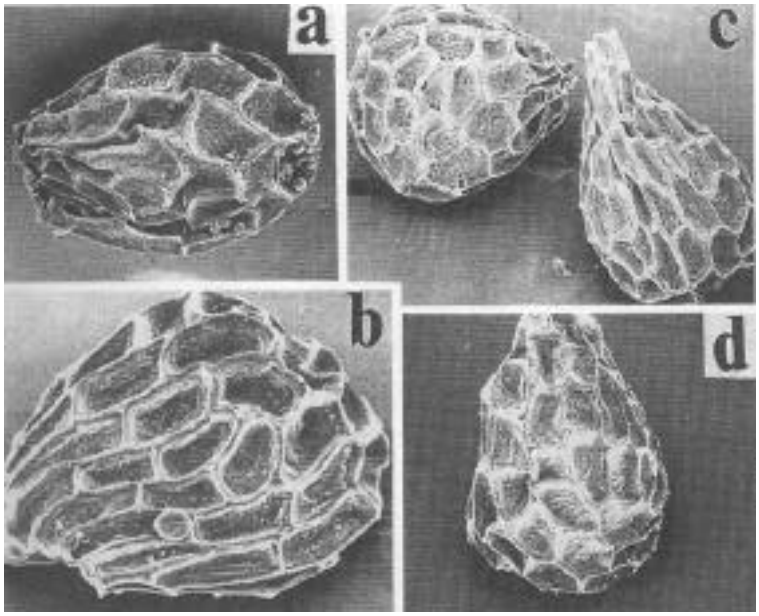

Figure 1.1.5. *Orobanche* spp. seeds. (Abu Sbaih and Jury 1994).

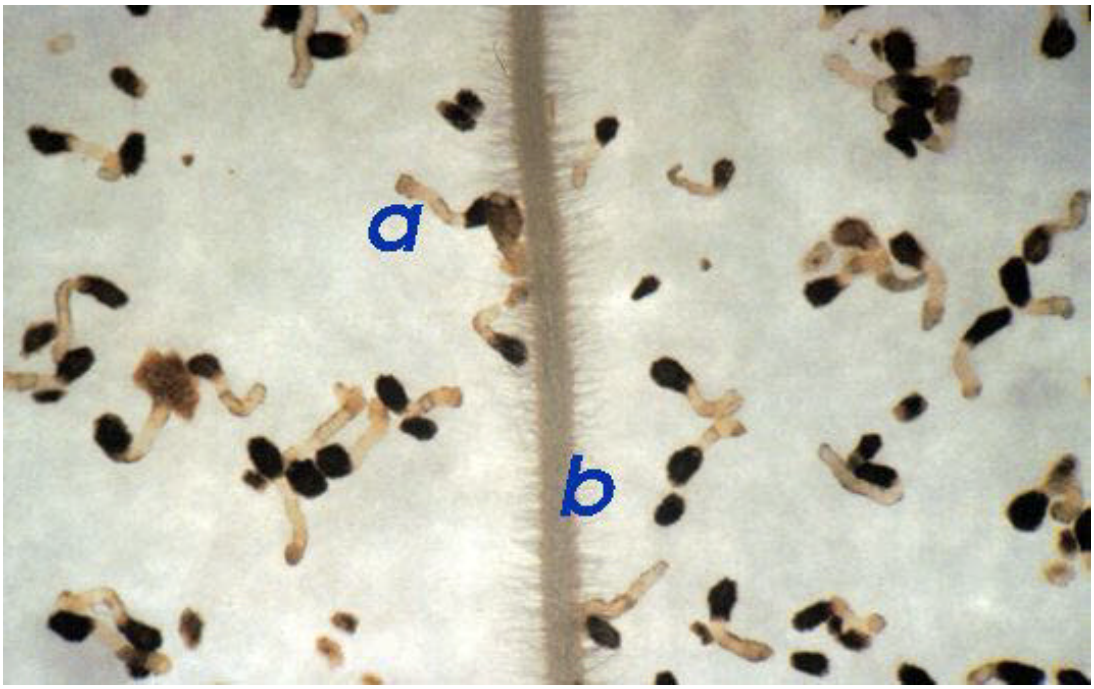

Figure 1.1.6. Germinating *Orobanche aegyptiaca* seeds. a – germinating seeds; b – red clover root.

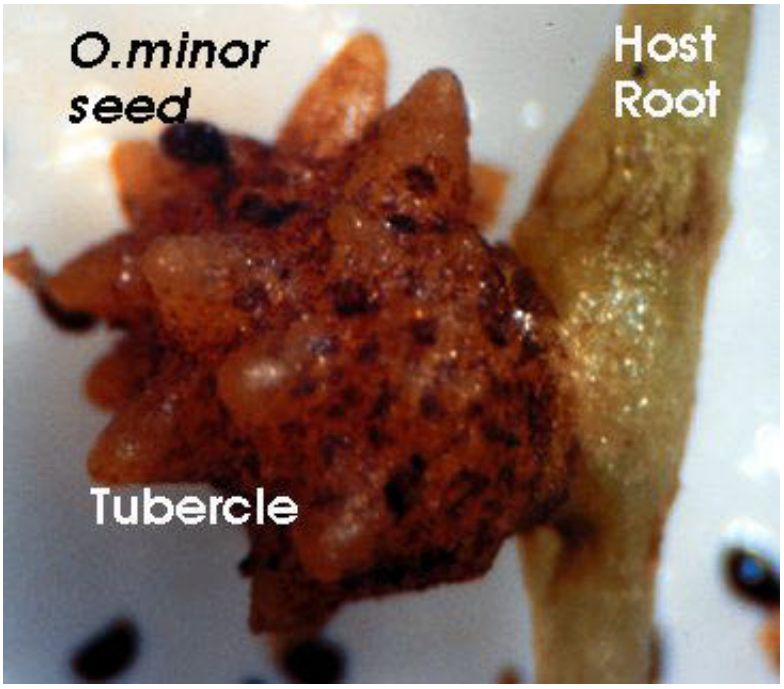

Figure 1.1.7. *Orobanche minor* tubercle on red clover (*Trifolium pratense* L.) roots.

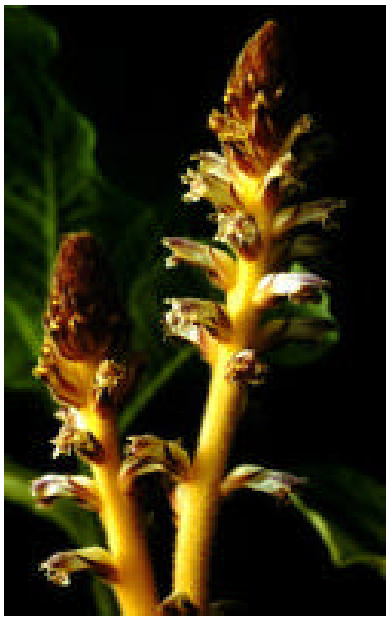

Figure 1.1.8. Flower spike of *O. minor* parasitizing tobacco (*Nicotiana tabacum* L.).

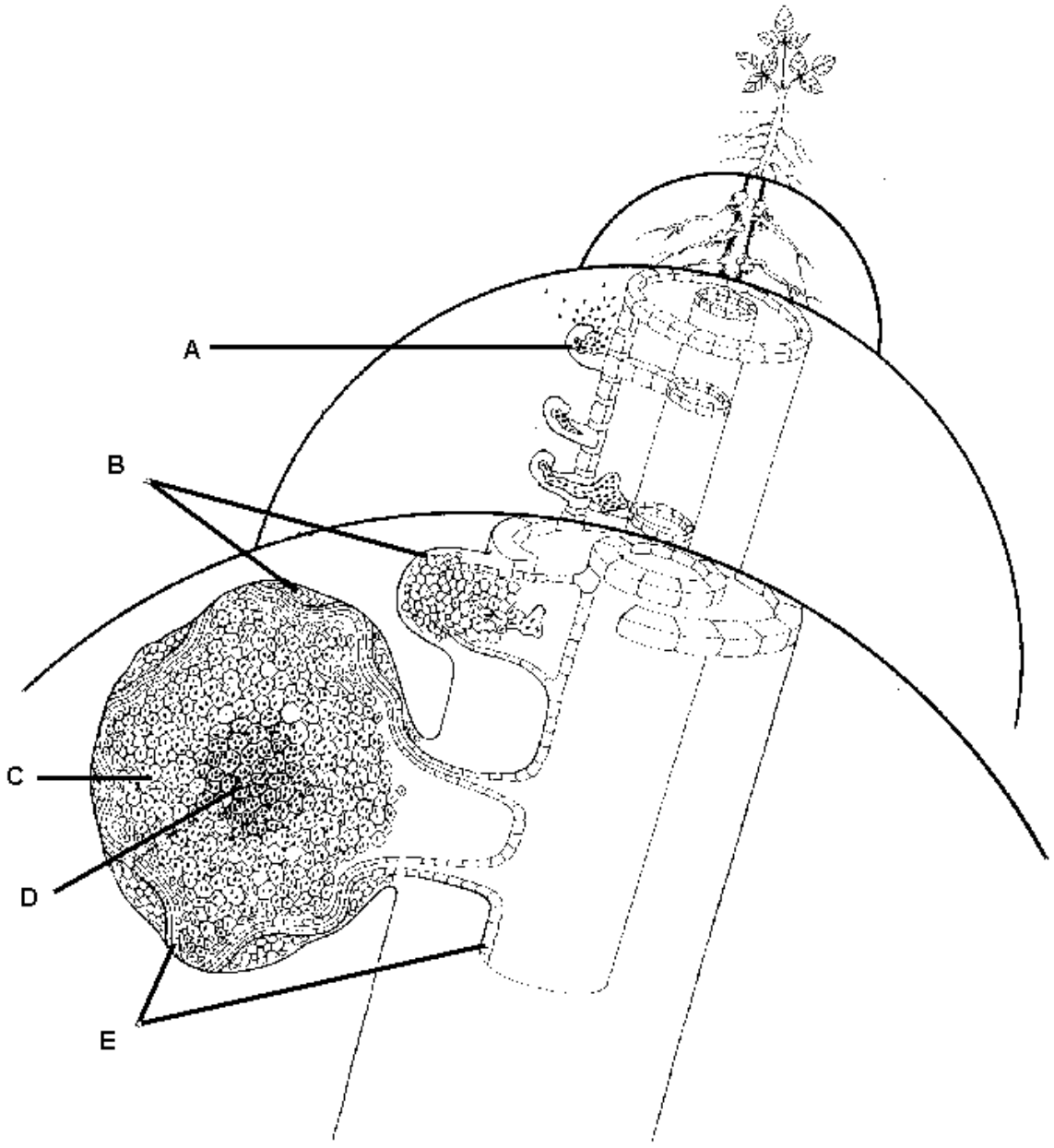

Figure 1.2.1. Nodule initiation and development on soybean [*Glycine max* (L.) Merr.] root. A. Infection thread forms inside the curled root hair; B. Meristem made up of uninfected cells and that provide for the growth of the nodule; C. Nitrogen-fixation zone containing cells with bacteroids and tinted red by leghemoglobin; D. Degeneration zone filled with senescent cells which do not participate in nitrogen fixation; E. Vascular system outgrowths derived from the host plant. (FAO 1984).

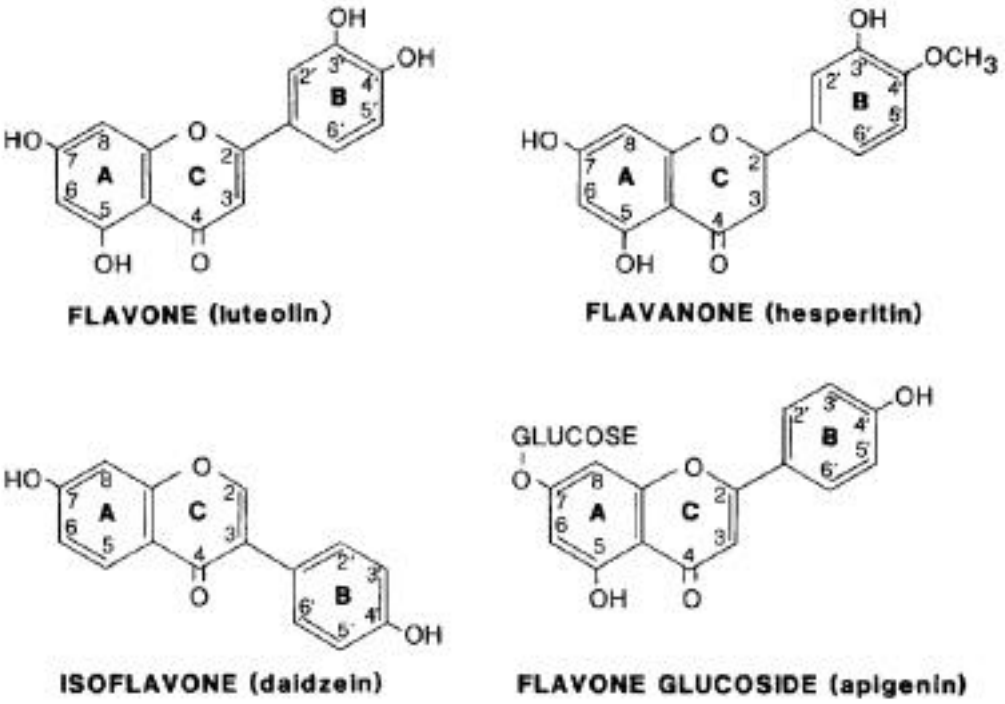

Figure 1.2.2. Flavonoid and isoflavonoid compounds exuded from the legume roots that activate and /or inhibit transcription of nod genes in rhizobia. B - ring derived from phenylalanine; A and C - derived from malonate. (Vance 1997).

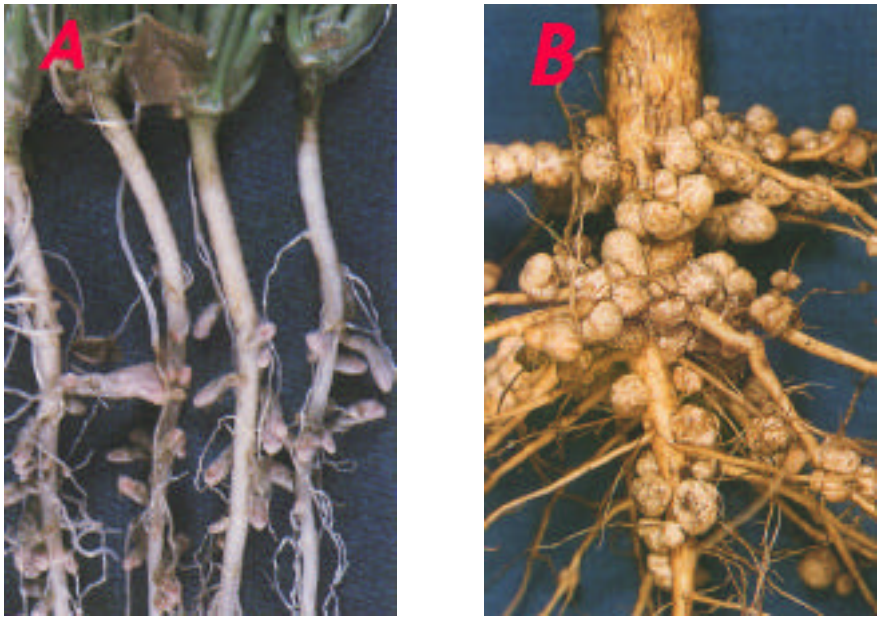

Figure 1.2.3. Nodule types. A. indeterminate nodules on white clover (*Trifolium repens* L.); B. determinate nodules on soybean [*Glycine max* (L.) Merr.]. (FAO 1984).

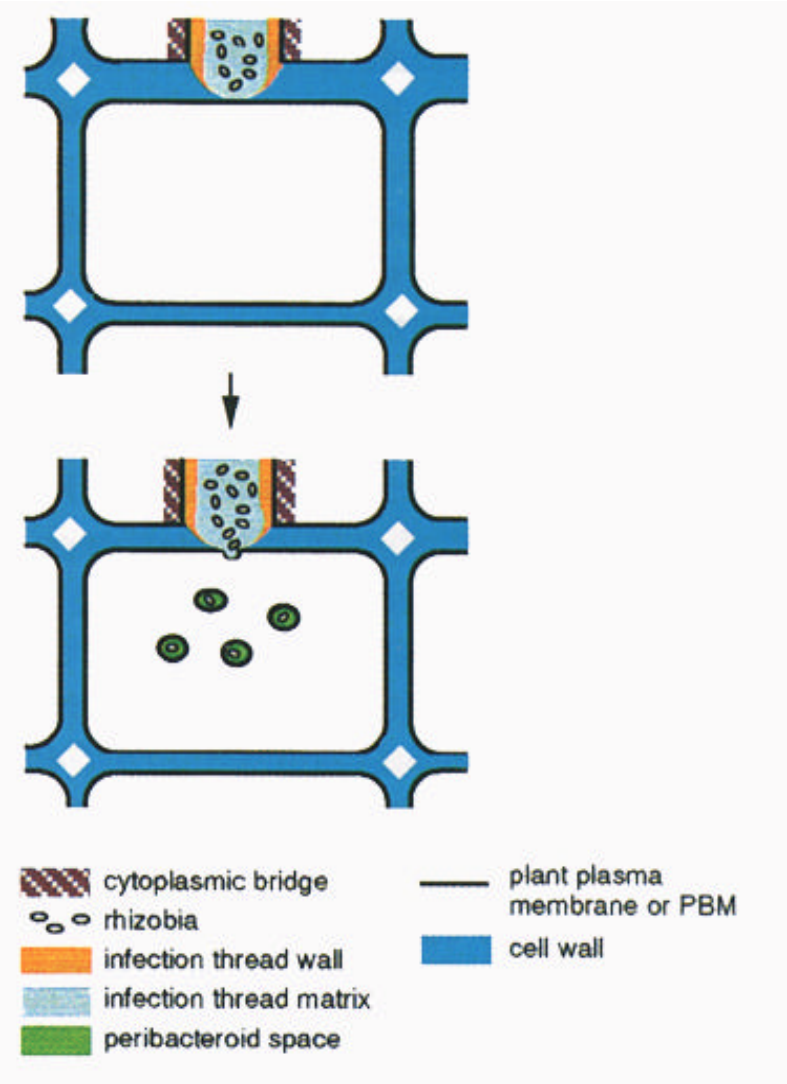

Figure 1.2.4. Uptake of endosymbionts into plant cells. Infection of a legume nodule primordium cell. The intracellular pathway of infection is shown. (Top) Within the infection thread, rhizobia are embedded in the infection thread matrix and surrounded by the fibrillar infection thread wall. (Bottom) When an infection thread reaches a nodule primordium cell, rhizobia are released into host cells from the unwalled tip of the infection thread via an endocytotic process (Newcomb 1976). Within the host cytoplasm, the bacteria are surrounded by the PBM, forming symbiosomes (Bassett et al. 1977). The symbiosomes multiply and enlarge (not shown). (Pawlowski and Bisseling 1996).

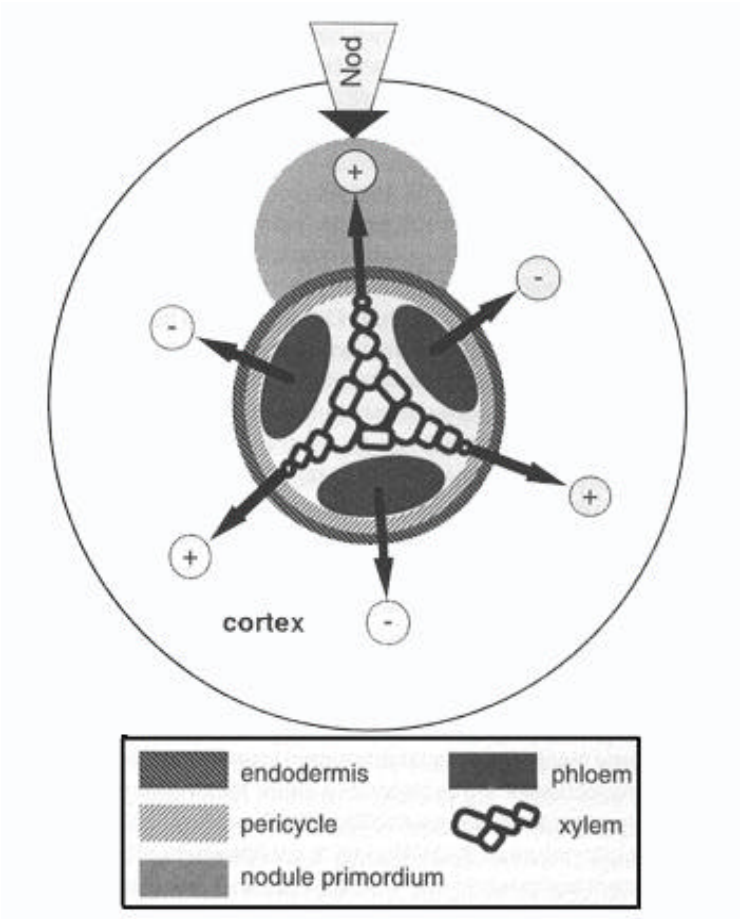

Figure 1.2.5. Determination of the position of mitotically reactivated cells. Mitotic reactivation normally takes place opposite protoxylem poles. Thus, factors determining the susceptibility of cortical cells to mitotic activation must be exuded by the stele, that is, positive factors from the xylem (+) and/or negative factors (-) from the phloem. These factors should form a gradient, which in coordination with Nod factors produced by the bacteria and taken up by the root (arrowhead) will determine the location of cell divisions (Heidstra et at. 1994). Because uridine preferentially stimulates cortical cell divisions in pea root explants opposite protoxylem poles, it is a candidate for a positive factor. However, it is not known whether uridine is in fact exuded by the protoxylem. (Pawlowski and Bisseling 1996).

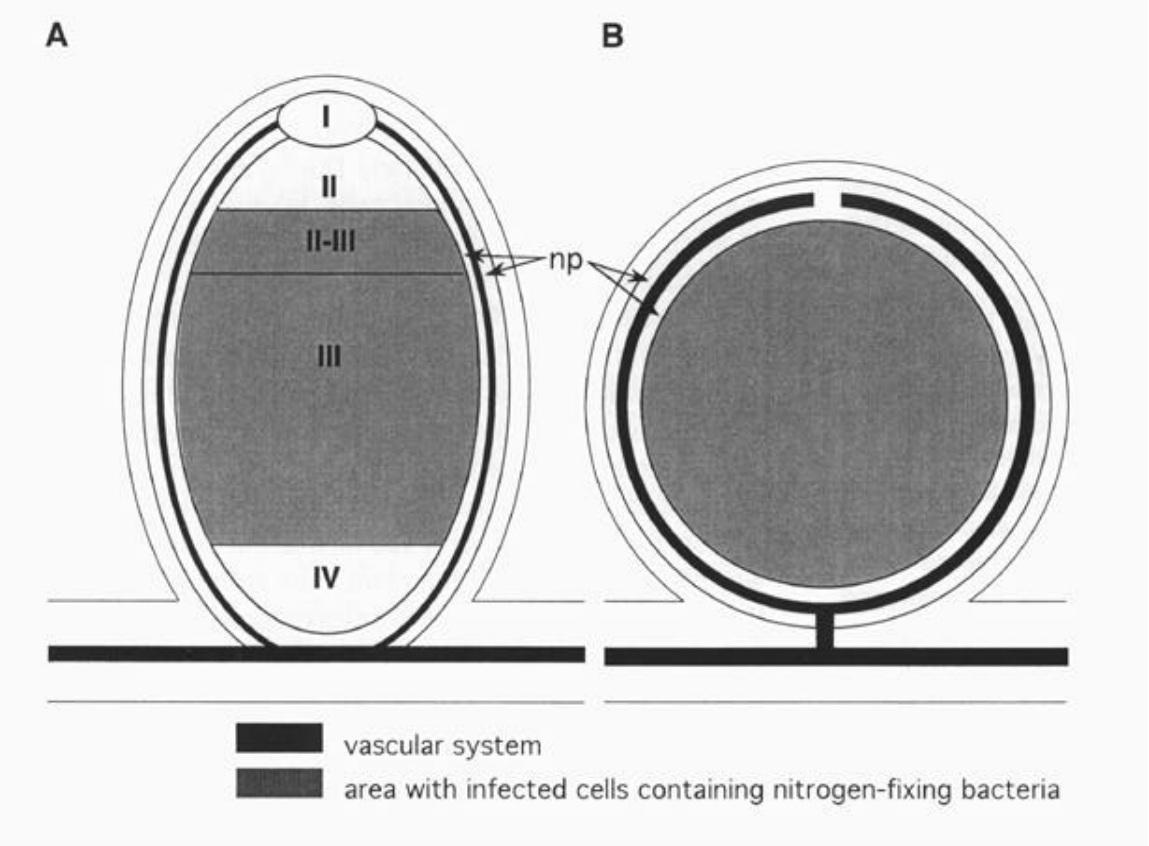

Figure 1.2.6. Nodule structure. (A) Indeterminate legume nodule. The central tissue can be divided into five zones (Vasse et al. 1990). Directly below the meristem (I), in the prefixation zone (II), cells become infected. Rhizobia are enclosed by PBMs and start to differentiate into their symbiotic form, the bacteroids. In the interzone bacterial nitrogen fixation starts (Yang et al. 1991) and takes place throughout the nitrogen fixation zone (III). In the senescent zone (IV), bacteria are degraded. The oxygen diffusion barrier is formed by the nodule parenchyma (np). (B) Determinate legume nodule. All cells of the central tissue are more or less in the same developmental stage. (Pawlowski and Bisseling 1996).

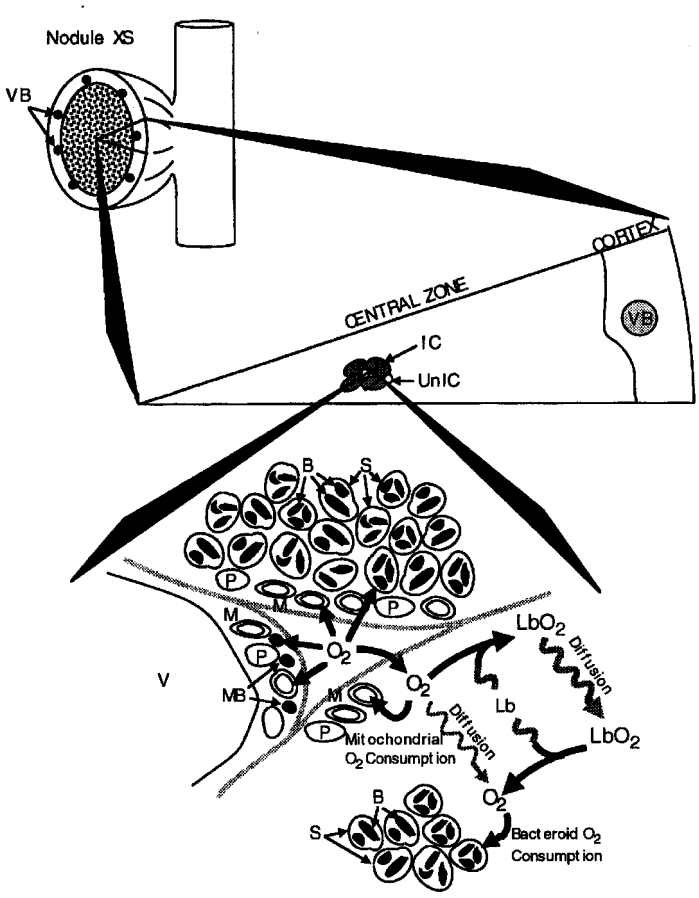

Figure 1.2.7. Structure of the soybean determinate nodule. Diagrammatic representation of a cross section of a legume nodule (in this case, a ureide producing nodule such as soybean) showing the nodule cortex with vascular tissue surrounding the central, infected zone. In the exploded view of the nodule, both infected (IC) and uninfected (UnIC) cells are depicted within the central zone, and a vascular bundle (VB) is shown within the nodule cortex. At the bottom of the figure, a gas filled space is shown along with portions of two infected cells and one uninfected cell. The uninfected cell contains a large central vacuole (V), plastids (P), mitochondria (M) and peroxisomes or microbodies (MB). The bacteria-infected cells lack microbodies, but contain large numbers of symbiosomes (S) that comprise bacteroids (B) enclosed within a plant membrane. Minimal subcellular details are depicted in one of the infected cells to show the path of leghemoglobin (Lb)-facilitated O_2 diffusion into the cell. Note that the plant organelles (mitochondria, plastids, peroxisomes) tend to be clustered around the gas-filled intercellular space (Millar et al. 1995). (Layzell and Atkins 1997).